DOCTOR OF PHILOSOPHY

The physiology of resource acquisition by Agrostis Stolonifera L. in heterogenesis environments

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Award date:
2000

Awarding institution:
Bangor University

THE PHYSIOLOGY OF RESOURCE ACQUISITION BY *AGROSTIS STOLONIFERA* L. IN HETEROGENEOUS ENVIRONMENTS

A Thesis submitted to the University of Wales

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2000

Dedication

To my late parents

Thomas William and Irene Margaret
Orchard

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Acknowledgements

I am grateful to both my supervisors, Professor John Farrar and Dr Chris Marshall for all their guidance and encouragement during the course of my studies and in the writing of my thesis.

My sincere thanks go to Gareth Williams for technical help and advice, especially with the radioactivity experiments, to Louise Thurlow for her willing assistance and to Dr John Gorham for the use of the porometer and for down-loading the data for me. I have very much enjoyed the friendship, stimulation and advice of all the members of the laboratory – Sarah and Harry, Lynne, Dave, Charlotte, Olga and Andre, Jos, PD, Sami and Bob and I am particularly grateful to Sheila for discussions on statistics.

I have had great encouragement from my family, from Anne Louise and Michael, and especially from my husband, John, without whose love and support during my time at Bangor – not least in proof-reading this thesis – this project would not have been possible.

ABSTRACT

An integrated approach to the study of the distribution of resources in the clonal grass *Agrostis stolonifera* L. is described. Paired, connected ramets of *A. stolonifera* were grown hydroponically with high (+P) or low (-P) phosphate treatments. Ramets in -P had a lower dry weight than +P with fewer leaves, branches and tillers and longer, less-branched roots. The length of the main stolons, the rates of production and elongation of leaves, photosynthesis and stomatal conductance were unaffected. Paired ramets given heterogeneous phosphate environments, with younger ramets given +P, were heavier than those grown in homogeneous +P. Vascular connections, investigated using contrasting dyes, revealed a bidirectional flow between ramets in the xylem of different vascular bundles. The rate of flow was greatest in the direction of the transpiration stream and shading the parent ramet had no effect on this. When one partner in a pair of ramets was given a P-treatment and the other either high, low or no water, droughted +P ramets showed reduced rate of stomatal conductance whereas droughted -P ramets did not. The uptake of ^{32}P, within paired ramets given contrasting phosphate treatments, was greatest by the roots of high P ramets. Transfer of P was faster towards +P than to -P ramets. P was carried in the xylem, circulated out of the leaves and translocated basipetally in the phloem. Approximately 95% P was in the vacuoles and 5% in the cytoplasm with the ratio of cytoplasmic to vacuolar P higher in the oldest leaves. The allocation of ^{14}C assimilate showed that little was translocated from a fed shoot but more was allocated to -P than to +P roots. The results show that adjacent ramets are physiologically integrated as regards water and phosphate but have independent carbon economies.

Chapter 1

General Introduction

Why do plants need phosphorus?

Plants need phosphorus for structural and metabolic purposes. A phosphorus concentration of about 0.2 - 0.5% dry weight (Epstein, 1972; Delhaize & Randall, 1995; Marschner, 1995; Jeschke *et al.,* 1996; Schachtman *et al.,* 1998) is sufficient to ensure normal growth. Phosphorus is the second (after nitrogen) most important macronutrient for plant growth and yet is the least available from the soil (Raghothama, 1999). The concentration of phosphate in fertile soils is lower than 10 μmol dm^{-3} - typically 2 μmol dm^{-3} - whereas plants contain up to 20 mmol dm^{-3} (Bieleski, 1973). The low concentration of inorganic phosphorus (Pi) in soil solution is possibly a major factor limiting growth in many ecosystems (Raghothama, 1999; Rao *et al.,* 1999). Above 1% phosphorus becomes increasingly toxic, although lower concentrations may be toxic in some tropical food legumes (Marschner, 1995).

Phosphorus is used structurally in the phospholipids of cell membranes. Phospholipids confer hydrophilic properties which are important in the interactions between the cell membranes and the surrounding ions. Phosphate groups also form the bridges linking nucleoside monomers into polymers of nucleic acids (Marschner, 1995). Phosphorus is required for the formation of energy storage compounds such as ATP, GTP and UTP. On hydrolysis of these molecules the phosphate group, along with the energy-rich pyrophosphate bond, can be transferred to other compounds such as glucose-6-phosphate and phosphoglyceraldehyde. ATP provides the energy for the synthesis of starch and the transfer, by carriers, of molecules across cell membranes (Marschner, 1995). ATP is used in the reversible phosphorylation of enzymes for the regulation of metabolic pathways (Theodorou & Plaxton, 1993). Plaxton (1996) found that ATP and other nucleotides were reduced under conditions of phosphate deprivation whereas pyrophosphate levels remained high. The respiratory

metabolic pathway altered as the glycolytic pathway enzymes were bypassed in favour of enzymes produced *de novo,* which did not require ATP or Pi to permit respiration to continue (Duff *et al.,* 1989).

How do plants acquire phosphate?

Barber (1995) listed the forms of phosphorus in soil as (1) ions and phosphorus compounds in the soil solution, (2) phosphorus adsorbed on the surfaces of soil particles, (3) phosphorus minerals and (4) P incorporated in soil organic matter. The commonest form of organic phosphorus in soil is phytic acid (inositol hexaphosphate) followed by nucleic acid from organic material. Inorganic phosphate is present as $H_2PO_4^-$ in soils with low pH and as HPO_4^{2-} in (less usual) alkaline conditions. These ions are in equilibrium at soil pH 7.2 (Barber, 1995). Phosphate removed from the soil solution by plant roots is replenished in the soil solution from the adsorbed phosphate (Barber, 1995) but phosphate is of very low mobility in soil (Bieleski, 1973; Clarkson & Lüttge, 1991; Fairhurst *et al.,* 1999).

When the roots of a plant first penetrate a new patch of soil they take up nutrients at a rate which depends on the concentration of that mineral in the soil (Ridge, 1991). As phosphates are both immobile and in limited supply in soils (Clarkson & Luttge, 1991) this rapidly results in depletion in the vicinity of the roots resulting in a zone of depletion with a radius of only 1 mm (Ridge, 1991). Plants therefore have a range of mechanisms for overcoming the problem of depletion of minerals around their roots. The simplest is to grow new roots into new patches of soil and forage for minerals. Phosphate-starved plants have longer and often heavier roots systems than P-sufficient plants (Friesen *et al.,* 1997).

Increased exudation of organic acids, particularly malic and citric, occurs from the roots of plants experiencing low phosphate (Raghothama, 1999) notably those lacking mycorrhizal association (Jones & Farrar, 1999). These acids decrease the pH of the soil in the rhizosphere, releasing Pi which is available to

plant roots. Whether these exudations increase the uptake of phosphate by plant roots, or whether they are released because low internal phosphate causes increased leakiness of plasma membranes is unclear (Jones & Farrar, 1999). Plants may also exude citric and malic acids into the soil to chelate iron and aluminium compounds on which phosphate may be adsorbed (Föhse *et al.,* 1991). The presence in the rhizosphere of organic carbon originating in the roots of plants increases the number of bacteria compared with the number in the bulk soil. The types of micro-organisms present and their physiological characteristics vary between plant species but they have a role in recycling organically bound phosphorus and making it available to plant roots (Marschner, 1995).

Mycorrhizas are the most widespread of associations between plants and micro-organisms and are considered to be an integral part of the Pi absorption and translocation pathways in a variety of plant genera (Raghothama, 1999). There is a high degree of correlation between the formation of mycorrhizas and the Pi status of the soil (Raghothama, 1999). Ectomycorrhiza (ECM) enclose the root surface and penetrate between the cells to form a network, the Hartig net. ECM are unusual on grasses (Wilcox, 1991). Vesicular-arbuscular mycorrhizas (VAM) are endo-mycorrhizas which penetrate the root cortex cells. VAM occur in association with many plants including *Agrostis stolonifera* (Grime *et al.,* 1988) and the frequency of association with mycorrhizal fungi is increased if plants are deprived of phosphate (Raghothama, 1999). The fungal hyphae have an important role in the acquisition of phosphate from the soil, increasing the rate of uptake three- to five-fold compared with non-mycorrhizal roots (Schachtman *et al.*, 1998). By extending beyond the depletion zone created by the plant roots, the hyphae increase the diameter of the zone of depletion of phosphate round each root (Smith & Read, 1997). Nielsen *et al.* (1998) found a higher concentration of phosphorus in the roots and leaves of low-P bean plants in the presence of mycorrhizal infection than in noninfected plants. Increased rates of uptake of phosphate in the presence of mycorrhiza have been reported in tomato (Bryla & Koide, 1998), in *Capsicum annum* (Aguilera-Gomez *et al.,* 1999) and in *Citrus* spp. (Syvertsen & Graham, 1999).

Phosphate (and other) ions are small enough to diffuse into the free space of the walls of root cells and this allows solutes to enter cells of the root cortex from the external solution (Marschner, 1995). However, interactions with cations in the cellulose cell wall may reduce the phosphate concentration to 10 times lower than in the external medium. Ullrich-Eberius *et al.* (1981) and Okihara *et al.* (1995) showed that phosphate is taken into the cortical cells by co-transport with protons (H^+) resulting in an increased pH of the surrounding medium, the number of co-transporting protons increasing up to 4 as the concentration of Pi in the medium decreases (Raghothama, 1999). The motive force is provided by a gradient of protons generated by H^+-ATPase in the plasma membrane. There are two systems involved in the uptake of phosphate by plant roots (Bieleski, 1973; Ullrich-Eberius *et al.*, 1981). These two systems consist of a constitutive high-affinity transporter which operates when concentrations of phosphate in the surrounding medium are low ($\mu mol\ dm^{-3}$ – the normal concentration of Pi in soil) and an inducible (or more likely, de-repressed) low-affinity transporter which may come into operation in high concentrations of phosphate ($mmol\ dm^{-3}$) (Bieleski & Läuchli, 1992; Dunlop *et al.,* 1997; Raghothama, 1999). Drew *et al.* (1984) hypothesised that the two systems working together could account for the increase in the rate of uptake of inorganic phosphate by the roots of plants with phosphate deficiency, an adjustment believed by Raghothama (1999) to be based on the internal phosphate status. An increase in the rate of uptake of phosphate by barley (*Hordeum vulgare)* results from shortage of phosphate within the plant tissues (Clarkson & Scattergood, 1982; Drew & Saker 1984). In contrast, the capacity of roots of *Artemesia* and *Pesudoroegneria* for uptake increases in local fertile areas (Caldwell *et al.* 1991). Roots proliferate in patches enriched with phosphorus and increase their rate of uptake to compensate for the low availability of the mineral to other parts of the root system (Drew 1975).

Lee (1988) reported that the influx of phosphate and the activity of extracellular phosphatase in barley were closely correlated. Lefebvre *et al.* (1990) investigated the role of acid phosphatase produced under conditions of P-deprivation, arguing that production of extracellular acid phosphatase in an

organic medium was an advantage to heterotrophic yeasts and bacteria but that higher plants, fixing their own carbon, would compete with microbes for inorganic phosphorus in a rich organic soil. Acid phosphatase in higher plants therefore may not have the same function of hydrolysing organic phosphorus (Po) to Pi as in micro-organisms, since when organic phosphorus was supplied to higher plant cells much inorganic phosphorus was found in the medium. Lefebvre *et al.* (1990) found that in *Brassica nigra* cells in suspension the rate of uptake of inorganic phosphate correlated more closely with the concentration of phosphorus within the cells than the activity of secreted phosphatases. They concluded that P-starvation induced production of acid phosphatases but that these are not directly related to the rate of uptake of phosphorus into the cells. Inhibitors of phosphatase did not slow down the rate of influx of phosphorus. They concluded that the role of phosphatases was to convert Po to Pi in which form it is available for uptake by plant roots. External acid phosphatase was produced by rice, wheat and tomato under conditions of low phosphate (Trull & Deikman, 1998; Rao *et al.,* 1999). Li *et al.,* (1997) found that the region of increased activity of acid phosphatase round the roots of lupin corresponded to a region of lower organic phosphorus. However this mechanism for increasing the availability of phosphates from the soil is not universal as Yan *et al.* (1995) found that Po was not a source of phosphorus for beans growing in tropical soils.

The external concentration of phosphate influences the efflux of P from the roots. This increases with increasing external concentrations of phosphate until it almost equals the influx (Raghothama, 1999). The rate of efflux of phosphate is also influenced by the internal concentration; although the total amount of phosphate lost from the roots is greater from plants with a high internal concentration of phosphate, a higher proportion of the phosphate taken in by low-P plants is lost in efflux (McPharlin & Bieleski, 1989) possibly related to increased permeability of phospholipid membranes under conditions of phosphate shortage.

How is phosphate distributed within plants?

Marschner (1995) states that the Pi taken up by roots is almost immediately incorporated into Po as ATP and sugar phosphates. In this form it is transported, mainly across the symplasm of the root cortex, and is reconverted to Pi for transfer into the xylem (Milthorpe & Moorby, 1969; Bieleski, 1973; Marschner, 1995). On arrival at the junction of the symplasm with the xylem, Pi is released, possibly by the action of glucose-6-phosphatase (Marschner, 1995), and loaded into the xylem. Phosphorus moves through the xylem mainly as inorganic ions (Pi), translocated by mass flow and moving at the same velocity as the xylem water whereas in the phloem there are significant quantities of Po (Läuchli, 1972; Bieleski, 1973). The activity of the P-transport system increases as the cellular inorganic phosphate and other P-containing compounds become diluted by growth of the plant (Jeschke *et al.,* 1997). Parenchyma cells associated with the termini of xylem and companion cells in the phloem of minor veins release large amounts of solutes into the translocation channels and the activities of these parenchyma cells, rather than the mass flow of water, determine the bulk movement within the channels (Van Bel, 1995).

Phosphate use efficiency, defined as unit dry weight of plant material produced per unit of phosphate present in the dry matter (Marschner, 1995), also increases in low-P plants (Raghothama, 1999). Increased phosphate use efficiency is manifested by 'the ability to realise an above-average yield under conditions of sub-optimal P supply' (Rao, *et al.,*1999). More phosphate is mobilised in plants with P-deficiency, hence there is an increase in the rate of transfer of stored phosphate from vacuoles to cytoplasm (Mimura, 1995) and phosphate is translocated round the plant more rapidly (Raghothama, 1999).

The activity of internal phosphatases increases under phosphate deficiency to permit remobilisation of phosphate from P-esters in the cytoplasm of the cells of senescing leaves (Bieleski, 1973; Duff *et al.,* 1994; Marschner 1995) leading to a faster turnover rate of phosphorus within the plant and an increased rate of export

from the phloem. Trull & Deikman (1998) showed most acid phosphatase activity to be in the tip of *Arabidopsis* roots, diminishing further back from the tip.

Phytase production also increases to release phosphate from phytic acid (Marschner, 1995). Several genes are expressed under conditions of phosphate deficiency. Raghothama (1999) suggested that more than 100 genes may be involved in the adaptations of plants to low phosphate including genes which code for proteins such as RNases, phosphatases and Pi transporters.

What effects are brought about by high and low concentrations of phosphorus in plants?

Since phosphate is required for structural and metabolic activities and is utilised in organic and inorganic forms (Mimura, 1995) it is to be expected that deficiency in the supply of phosphate has observable effects on the growth of plants. A comparison of cultivated and wild varieties of barley *(Hordeum spp.)* showed that the relative growth rate (RGR) was reduced by reduced supply of phosphorus in some varieties but not in others (Chapin *et al.,* 1989). Seedlings of birch (*Betula pendula)* deprived of phosphorus produced less shoot biomass than those grown with adequate phosphorus (Ericsson & Ingestad, 1988) as did tobacco seedlings (Paul & Stitt, 1993) and castor bean (Jeschke *et al.,* 1996). The shoots of cotton tended to be shorter (Radin & Eidenbock, 1984) with fewer and smaller leaves (Fredeen *et al.,* 1989) and to have fewer branches than plants given adequate phosphate (Slade & Hutchings, 1987) and to develop red colouration of stems due to accumulation of anthocyanin. The leaves were darker green than in plants with access to adequate phosphate because although the production of chloroplasts was unaffected by reduction in phosphorus supply, the mesophyll cells were often smaller (Marschner *et al.,* 1996). However Ryser *et al.* (1997) considered that a positive correlation between P supply and chlorophyll concentration in three species of grass may have been due to the provision of excess nitrogen to experimental plants also given high phosphate.

In plants given low phosphate the root:shoot ratio of dry weight (R:S) increases. Phosphate deficiency causes plants to allocate more carbohydrate to their roots (Fredeen, *et al.*, 1990; Paul & Stitt, 1993; Cakmak *et al.*, 1994; Jeschke *et al.*, 1997) and to retain a greater proportion of the absorbed phosphate in their roots (Bieleski, 1973; Raghothama, 1999). Increased allocation of carbohydrate to the roots of P-deprived tobacco seedlings not only decreases growth of roots less than growth of shoots but also increases growth of the roots compared with roots of plants given full nutrient medium (Paul & Stitt, 1993). Sugar concentration and growth of roots increase in *Phaseolus vulgaris* grown in low phosphate (Cieresko *et al.*, 1999). Where plants are grown in soil short of phosphate more and longer root hairs are produced (Raghothama, 1999).

What effect does shortage of phosphate have on photosynthesis?

Paul & Stitt (1993) and Theodorou & Plaxton (1993) reported that low phosphate does not affect the rate of photosynthesis but alters the utilisation of carbohydrate, whereas Fredeen *et al.* (1990) and Jeschke *et al.* (1997) showed that there is a reduction in the activity of enzymes required for photosynthesis. Fredeen *et al.* (1989); Lynch *et al.* (1991) and Jeschke *et al.* (1997) all noted a reduction in the rate of production of new leaves and a reduction in the leaf area of plants short of phosphate because of fewer and smaller leaves. These low phosphate plants had reduced rates of photosynthesis, a reduction which was also noted by Heineke *et al.* (1989), Marschner (1995), Dietz & Foyer (1986) and Jeschke *et al.* (1997) and attributed by Rao & Terry (1995) to diminished regeneration of ribulose bisphosphate. Dietz & Foyer (1986) pointed out that species vary in the sensitivity of their photosynthesis systems to shortage of phosphorus. Ciereszko *et al.* (1996) found that although the growth of shoots of *Phaseolus vulgaris* was less in low phosphate, the rate of photosynthesis was not reduced. However in the range of experiments referred to above not only were different plant species used but the concentrations of phosphate supplied varied such that high P was from 0.5 to 3.0 mmol dm^{-3} and low phosphate from 0 phosphate to 0.3 mmol dm^{-3}.

Does the phosphate status of a plant alter the distribution of assimilates?

Inorganic phosphate (Pi) may regulate the activity of the enzymes which catalyse starch and sucrose metabolism. Thus the starch content was greater and the sucrose content less in the leaves of soybean (*Glycine max)* (Fredeen *et al.,* 1989) and in sugar beet (*Beta vulgaris)* (Rao & Terry, 1995) in low phosphate plants than in plants given high phosphate. The increase in the R:S dry weight ratio under phosphate deficiency reported by Fredeen *et al.* (1989), Rao & Terry (1989), Paul & Stitt (1993), Jeschke *et al.* (1996) and Ciereszko *et al.* (1999) is possibly due in part to retention within the root of phosphate taken up by roots and in part to recirculation of phosphorus from the shoot back to the root. The roots of P-deficient plants may also have a higher carbohydrate content than those of P-sufficient plants (Fredeen *et al.*, 1989; Cakmak *et al.,* 1994; Jeschke *et al.,* 1996). Fredeen *et al.* (1989) suggested that P-deficient plants allocated more photosynthate to the roots to increase their capacity to absorb phosphate when this was in short supply. The phosphate status also has an effect on the uptake of nitrogen – a low concentration of phosphate reduces the rate at which nitrogen is taken up (and *vice versa*) (Schjörring, 1986; Jeschke *et al.*,1996).

The strength of a sink, defined as the ability to consume carbon in a 24 h period when the supply of carbohydrate is not limiting, may be altered by changes occurring in the source leaves bringing about changes in the supply of carbohydrates. Infection with fungal parasites may deplete the phosphorus supplies in the leaves and thus alter the rate of photosynthesis and subsequent partitioning to sinks. Zulu *et al.* (1991) showed that whilst the concentration of phosphate in the external medium has little effect on the rate of photosynthesis in healthy wheat (*Triticum aestivum)* leaves, infection with *Erysiphe graminis* reduces the rate of photosynthesis, an effect which is enhanced by low supplies of phosphate. However, low phosphate status does not directly affect photosynthesis and, since the concentration of non-structural carbohydrate overall is increased in the leaves of infected plants, the conclusion is that disease reduces the ability of the phloem to translocate sugars. This reduced ability is also observed in high-P barley infected with *Puccinia hordei* where it may be

due to the reduction in the pH of the apoplast of infected leaves reducing the proton gradient between apoplast and symplast and thus inhibiting the ability for sugar to be loaded into the phloem (Tetlow & Farrar, 1993). Carbohydrate is allocated more to the roots of low-P plants than to high-P soybean plants (Fredeen *et al.*, 1989). The ability of the roots to utilise sugar is altered by the P status (Paul & Stitt, 1993; Theodorou & Plaxton, 1993; Cierezsko, 1996; Jeschke, 1997). Low P results in reduction in the rate of growth of shoots (Rao & Terry, 1995) and since the rate of photosynthesis is unaffected there is more carbohydrate available for the growth of roots and hence the R:S ratio increases (Fredeen *et al.*, 1989). Thus changes in the R:S are directly attributable to the P status of plants.

How do plants respond to heterogeneous supplies of phosphate when grown in patchy environments?

The soil is a patchy environment with some pockets rich, and others poor, in nutrients. High-nutrient pockets may be large and coarse-grained or they may be small, giving a fine-grained distribution of patches (Fitter, 1994). Jackson & Caldwell (1993) found that an area of 0.5 m^2 soil had 40% less phosphate at one side than the other and that within the area the pockets of high phosphate were less than 10 cm in diameter. Plants themselves contribute to soil heterogeneity – adding nutrients by deposition of leaf litter and depleting the soil as their roots take up water and minerals (Robinson & Van Vuuren, 1998). Heterogeneity can be both temporal and spatial and plant roots may react plastically to maximise their uptake of nutrients and water (Marschner, 1995). Kovar & Barber (1989) used modelling to predict that in agricultural soils the uptake of nutrient by plants in a patchy environment would increase whereas Jackson & Caldwell (1996) using model simulation for calcareous soil, predicted that here the uptake of phosphate would be less in heterogeneous than in homogeneous distribution. Neither of these simulations took into account the ability of roots to proliferate in rich patches of nutrient but not all species show such proliferation (Grime, 1994). The uptake of resources may be different depending on whether the resource is exploited by fast-growing, large-diameter roots or by slower-

growing, fine roots (Fitter, 1994). For slow-growing plants soil-nutrient heterogeneity in time rather than in space may be more significant (Grime, 1994). A number of authors have shown, by means of split root experiments, that allocation of resources to roots in a rich patch involves a reduction in the growth of roots elsewhere (Drew *et al.*, 1975; Granato & Raper, 1989) and will only lead to greater growth of the whole plant if the gains made in the rich patch are greater than the losses incurred by less root growth in poor patches (Grime, 1994; Robinson, 1994, 1996).

How are clonal plants well adapted for the exploitation of patchy resources?

For a non-clonal plant the soil in which it is rooted, favourable or not, is the habitat in which it has to remain whereas for a clonal plant the possibility exists of daughter plants, ('ramets' Harper, 1977) finding better, or worse, habitats. Van Groenendael *et al.* (1996) define a clonal plant as one capable of naturally producing offspring by means of vegetative growth. Clonal growth is regarded as an evolutionarily primitive character in vascular plants (Jonsdottir & Watson, 1997). A clone may operate as a physiologically integrated system i.e a system in which resources such as carbohydrate, water and minerals are freely transported from one part of the clone to another along persistent connections between ramets. Alternatively, ramets may develop varying degrees of physiological independence, in which case resources are retained within individual ramets with limited or no sharing of resources between them (Pitelka & Ashmun, 1985; Marshall, 1990). Physiological connection between the ramets in a clone is also considered to be a primitive trait (Jonsdottir & Watson, 1997). Stress may bring about re-integration of ramets (Nyahoza, *et al.*, 1973; Alpert & Mooney, 1986). Jonsdottir & Watson (1997) distinguish 'disintegrators', in which connections between ramets rapidly break down, from 'integrators' in which the connections are maintained. They further sub-divide the integrators on the extent to which ramets are naturally interdependent when unstressed, either as clonal fragments (which they define as part of a clone with intact connections between two or more ramets) or as large clonal systems. In the classification of Jonsdottir & Watson (1997) *Aster spp.* show restricted integration in clonal

fragments (Schmid & Bazzaz, 1987) whereas *Trifolium repens* has restricted integration in large clonal systems (Kemball *et al.*, 1992; Chapman & Hay, 1993; Kemball & Marshall, 1995; Stuefer *et al.*, 1996). Jonsdottir & Watson (1997) classify *Agrostis stolonifera* in the group with restricted integration in large clonal systems. Examples of clonal species with full integration include the small ramet systems of *Solidago altissima* (Hartnett & Bazzaz, 1983, 1985) and the large ramet systems of *Carex arenaria* (Noble & Marshall, 1983). The ramets of clonal plants may share supplies of water, minerals and photosynthate and movement of resources is predominantly acropetal i.e. from older to younger parts of the clone.

.Table 1.1 *Some resources shared by clonal plants*

Resource	Translocation	Species	Author
Phosphate	From parent root to parent stolon, to young roots and shoots, to rhizome apex.	*Carex arenaria*	Noble & Marshall, 1983
^{14}C-assimilate	From fed shoot to defoliated shoots, entire root system, rhizome apices.		
Water	From ramets supplied with water to ramets supplied with saline.	*Ambrosia psilostachya*	Salzman & Parker, 1985
Carbohydrate	From a ramet in light to a sibling in shade.	*Fragaria chiloensis*	Alpert & Mooney, 1986
Water	From watered parent to a string of unrooted offspring.		
Nitrogen	From one of a pair of ramets to the other lacking nitrogen.		

Table 1.1 *cont'd*

Resource	Translocation	Species	Author
N-P-K fertiliser	From parent.	*Aster sp.* *Solidago sp.*	Schmid & Bazzaz, 1987
'Phostrogen' fertiliser	From parent. Increased the rate of production of new ramets.	*Glechoma hederacea*	Slade & Hutchings, 1987
Nitrogen	From parent. Increased the rate of production of new ramets.	*Fragaria chiloensis*	Alpert ,1991
Carbohydrate Nitrogen	Exchange of resources between ramets so shaded ramet acquired carbohydrate and N-deprived ramet acquired nitrogen.	*Fragaria chiloensis*	Friedman & Alpert, 1991
Mineral nutrients	Translocation to unrooted (nutrient-stressed) ramets.	*Agrostis stolonifera*	Marshall & Anderson-Taylor, 1992
Carbohydrate	From unshaded to shaded ramets.	*Potentilla anserina*	Stuefer *et al.,* 1994
Soil nutrients	To all parts of the clone resulting in greater biomass accumulation from heterogeneous than from homogeneous sources.	*Glechoma hederacea*	Birch & Hutchings, 1994
Carbohydrate Water	Division of labour; ramets grown in light/dry conditions maximised leaf growth whilst shaded/well-watered ramets maximised root growth.	*Trifolium repens*	Stuefer *et al.,* 1996

Clegg (1978) used the term "phalanx" to describe clonal plants whose growth form is compact, with many branches and closely packed ramets e.g. *Trifolium repens*. This growth-form tends to preclude the growth of competitors in a habitat and, if the site is nutrient-rich, the clone can utilise these nutrients. The same author used the term "guerrilla" to describe the more straggling growth of clonal plants such as *Ranunculus repens, Carex arenaria* and *Fragaria vesca* in which the internodes are long and the stolons relatively unbranched. Clegg (1978) also suggested that guerrilla growth would allow plants to "forage" for resources. Hutchings & Slade (1988) defined "foraging" as the process whereby an organism searches or ramifies within its habitat in the activity of acquiring essential resources. Such activity is genetically controlled but the plasticity which permits foraging is environmentally induced (Mogie & Hutchings, 1990).

Plasticity

Changes in the availability of resources such as light, water and minerals may bring about changes in the morphology of clonal plants. Hutchings & Mogie (1990) listed the environmental factors which could cause the growth form of clonal plants to become either more phalanx-like or more guerrilla-like. They suggested that high photon flux density, high ratio of red:far-red light, nutrient-rich soil and blue light all led to a more guerrilla-type growth form whilst low light, low red:far-red ratio and nutrient-poor soil all resulted in phalanx-like growth. Hutchings & de Kroon (1994) concluded that plasticity is not used by clonal plants for site selection but for site exploration. Slade & Hutchings (1987) showed that *Glechoma hederacea* increased the rate of production of branches, but the mean length of the stolon internodes was significantly less for plants grown in nutrient-rich sites. Other workers have reported no difference between the lengths of internodes from plants in nutrient-rich compared with those from nutrient-poor habitats (de Kroon & Knops, 1990; Schmid & Bazzaz, 1992). Increased branching, however, is a frequent occurrence in nutrient-rich habitats (Evans, 1988; Hutchings & Slade, 1988; Alpert, 1991). Caraco & Kelly (1991) point out that the increased growth of the clone as a whole must outweigh the

cost of maintaining connections between ramets by which sharing is made possible.

Division of labour in clonal plants

For non-clonal plants, shortage of a resource often results in increased growth of the part of the plant responsible for acquisition of that resource in order to maximise its capture (Minchin *et al.*, 1994). Thus shading results in an increase in the growth of the shoot relative to the root, in order to compensate for reduced photosynthesis *per* unit of leaf (Thornley & Johnson, 1990). For clonal plants there is evidence that division of labour between ramets results in plant organs specialising in the uptake of a rich resource, with mutual exchange of resources between ramets (Stuefer *et al.*, 1996; Stuefer, 1998). Stuefer *et al.* (1996) demonstrated that if part of a clone of *Trifolium repens* was subjected to low light but provided with water whilst another part of the clone had high light but little water, the roots of the well watered ramets proliferated even though their shoots were shaded and the shoots of the ramets given high light were larger than those grown in low light. Growth of clones given homogeneous treatment in which all the ramets had low light and high water was of the pattern described by Minchin *et al.* (1994) in which shading reduced the proportion of photosynthate exported to the root (Stuefer *et al.*, 1998). The extent to which clonal plants can share or exchange resources is limited by the vascular architecture of the plants, although such vascular constraints may be a feature of dicotyledonous plants and largely absent in monocotyledons (Marshall & Price, 1997).

How do clonal plants allocate phosphate from patchy environments?

Several studies have been made of the allocation of phosphate in clonal plants. These are summarised in Table 1.2 and indicate that most is allocated to the apex of the mains stolon with little transported basipetally.

Table 1.2 *The distribution of phosphate fed to the roots of clonal plants*

Source	Sink	Species	Reference
Roots of youngest ramet (nearest apex). Ramet halfway along main stolon. Parent	Most to apex of main axis; a trace basipetally. Bidirectional with most to the apex. Remained in parent.	*Agrostis stolonifera*	Anderson –Taylor, 1982
Large roots of rhizome.	To subtending shoot or to young roots and shoots and rhizome apex; trace basipetally.	*Carex arenaria*	Noble & Marshall, 1983
Roots of parent.	To apex of main axis but variations in distribution between genotypes.	*Trifolium repens*	Chapman & Hay, 1993
Roots of ramets near apex.	Towards apex or remained in ramet; trace basipetally.	*Trifolium repens*	Kemble & Marshall. 1995

The aims of this thesis

This thesis examines the uptake and distribution of resources by the clonal grass *Agrostis stolonifera* when it is grown with different parts of the clone supplied with different levels of resources. The distribution of materials within the clone is studied by means of dyes to determine the vascular connections and, by means of radiolabelled phosphorus and carbon, to determine the sinks for these resources within the clone. Experiments in the laboratory and greenhouse are described in subsequent chapters. The native clonal grass *Agrostis stolonifera* was chosen as the study plant throughout because the same genotype has been used for comparable studies so background information is available (Anderson-Taylor, 1982; Marshall & Anderson-Taylor, 1992; Agha, 1999).

Chapter 2 The aim is to establish the reduction in the supply of phosphate required to produce a significantly lower dry weight than a control given adequate phosphate. Studies using whole stolons showed that phosphate was

translocated acropetally; use of a clonal fragment (part of a clone with intact connections between two or more ramets (Jonsdottir & Watson, 1997) consisting of two ramets reduces the effect of the age of ramets and removes many competing sinks from the system. The hypotheses tested are (i) that phosphate taken up by the roots of one ramet will support a sibling ramet if the latter is deprived of phosphate; (ii) that support will occur whether the deprived ramet is the younger or the older; (iii) that shortage of phosphate will increase the R:S ratio of dry weight (iv) that shortage of phosphate results in reduction in the rate of production of new leaves, branches and basal tillers.

Chapter 3 This describes the use of dyes to demonstrate the vascular connections between ramets by which phosphate could be distributed within the clone. The hypothesis is tested that although the transpiration stream may be important in the translocation of phosphate towards the apices, since traces of dye have been observed to move basipetally xylem connections must be present and offer a route from younger to older ramets.

Chapter 4 This tests the hypothesis that phosphate is transported in the xylem and its movement is a function of transpiration, by subjecting parts of a clone to low water whilst other parts are given high phosphate.

Chapter 5 In this chapter ^{32}P is used to show the circulation of phosphate within a pair of connected ramets. It determines the allocation of phosphate, taken up by the roots of one of a pair of connected ramets, to the rest of the pair. It tests the hypothesis that if a plant is well-supplied with phosphate the rate of uptake and distribution of phosphate are faster than in a P-deprived plant.

Chapter 6 This describes experiments with ^{14}C to test the hypothesis that the allocation of photoassimilates alters in plants depending on their phosphate status.

Chapter 7 Discusses the results in the context of previous studies.

These chapters provide an integrated approach to the study of the distribution of resources in *A. stolonifera,* a study which has not previously been made for a stoloniferous grass.

***Agrostis stolonifera* L. Gramineae** (Creeping Bent, Fiorin)

A. stolonifera is a fast-growing stoloniferous grass common in a wide range of fertile habitats where it exploits pockets of nutrient enrichment. It occurs in aquatic habitats and mire, at woodland margins and on roadsides, on spoil heaps, in moist grassland, on arable land and in maritime habitats such as dune slacks, sea cliffs and salt marshes (Grime *et al.,* 1988). Salt-tolerant roadside forms, and a form resistant to heavy metals, found on contaminated soils in N.Derbyshire, are distinct ecotypes.

The grass consists of a main stolon with internodes approximately 10 cm long. Each node bears a single leaf and axillary bud from which a tiller develops. As the main stolon elongates it becomes horizontal and, where the nodes contact the substrate, roots emerge so each tiller rapidly establishes itself as a ramet. Basal tillers emerge at each node and these too elongate and produce secondary tillers at each node. The main stolon and each of the branches may extend for 1 m or more so that a large clone may be established. The strategy of the species is designated C-R (competitor-ruderal) by Grime *et al.* (1988).

The clone used in the experiments described in this thesis has been established in the University of Wales, Bangor Field Station glasshouses for several years. Although *A. stolonifera* flowers in the field in July and August, reproduction in the glasshouse is vegetative. Inflorescences are not produced, possibly because long day-length is maintained by artificial light and the temperature in the glasshouse is too high to initiate flowering.

Chapter 2

The effects of contrasting phosphate treatment on the growth of connected ramets of *Agrostis stolonifera*

INTRODUCTION

Sub-optimal supplies of phosphate reduce the acquisition of dry matter by plants (Burd, 1947; Asher & Loneragan, 1967; Milthorpe & Moorby, 1969; Clarkson *et al.*, 1978; Ericsson & Ingestad, 1988) and often result in darker green leaves and reddish stems (Marschner, 1995, Halsted & Lynch, 1996). Growth may be reduced because low Pi limits leaf expansion, although this is controversial. The total leaf area in soybean (*Glycine max)* grown in low phosphate was decreased to less than 15% of the value for high P plants after 19 d treatment (Fredeen *et al.*, 1989). The mechanism for reduction in the rate of leaf elongation might be that hydraulic conductance of root cortical cells is reduced in the absence of adequate supplies of phosphate (Radin & Matthews, 1989; Radin, 1990). The effect of reduced rate of elongation of leaves is that there is an overall reduction in total leaf area with a consequent reduction in the rate of growth (Radin & Eidenbock, 1984; Fredeen *et al.*, 1989). However, other authors have found no evidence for reduction in the length of leaves in phosphate-deprived plants (Milthorpe & Moorby, 1969). Milthorpe & Moorby (1969) concluded that phosphate deprivation does not reduce the rate of expansion of leaves but causes their early senescence in *Ricinus communis* L., *Hordeum* spp. and sugar cane (*Saccharum officinarum*) whereas Chapin *et al.* (1989) found a decrease in the rate of elongation of new leaves and a diminution in the relative growth rate of barley plants in the absence of phosphate. The phosphorus concentration in leaves of *Ricinus* decreases strongly with leaf age (Jeschke *et al.*, 1997). Halsted & Lynch (1996) stated that monocotyledon plants are better at resisting phosphate deficiency than are dicotyledon plants. Monocotyledons have less phosphorus in their leaves and maintain leaf production and branching when phosphate-deprived (Halstead & Lynch, 1996).

Another reason for reduced growth is that photosynthetic activity is often reduced by shortage of phosphorus (Milthorpe & Moorby, 1969; Herold, 1980; Dietz & Foyer, 1986; Heineke, *et al.* 1989). The rate of photosynthesis is reduced in sugar beet (*Beta vulgaris)* grown without phosphate (Rao & Terry, 1995) but not in soybean (*Glycine max)* (Fredeen, *et al.,* 1989). Similarly Fredeen *et al.* (1989) and Zulu *et al.* (1991) observed no reduction in the rate of photosynthesis in soybean and wheat plants respectively, grown in low phosphate.

Furthermore, reduced growth could be due to preferential partitioning of assimilates to roots in low phosphate, thus reducing the production of new leaves. A relative increase in the dry weight of roots, resulting in a greater root:shoot dry weight ratio, is a common result of low nutrient supply (Minchin *et al.*, 1994). This effect of low phosphate occurs, for instance, in soybean (Marschner *et al.,* 1996) and six cultivars of barley and associated wild grasses (Chapin *et al.,* 1989). Although the mechanism is not known it has been suggested that this may be due to phosphorus partitioning. There may be retention of phosphorus in the roots and perhaps some of the phosphate translocated to the shoot is rapidly recycled to the roots. Certainly some plants grown in low phosphate retain a greater proportion of phosphorus in their roots than in those of plants grown in high phosphate (Bieleski, 1973; Marschner *et al.,* 1996; Jeschke *et al.,* 1997). Cakmak *et al.* (1994) suggested that in *Phaseolus vulgaris* the increased root:shoot ratio is due to inhibition of the shoot apex and consequent reduction in growth of the shoot. Hackett (1968) found that phosphate concentration has little effect on the length of barley roots whereas Jackson *et al.* (1990) reported that root length varies not only with phosphate concentration in the soil but from species to species. The same authors showed that slower-growing species are able to exploit short-duration pulses of resources more effectively than do faster-growing species. Radin & Eidenbock (1984) found a reduction in the rate of elongation of roots in cotton (*Gossypium hirsutum* L.) in low phosphate but no effect on the dry weight of the root. Roots proliferate more in nutrient-rich patches than in nutrient-poor ones (Passioura &

Wetselaar, 1972) and show an increased ability to take up phosphate should they encounter a patch rich in phosphate (Jackson *et al.,* 1990).

Ryel & Caldwell (1998), using simulation models, predicted that the uptake of P would be lower from a patchy than from a uniform environment and that the coarser the patches the lower the uptake. However, the nutrient uptake overall was increased in a patchy environment (Kovar & Barber, 1989). This would be possible if changes in uptake kinetics allowed exploitation of the rich patches and uptake would then be greater than would be expected from a uniform soil (Robinson & Van Vuuren, 1998). Fast and slow-growing plants are likely to differ in the extent to which their roots can respond physiologically to a nutrient patch (Robinson & Van Vuuren, 1998) and *A. stolonifera* is said to have an intermediate relative growth rate (RGR) (Crick & Grime, 1987). Using a model to simulate uptake of NO_3^- and phosphate, it was found that whereas for nitrate the rate of uptake per unit length of root is more important than the rate of proliferation of the roots, the reverse is true for the uptake of phosphate (Jackson & Caldwell, 1996).

The effects of low phosphate described so far are for non-clonal plants with a single shoot and a single root system. Clonal plants may transport resources from one ramet to another to support ramets which are deprived of that resource. (Pitelka & Ashmun, 1985; Marshall, 1990). Differences in growth of P-sufficient and P-deprived, but connected, ramets indicate the extent to which phosphorus is transported to parts of a clone where it is in short supply and thus the extent to which ramets are physiologically independent or dependent on other ramets within the clone not only for shared resources but also for integrated control of the growth within the clone e.g. allowing proliferation of roots in rich patches. Marshall & Anderson-Taylor (1992), working with *Agrostis stolonifera,* found that clones given water have shorter stolons than those supplied with full Long Ashton nutrient solution. However, for *Glechoma hederacea,* shortage of resources results in longer internodes along the stolon so ramets are fewer and more widely spaced (Slade & Hutchings 1987; De Kroon & Knops, 1990; Price

et al., 1996). Other experiments with clonal plants show that the observed response depends on the species, reflecting the nature of the connections between ramets (Pitelka & Ashmun, 1985) and that extrapolation from one species to another is not valid (Van Groenendael *et al.,* 1996). Chapman and Hay (1993) found wide differences in the response to high and low phosphate even within two genotypes of the same species of white clover (*Trifolium repens).*

The growth of non-clonal plants is reduced in low phosphate. Clonal plants may share resources while their internodes remain intact and the adverse effects of phosphate shortage may thus be ameliorated. This chapter investigates the effects of low phosphate on paired ramets cut from stolons of *A. stolonifera* in which both the partners are given low phosphate. These ramets are compared with pairs in which the partners are given homogeneous high phosphate and with pairs in which one partner has high phosphate and the other low. Experiments were carried out on *Agrostis stolonifera* L., first to establish what reduction in concentration of phosphate was required to bring about a reduction in the growth, then to test the hypotheses that

- the response to reduced concentrations of phosphate is dose-dependent
- provision of adequate phosphate to one of a pair of attached ramets of *Agrostis stolonifera* L. will increase the dry weight of a phosphate-deprived partner when this is compared with the dry weight of partners in which both are P-deprived
- the dry weight of ramets will vary with the internal concentration of P
- low phosphate will increase the root: shoot dry weight ratio
- low phosphate will reduce the rate of production of branches, basal tillers and leaves, the rate of elongation of leaves, and the final area, length and dry weight of leaves in *A. stolonifera* L.
- low phosphate will decrease the rate of carbon assimilation and stomatal conductance in *A. stolonifera* L.

MATERIALS AND METHODS

Agrostis stolonifera L. plants (from the a clone maintained at Pen y Ffridd field station, University of Wales, Bangor) were grown in John Innes No1 potting compost in a glasshouse until they had produced a main stolon and basal tillers. The nodes of the main stolon and tillers were gently scarified with fine grade (P360) wet-or-dry Carborundum™ paper (Lloyd and James, Bootle, UK) to encourage rooting. The stolons and tillers were then laid on wet paper and the nodes covered with strips of paper which were kept damp until roots and shoots about 5 cm long had developed at the nodes.

Paired ramets, still attached by the internode of the original parent main stolon, were cut from the main stolon and their roots inserted through split discs of plastic foam into 1cm holes drilled in the snap-on lids of paired, 0.5 dm^3 plastic containers. The older ramet was designated R1, the younger R2. The plastic pots and their lids were covered with black adhesive tape to exclude light.

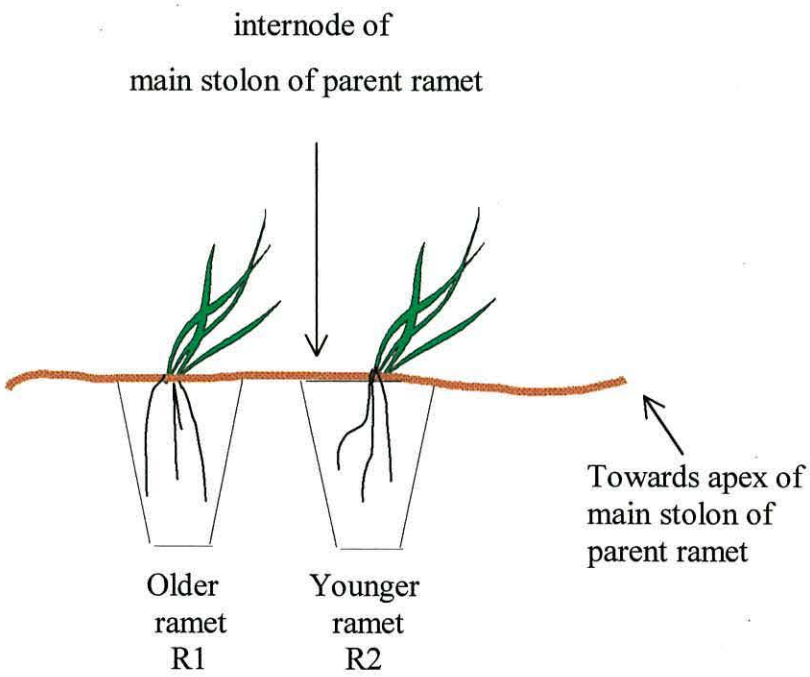


Fig. 2.1 *The arrangement of paired ramets of Agrostis stolonifera attached by the original main stolon internode of the parent ramet*

Five hundred cm^3 Long Ashton nutrient solution (Hewitt, 1966) was added to each pot. Full strength Long Ashton solution contains 4 mmol dm^{-3} potassium nitrate, 4 mmol dm^{-3} calcium nitrate, 1.33 mmol dm^{-3} sodium dihydrogen orthophosphate, 1.5 mmol dm^{-3} magnesium sulphate, 0.1 mmol dm^{-3} EDTA iron sodium salt solution and micronutrients consisting of 0.01 mmol dm^{-3} manganese sulphate, 0.001 mmol dm^{-3} copper sulphate, 0.001 mmol dm^{-3} zinc sulphate, 0.05 mmol dm^{-3} boric acid, 0.0004 mmol dm^{-3} sodium molybdate and 0.1 mmol dm^{-3} sodium chloride.

Experiment 2.1 To test the hypothesis that the response of paired ramets of <u>Agrostis stolonifera</u> L. to reduced concentrations of phosphate is dose-dependent and to establish what reduction in supply of phosphate is required to produce a reduction in the dry weight of ramets

Pairs of ramets were prepared as described above. Four Long Ashton nutrient solutions were used, the full formulation, with three different concentrations of inorganic phosphate and the fourth with no phosphate:

(1) 1.33 mmol dm^{-3} sodium dihydrogen orthophosphate

(2) 0.133 mmol dm^{-3} sodium dihydrogen orthophosphate

(3) 0.013 mmol dm^{-3} sodium dihydrogen orthophosphate

(4) no added phosphate.

The roots of both of a pair of ramets received the same phosphate treatment and four replicates of each treatment were set up. The plants were grown for 21 days in a Conviron E15 constant environment cabinet with a light intensity (measured using a Skye Quantum sensor, Model SKP 200) of 500 μmol m^{-2} s^{-1} at plant height, supplied by three 250 watt HQI/NDL lamps. A photoperiod of 16 h light/8 h dark was given with a temperature of 20 °C in the light period and 16 °C in the dark and a vapour pressure deficit (VPD) of 0.83 kPa at 20 °C and 0.64 kPa at 16 °C. The plant roots in each container were aerated by means of a length of 1 mm diameter plastic tubing attached to a 0.6 mm diameter hypodermic needle stuck into a length of aquarium aeration tubing leading from an aquarium air pump. This system provided 30 cm^3 air min^{-1} to each 500 cm^{-3}

container. The solutions were changed twice a week at first, then three times each week, to avoid depletion of nutrients. During the course of the experiment the total number of leaves was counted and the lengths of the main stolon and the longest root were measured. After harvest the dry weights of the roots and shoots were measured.

Experiment 2.2 ***To test the hypothesis that provision of adequate phosphate to one of a pair of attached ramets of Agrostis stolonifera will increase the dry weight of a phosphate-deprived partner when this is compared with the dry weight of partners both of which are P-deprived***

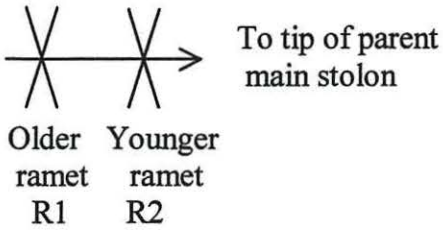
Pairs of ramets of *A. stolonifera* were prepared as before. In an attempt to reduce the variability in growth only the 3rd and 4th nodes of basal tillers were used to produce daughter ramets. The pairs were given the contrasting phosphate treatments summarised in Table 2.1.

Table 2.1 *Contrasting phosphate treatments used to test the hypothesis that provision of adequate phosphate to one of a pair of attached ramets of A. stolonifera will increase the dry weight of a phosphate-deprived partner when this is compared with the dry weight of partners in which both are P-deprived*

Phosphate treatments	
+P	-P
1.33 mmol dm^{-3} sodium hydrogen orthophosphate	0.007 mmol dm^{-3} sodium hydrogen orthophosphate
41 μg P cm^{-3}	0.2 μg P cm^{-3}
Full strength	1/200th strength

Four replicates of each of the heterogeneous and homogeneous phosphate treatments were set up (Table 2.2).

Table 2.2 *The treatments provided to four pairs of connected ramets of* <u>*A. stolonifera*</u> *to test the hypothesis that provision of adequate phosphate to one of a pair of attached ramets will increase the dry weight of a phosphate-deprived partner when this is compared with the dry weight of partners in which both are P-deprived*



Treatment of pair	Phosphate treatment given to R1	Phosphate treatment given to R2
+P -P	+P	-P
-P +P	-P	+P
+P +P	+P	+P
-P -P	-P	-P

The pH was checked daily; it remained between 5.5 and 6.5. After 29 days the rates of stomatal conductance and carbon dioxide assimilation were measured on the youngest fully expanded leaf on the main stolon of each ramet by means of an open gas exchange system (CIRAS-1; PP Systems, Harpenden, Herts). Readings were taken on the plants within the cabinet in which they had been grown and under the same growth conditions starting three hours into the light period. The plants were harvested and the roots washed in two changes of distilled water to remove nutrient solution from their surface. The roots and shoots were dried for 48 h at 80 °C in a ventilated oven and their dry weights and root:shoot dry weight ratios obtained. Finally the phosphorus content of roots and shoots was established using the phospho-molybdenum blue technique (Sandell and Onishi, 1978) following acid digestion of subsamples (Scholes and Shattock, 1984). The variability of the plants was such that the experiment was repeated using five replicates. Figures and tables show the results for both runs of the experiment

Experiment 2.3 ***To test the hypothesis that low phosphate will reduce the rate of production of branches, basal tillers and leaves, the rate of elongation of leaves, and the final area, length and dry weight of leaves in <u>A. stolonifera</u>***

Paired ramets were grown for 13 days as described above with four replicates of each of the four treatments. During the course of the experiment the rate of production of ten successive leaves was recorded for each ramet along with the rate of extension of the new leaves from their appearance until the leaf was fully expanded when the leaf had unrolled and the ligule could be seen clearly. The leaf was then measured daily until fully expanded. The length was measured from the tip of the developing leaf to the node of the next-but-one leaf as shown (Fig. 2.2) inclusive of the leaf sheath since the node was concealed within the leaf sheath of the next older leaf.

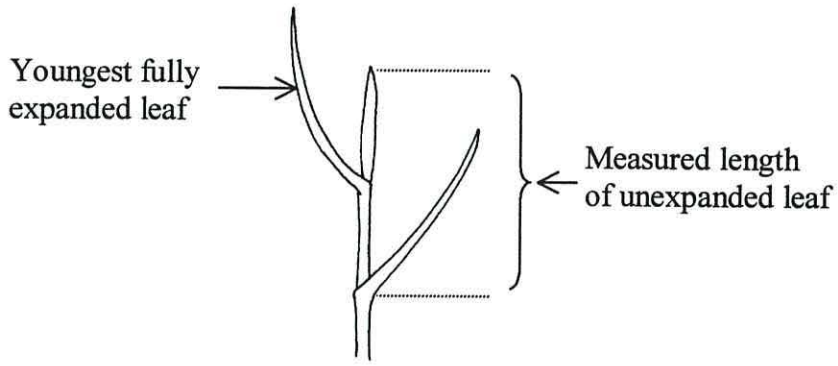


Fig. 2.2 *Method of measuring the length of each leaf from the tip of the developing leaf to the node of the next-but-one leaf to ensure that the measured increase in length included extension of the leaf sheath since the node was concealed within the leaf sheath of the next older leaf*

The number of basal tillers and the number of main stolon branches were recorded on every second or third day.

The plants were harvested after 13 days and the roots washed in two changes of distilled water to remove nutrient solution. The area of the leaves was found by scanning with a Hewlett Packard Deskscan 2, v. 2.3 and measuring the scanned image using Delta-T Logger Analysis Software, Version 2, (Delta-T Devices,

Cambridge, UK). The leaves were weighed and their phosphorus concentration established separately from the rest of the shoots. The roots and shoots were dried and their dry weights and root:shoot ratios obtained. Finally the phosphorus content of the roots and shoots was established using the phospho-molybdenum blue technique as before and the distribution of phosphorus between roots and shoot calculated.

STATISTICAL ANALYSIS

Results were tested for homogeneity using Cochran's Test for Equality of Variance then analysed statistically by SPSS 8.0 for Windows 1998 using ANOVA followed by the Least Significant Difference (LSD) *post hoc* test. The mean difference was accepted as significant at the 0.05 level unless otherwise stated. Where data were not homogeneous they were transformed to natural logs before statistical analysis. The LSD is a liberal statistic and thus more liable to Type-1 errors than the more conservative Tukey's Honestly-significant test. However Tukey's test is liable to Type-2 errors with small samples such as the four replicates used in most of these experiments (Dytham, 1999). Where data were pooled to give larger samples Tukey's *post hoc* test was used.

RESULTS

Experiment 2.1

This tested the hypothesis that the response of paired ramets of *Agrostis stolonifera* to reduced concentrations of phosphate is dose-dependent and established what reduction in supply of phosphate is required to produce a reduction in the dry weight of ramets. After 21 days both shoots and roots had a lower dry weight in low concentrations of phosphate (Fig. 2.3). Ramets given 0.013 mmol dm^{-3} phosphate or no phosphate had shoots with a significantly lower dry weight than that of the ramets given 1.33 mmol dm^{-3} and ramets given no phosphate had shoots with a significantly lower dry weight than ramets given phosphate in any concentration. Only the ramets given no phosphate had roots with a significantly lower dry weight than those given phosphate.

The mean number of leaves for each treatment (Fig. 2.4) was greater ($p<0.1$) for the 1.33 mmol dm^{-3} phosphate treatment than for the 0.13 mmol dm^{-3} treatment and significantly greater ($p<0.05$) than ramets given 0.013 mmol dm^{-3} or no phosphate. The rate of production of new leaves and the relative growth rate RGR (leaves) is shown in Table 2.3. There was no significant difference in the lengths of the stolons for any of the four treatments (Fig. 2.5).

Although the plants supplied with high phosphate had the longest roots for the first 15 days, by the end of the experiment there was no difference in length between the roots of the plants for any of the four treatments (Fig. 2.6). The rate of elongation of the roots in low phosphate treatments was greater than that of the high phosphate plants between 7 d and 21 d. Over a three-week period the rate of increase in the length of the longest root was reduced in the highest phosphate concentration.

The root:shoot dry weight ratio increased from 0.12 (1.33 mmol dm^{-3} P) to 0.13 (0.1 mmol dm^{-3} P), 0.14 (0.01 mmol dm^{-3} P) and 0.18 (no P). Although these values may indicate a trend they are not significantly different. The growth of pairs of ramets in the four treatments is summarised in Table 2.3.

Table 2.3 *Summary of growth data for pairs of ramets of A. stolonifera grown for 21 days. The concentrations of phosphate shown were supplied to the roots. The ramets within each pair were given the same treatment*

	1.33 mmol dm^{-3} P	0.133 mmol dm^{-3} P	0.013 mmol dm^{-3} P	No P
Total leaves on pair of ramets	149 ± 22.8	124 ± 17.9	70 * ± 6.2	31 ** ±7.9
Rate of production of new leaves (No. d^{-1})	6.94 ± 1.1	5.73 ± 0.8	3.15 * ± 0.5	1.31 ** ± 0.4
RGR (leaves)	0.17 ± 0.02	0.17 ± 0.02	0.13 * ± 0.03	0.09 ** ± 0.03
Sum of lengths of main stolons of R1 + R2 (cm)	59.6 ± 4.0	48.4 ± 5.0	44.4 * ± 6.2	47.4 ± 5.2
Sum of lengths of longest roots of R1 +R2 (cm)	24.5 ± 1.7	25.1 ± 1.1	26.8 ± 2.7	23.4 ± 2.8
Sum of dry weight of shoots of R1 + R2 (g)	1.40 ± 0.2	1.17 ± 0.2	0.76 * ± 0.2	0.35 ** ± 0.1
Sum of dry weight of roots of R1 + R2 (g)	0.16 ± 0.02	0.15 ± 0.03	0.11 ± 0.02	0.06 * ± 0.02
Sum of dry weight of R1 + R2 (g)	1.56 ± 0.2	1.32 ± 0.2	0.87 * ± 0.2	0.41 ± 0.1
R:S dry weight ratio of R1 + R2	0.12 ± 0.01	0.13 ± 0.03	0.14 ± 0.02	0.18** ± 0.03

* $p < 0.05$; ** $p < 0.005$

From Table 2.3 it can be seen that as the supply of phosphate to the roots was reduced the growth of the pairs of ramets reduced too. There was no significant difference in the lengths of the roots for any phosphate treatment. The pairs given no phosphate had significantly fewer leaves, dry weight of the shoot, dry weight of the roots and hence of the whole ramet than pairs given 1.33 mmol dm^{-3} phosphate. Ramets given 0.01 mmol dm^{-3} phosphate had fewer leaves, shorter main stolons, lower dry weights of shoots and whole ramets than the full phosphate pairs.

For subsequent experiments the supply of phosphate given to the high P ramets was 1.33 mmol dm^{-3} and that given to the low P ramets 0.007 mmol dm^{-3} (0.5% of the full amount).

Experiment 2.2

The hypothesis was tested that provision of adequate phosphate to one of a pair of attached ramets of *Agrostis stolonifera* will increase the dry weight of a phosphate-deprived partner when this is compared with the dry weight of partners both of which are P-deprived. The sum of the dry weights of the roots and shoots of each pair of ramets was compared to see the effect on dry weight of the clonal fragment if the root of even one ramet had access to high phosphate (Fig. 2.7). The mean dry weights for each treatment show that, for the pair of ramets, their total dry weight was greatest for the pair in which both were given high phosphate. The lowest combined dry weight was seen in the pair where both were given low phosphate. These dry weights are significantly different. Of the pairs of ramets given heterogeneous phosphate treatments, the dry weight of the pair in which the older ramet was given high phosphate and the younger given low phosphate (+P -P) was significantly less than that of the pair in which both received high phosphate. The combined dry weights of the pair given -P +P was not significantly different from that of the +P +P pair. Table 2.4 summarises the comparison in dry weight of the pairs given heterogeneous phosphate treatments (+P -P and -P +P) with the pairs given homogeneous high phosphate (+P +P) and low phosphate (-P -P).

Table 2. 4 *The dry weights of paired ramets given heterogeneous phosphate treatments (+P -P and -P +P) compared with the dry weights of the pairs of ramets given homogeneous treatments. ↑ shows a significantly greater dry weight, ↓ shows a significantly lower dry weight: n.s.d = no significant difference. $p<0.05$*

	+P -P	-P +P
Comparison with +P +P pair	↓	n.s.d
Comparison with -P -P pair	n.s.d	↑

The experiment was repeated to verify the unexpectedly high dry weights for the pair in which the R1 was given low phosphate and the R2 high phosphate. The mean dry weights of the roots and shoots within each pair in the two experiments are summarised in Table 2.5.

Table 2.5 *Mean dry weights of roots and shoots ± 1 standard error of the mean, of each of a pair of ramets given the contrasting phosphate treatments shown at the top of each column and grown for 30 days. (a) and (b) are replicates of the same experiment.* +P = *1.33 mmol* dm^{-3} *phosphate;* -P = *0.007 mmol* dm^{-3} *phosphate*

Experiment		Mean dry weights (mg)							
		R1 **+P**	R2 **-P**	R1 **-P**	R2 **+P**	R1 **+P**	R2 **+P**	R1 **-P**	R2 **-P**
(a)	Shoot	1297 ± 444	1292 ± 371	1042 ± 186	2393 ± 173	2140 ± 197	1919 ± 425	756 ± 113	720 ± 80
(n = 4)	Root	127 ± 68	108 ± 47	98 ± 85	213 ± 80	199 ± 86	173 ±83	76 ± 33	89 ± 61
	Ramet	1424 ± 510	1400 ± 388	1008 ± 346	2602 ± 198	2339 ± 239	2092 ± 464	832 ± 123	809 ± 81
(b)	Shoot	1301 ± 212	1358 ± 233	2063 ± 483	2288 ± 584	1979 ± 180	2253 ± 417	856 ± 68	1262 ± 103
(n = 5)	Root	98 ± 11	135 ± 41	205 ± 47	141 ± 38	154 ± 50	176 ± 15	141 ± 27	140 ± 140
	Ramet	1399 ± 217	1493 ± 265	2268 ± 518	2430 ± 618	2133 ± 227	2429 ± 428	997 ± 84	1402 ± 106

On both occasions the +P R2 ramet (shown in bold) in the pair given -P **+P** is heavier than the +P R1 ramet in the pair given **+P** -P. Attachment to a high P ramet increased the dry weight of the shoot of a ramet given low P (Fig. 2.8). Comparison of the low phosphate ramets in the heterogeneous pairs with ramets in the homogeneous -P -P control pair showed that the dry weight of the shoots of ramets attached to a high phosphate partner was significantly greater. Attachment to a low P ramet resulted in a lower dry weight of the high P partner if the latter was the older ramet, but not if it was the younger (Fig. 2.8).

In experiment (a) (Table 2.5) the roots of the R2 ramets in the heterogeneous pair given -P **+P** and the R1 ramets in the pair given **+P** +P treatment were significantly heavier than those of both ramets in the -P -P treatment (Fig. 2.9)

(Analysis of Variance followed by the *post hoc* test for Least Significant Difference). However there was no significant difference between the dry weights of the roots of any of the pairs when the result from both experiments (a) and (b) were combined and analysed by ANOVA followed by Tukey's Honestly Significant Difference *post hoc* test.

The ramets given low phosphate always had longer roots than those of ramets given high phosphate (Fig. 2.10) but not necessarily significantly so. The R1 ramet given +P in the **+P** -P pair was significantly shorter than its counterparts, the R1 ramet given low P in the **-P** +P pair and in the homogeneous **-P**-P pair.

The roots and shoots were analysed for their phosphorus content. The total phosphorus content (mmol dm^{-3}) of the shoots of single ramets is shown in Fig. 2.11. Ramets given high phosphate had more P in their shoots than did ramets given low P. The low P ramets in both heterogeneous treatments had similar amounts of P and in both cases this was greater than the amount found in the homogeneous low P pair. The high phosphate shoot of the R2 ramet in the pair given -P **+P** had the same amount of P as the homogeneous high P pair, and significantly more than the R1 of the **+P** -P pair.

The results were also expressed as a concentration of phosphorus *per* unit dry weight of plant material (Fig. 2.12). The phosphorus concentration in the shoots varied more for low phosphate ramets than for high phosphate ramets. The large standard error bars (Fig 2.12) for R1 of **-P** +P reflect a wide variation in dry weights of these ramets with one having a dry weight of only half that of the others even though its total phosphate content was not dissimilar from the other three replicates (Fig 2.11).

The results for the phosphate concentration could not be analysed directly by ANOVA because the results were not homogeneous. After transformation of data to natural logs ($\log_e$) the results were analysed using ANOVA followed by the LSD *post hoc* test. There was no significant difference between the P

concentration (nmol mg^{-1}) in any of the shoots of high P ramets (Fig. 2.12). The R1 ramet in the pair given **+P** -P was not significantly different from either the other high P shoots or from the low P shoots in the heterogeneous treatments. The P concentration was higher in the homogeneous +P +P pair and in the R2 ramet given high P in the -P **+P** pair than in the low P ramets in the heterogeneous pairs. The shoots of the -P -P pair had a lower concentration of P than the rest.

The roots of all the ramets given high phosphate had more phosphorus than the ramets given -P treatment (Fig. 2.13) and a higher concentration of phosphate (Fig. 2.14). As in the shoots the roots of the +P +P ramets had a significantly higher concentration of phosphorus than any other roots. There was no significant difference between the P concentration in the +P ramets in the heterogeneous treatments. There was no significant difference in the phosphorus concentration in the roots of ramets given low phosphate treatment.

The total phosphorus concentration in both ramets together is shown in Table 2.6. The pair given +P -P have a P concentration between that of the -P -P pair and the +P +P pair whereas the pair given -P +P have a P concentration the same as that of the +P +P pair.

Table 2.6 *Concentration of phosphorus (nmol mg^{-1}) in paired ramets given the phosphate treatments shown*

	+P -P	-P +P	+P +P	-P -P
[P] (nmol mg^{-1}) in both ramets	196.6 ± 11.8	278.9 ± 27.8	314.7 ± 38.8	59.5 ± 11.9

The dry weights of ramets correlated well with their total P content (Fig 2.15). The dry weight of the shoots of ramets given high phosphate correlated ($p<0.1$) with the phosphorus concentration in their roots. For ramets given low phosphate there was little correlation between the phosphate concentration and dry weight (Table 2.7).

Table. 2.7 *Correlation between the mean dry weights of root and shoot of ramets and the phosphorus concentration (n mol P mg^{-1} dry weight) in roots and shoots of each of a pair of ramets of A. stolonifera given the phosphate treatment shown and grown for 30 days in a Conviron constant environment cabinet.* +P = *1.33 mmol dm^{-3} phosphate;* -P = *0.007 mmol dm^{-3} phosphate. n = 5*

P treatment	P conc. in	Dry weight	R^2
+P	Root	Root	0.4758
	Root	Shoot	0.8330
	Shoot	Root	0.0002
	Shoot	Shoot	0.2693
-P	Root	Root	0.0692
	Root	Shoot	0.0388
	Shoot	Root	0.0762
	Shoot	Shoot	0.3961

The root:shoot ratio of dry weights of single ramets was altered by the concentration of phosphate in the growth medium. Low phosphate increased the ratio (Fig. 2.16) but only the R:S of R1 ramets in the **-P** -P treatment was significantly greater than that of others. The correlation between R:S dry weight ratios, the phosphorus within each plant organ and the ratio of the P content of roots:shoots is shown in Table 2.8.

Table 2.8 *The correlation between the R:S dry weight ratio with the phosphorus concentration (nmol P mg^{-1} dry weight) in the shoot ($[P]_s$), and in the root, ($[P]_r$) and with the phosphorus content in the shoot (P_s) and root (P_r) (mmol) for individual ramets. Values are the means of the dry weights and [P] for experiments (a) and (b) shown in Table 2.6.* n = 9. 7 d.f

	+P	-P	-P	+P	+P	+P	-P	-P	R^2
R:S (dry weight)	0.08 ±0.01	0.10 ±0.02	0.09 ±0.02	0.07 ±0.01	0.08 ±0.01	0.09 ±0.01	0.14 ±0.02	0.12 ±0.02	
$[P]_s$ nmol mg^{-1}	125 ± 7	72 ± 9	81 ± 21	198 ± 7	156 ± 22	159 ± 17	27 ± 4	32 ± 8	0.783 $p < 0.05$
$[P]_r$ nmol mg^{-1}	138 ± 17	42 ± 9	47 ± 7	213 ± 32	250 ± 38	222 ± 17	41 ± 5	35 ± 5	0.504 $p > 0.1$
mmol P_s	165 ± 33	102 ± 24	106 ± 31	472 ± 75	318 ± 51	324 ± 48	17 ± 3	28 ± 10	0.636 $p < 0.1$
mmol P_r	15 ± 4	4 ± 1	5 ± 2	33 ± 6	29 ± 8	41 ± 7	5 ± 1	6 ± 3	0.393 $p > 0.1$
R:S (P content)	0.09	0.04	0.05	0.07	0.09	0.13	0.29	0.21	

The R:S dry weight ratio of pairs of ramets is most closely correlated with the phosphorus concentration in the shoot and least closely correlated with the phosphorus content of the roots (Table 2.9).

Table 2.9 *The correlation between the R:S dry weight ratio with the phosphorus concentration (nmol P mg^{-1} dry weight) in the shoot ($[P]_s$), and in the root, ($[P]_r$) and with the phosphorus content (nmol) in the shoot (Ps) and root (Pr) for paired ramets. n = 9. 7 d.f*

	+P -P	-P +P	+P +P	-P -P	R^2
R:S	0.09 ± 0.004	0.09 ± 0.008	0.08 ± 0.005	0.12 ± 0.02	
[P]s nmol mg^{-1}	98 ± 6	139 ± 11	157 ± 17	30 ± 5	0.908 $p < 0.001$
[P]r nmol mg^{-1}	90 ± 15	136 ± 16	237 ± 20	38 ± 10	0.772 $p < 0.05$
Ps nmol	267326 ± 57408	577871 ± 87730	642321 ± 87487	46018 ± 10883	0.773 $p < 0.05$
Pr nmol	13375 ± 4139	48314 ± 6236	105375 ± 9034	11684 ± 3556	0.616 $p < 0.1$

As for individual ramets, the R:S dry weight ratio of the paired ramets was most closely correlated with the phosphorus concentration in the shoot and least closely correlated with the phosphorus content of the roots. The root:shoot ratio of the phosphorus content (Fig. 2.17) showed a much greater P content in the roots than in the shoots in ramets with no access to high phosphate. There was no significant difference between the root:shoot ratio of the phosphorus concentrations in ramets given +P+P and -P-P treatments (Fig. 2.18) and in the heterogeneous treatments low P ramets had a lower ratio of root:shoot P concentrations.

The rate of photosynthesis and the stomatal conductance were measured on the youngest fully expanded leaf on the main stolon of each ramet (Figs. 2.19 and 2.20). There was no significant difference in either the rate of assimilation of carbon or the stomatal conductance for any treatment.

Ramets grown in 0.007 mmol dm^{-3} phosphate, especially those given -P -P treatment had reddish stems and their leaves senesced more rapidly than did those with access to high phosphate.

Experiment 2.3

Plants grown with access to adequate phosphate had more main stolon branches than those grown in low phosphate (Fig. 2.21). Given access to high phosphate supplies, all the ramets in the heterogeneous pairs, whether or not their roots were given high phosphate, produced the same number of branches as the high phosphate, homogeneous control pair and significantly more than the homogeneous low phosphate pair. The +P +P pair produced more branches than the -P -P pair. Of the two heterogeneous pairs only the R2 ramet in the -P **+P** pair produced significantly more branches that its low P partner.

The production of basal tillers was similar to the production of main stolon branches (Fig. 2.22). Again the two ramets given homogeneous, low phosphate had significantly fewer basal tillers than in any ramet given +P treatment. The R1 ramet in the heterogeneous treatment **+P** -P had more tillers than the R1 ramet in the pair given homogeneous **+P** +P but not significantly more than the other two high phosphate ramets. The low phosphate ramets in the heterogeneous treatments +P **-P** and **-P** +P had a significantly greater number of basal tillers than either of the homogeneous low P ramets, -P -P.

There was no significant difference in the rate of expansion of leaves 5, 6 and 7 which correlated with phosphate supply (Fig 2.23). There was a tendency for the leaf of the younger ramet (R2) to expand slightly faster than that of the older ramet (R1). The rate was significantly faster only for leaf 5, of the R2 ramet of the +P **+P** pair and the R2 ramet in the -P **-P** pair, when these were compared with the rate for their R1 partners.

The rate of production of new leaves was the same for all treatments except for the R1 ramet in the **-P** -P pair, where rate of production of new leaves was

significantly slower than the rest (Fig. 2.24). There was no correlation between the dry weight of the shoots and the rate of production of leaves for either high phosphate ramets ($R^2 = 0.006$) or low phosphate ramets ($R^2 = 0.009$).

There was no significant difference in the final combined areas of leaves 5, 6 and 7 (Fig. 2.25). There was no correlation between the dry weight of the shoot and the area of leaves on the high phosphate ramets ($R^2 = 0.122$) but for the low phosphate ramets the dry weight of the shoots correlated more closely with the area of the leaves ($R^2 = 0.765$). The specific leaf areas (SLA) for leaves 5, 6 and 7 are shown in Fig. 2.26; the SLA of the R1 of **-P** +P is significantly less than that of the R1 of **+P** +P. The only significant difference in the total combined length of the three leaves was seen in leaves 5, 6 and 7 of the R1 ramet in the **+P** -P pair which were significantly longer than those of the R1 in the homogeneous **+P** +P pair (Fig. 2.27).

There was no significant difference in the combined dry weights of leaves 5, 6 and 7 for any of the treatments (Fig 2.28). For +P ramets there was some correlation between the dry weight of their leaves and the area ($R^2 = 0.59$) and between dry weight and the length of the leaves ($R^2 = 0.87$). Between dry weight and area the correlation coefficient, R^2, was 0.94 and between dry weight and length R^2 was 0 .90. There was a weak correlation between the area and the length of the leaves of +P ramets ($R^2 = 0.59$) whereas for -P ramets R^2 was 0.94.

The phosphorus concentration (nmol mg^{-1}) and also the phosphorus content (nmol) in leaves 5, 6 and 7 were highest in the +P +P pair and lowest in the -P -P pair (Table 2.10). Despite their greater phosphorus concentration the leaves of the +P +P control ramets were neither the heaviest nor longest and did not have the greatest area. The phosphorus concentrations are shown in Fig. 2.29.

Table 2.10 *The phosphorus content (nmol) and phosphorus concentration (nmol mg-^{1}) of leaves 5, 6 and 7 combined, after 29 days, for ramets given heterogeneous phosphate treatments compared with the mean phosphorus concentration in control ramets given homogeneous phosphate treatment*

	(R1) +P	(R2) -P	(R1) -P	(R2) +P	(R1) +P	(R2) +P	(R1) -P	(R2) -P
Phosphorus content (nmol)	3360 ± 570	2733 ± 909	1478 ±560	2643 ±586	3831 ±662	3629 ±570	403 0	739 ±202
Phosphorus concentration (nmol mg^{-1})	198 ± 27.7	161 ± 52.5	114 ± 60.2	215 ± 34.4	274 ± 19.1	262 ± 21.9	38 ± 4.1	61 ± 18.9

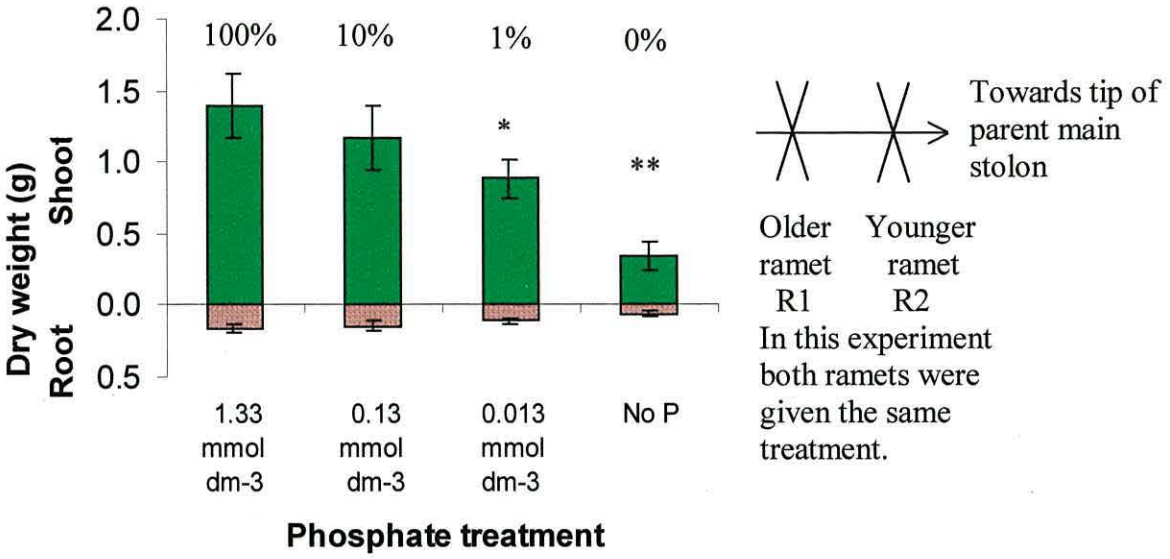


Fig. 2.3 *The dry weights of roots and shoots for R1 and R2 together in the pairs of ramets of A. stolonifera grown for 21 days with four contrasting phosphate treatments. Each bar is the mean of four replicates of each phosphate treatment. Error bars are ± 1 standard error of the mean.*
** = $p<0.05$; ** = $p<0.005$*

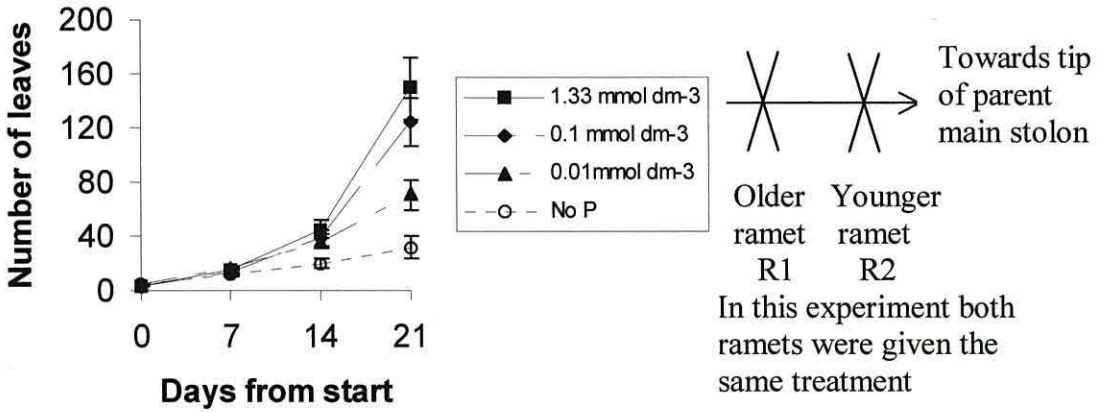


Fig. 2.4 *The increase in the number of leaves for R1 and R2 together in the pairs of ramets of A. stolonifera grown for 21 days with four contrasting phosphate treatments. Each point is the mean of the four replicates of each phosphate treatment. The concentrations of phosphate (mmol dm^{-3}) are shown on the graph. Error bars are ± 1 standard error of the mean.*

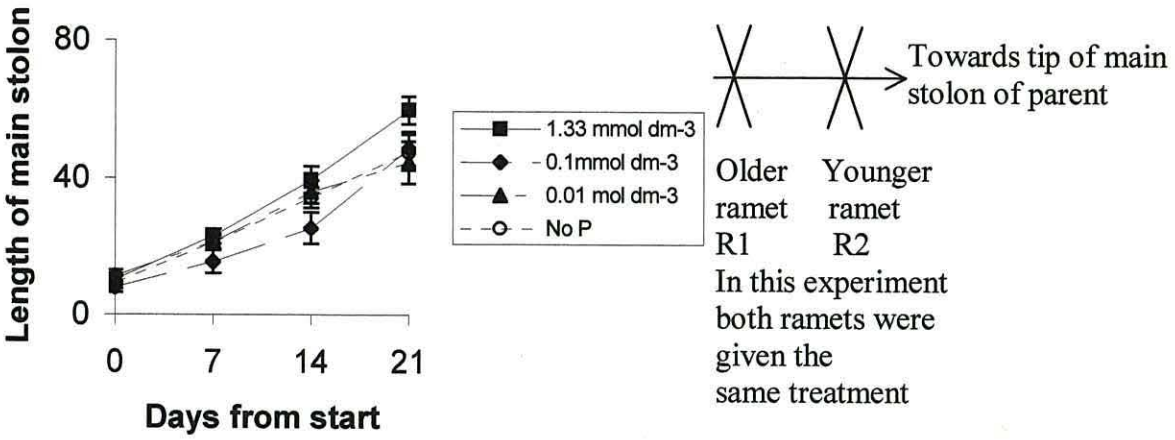


Fig. 2.5 *The increase in length of the main stolons for R1 and R2 together in the pairs of ramets of A. stolonifera grown for 21 days with four contrasting phosphate treatments. Each point is the mean of the four replicates of each phosphate treatment. The concentrations of phosphate (mmol dm^{-3}) are shown on the graph. Error bars are ± 1 standard error of the mean.*

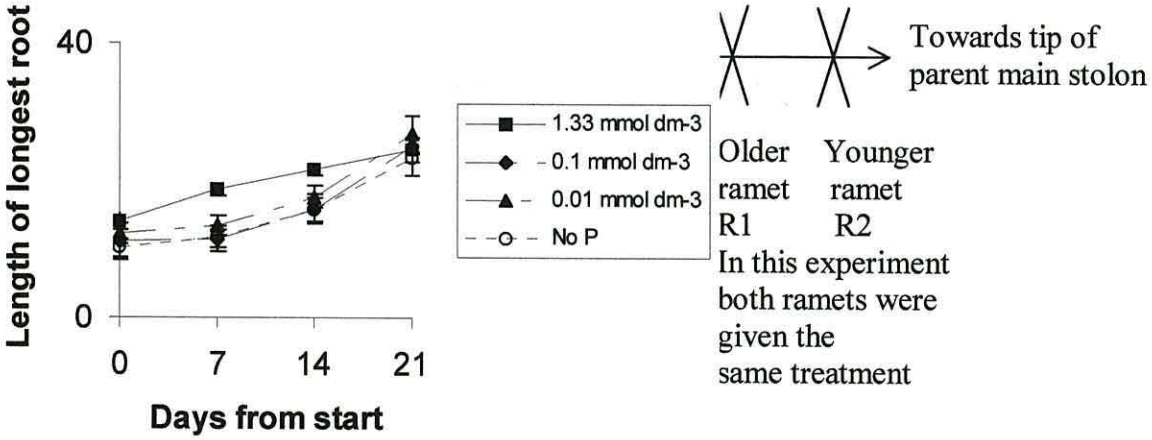


Fig. 2.6 *The increase in the length of the longest root for R1 and R2 together in the pairs of ramets of A. stolonifera grown for 21 days with four contrasting phosphate treatments. Each point is the mean of the four replicates of each phosphate treatment. The concentrations of phosphate (mmol dm^{-3}) are shown on the graph. Error bars are ± 1 standard error of the mean.*

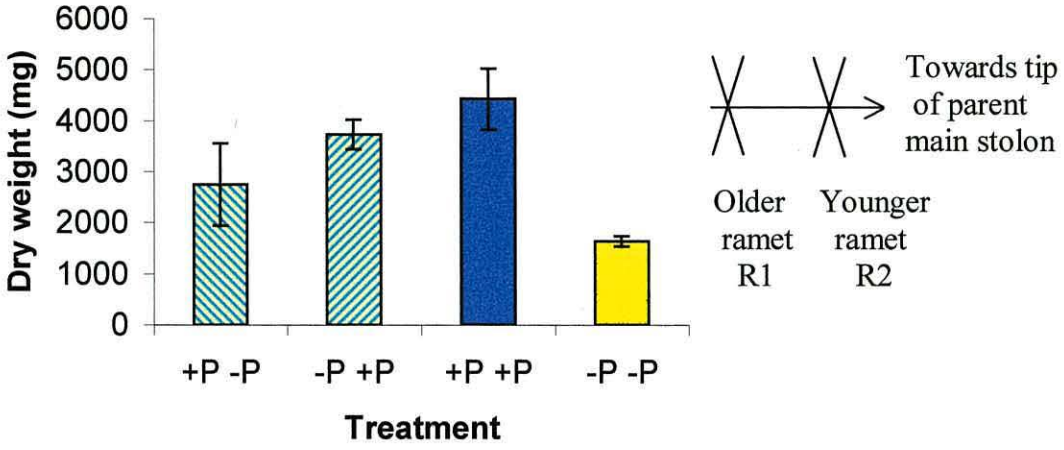


Fig. 2.7 *The combined mean dry weights of pairs of ramets of <u>Agrostis stolonifera</u> given the contrasting phosphate treatments shown and grown for 29 days. +P = 1.33 mmol dm^{-3} phosphate; -P = 0.007 mmol dm^{-3} phosphate. Error bars are ± 1 standard error of the mean. n = 4. Results from the first run of the experiment.*

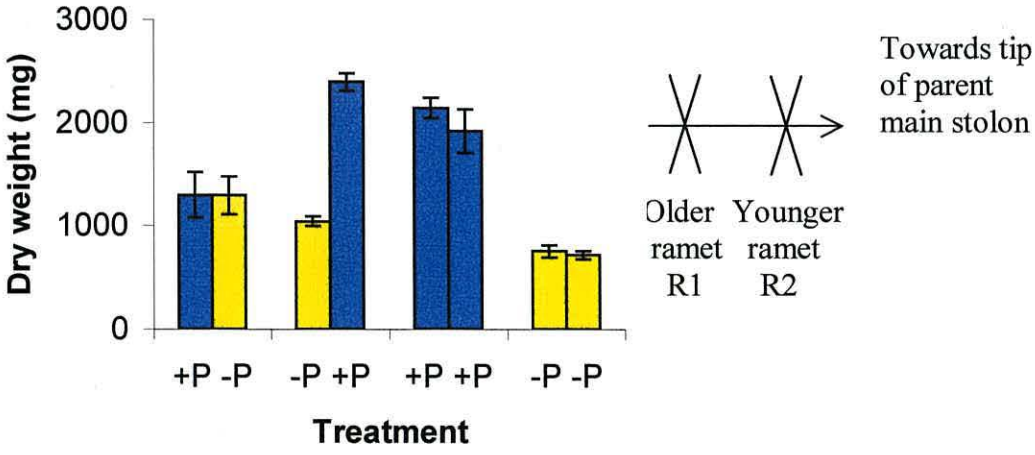


Fig. 2.8 *The mean dry weights of the shoots of each of a pair of ramets of <u>A.stolonifera</u> given contrasting phosphate treatments and grown for 29 days. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars show ± 1 standard error of the mean. n = 4. Results from the first run of the experiment.*

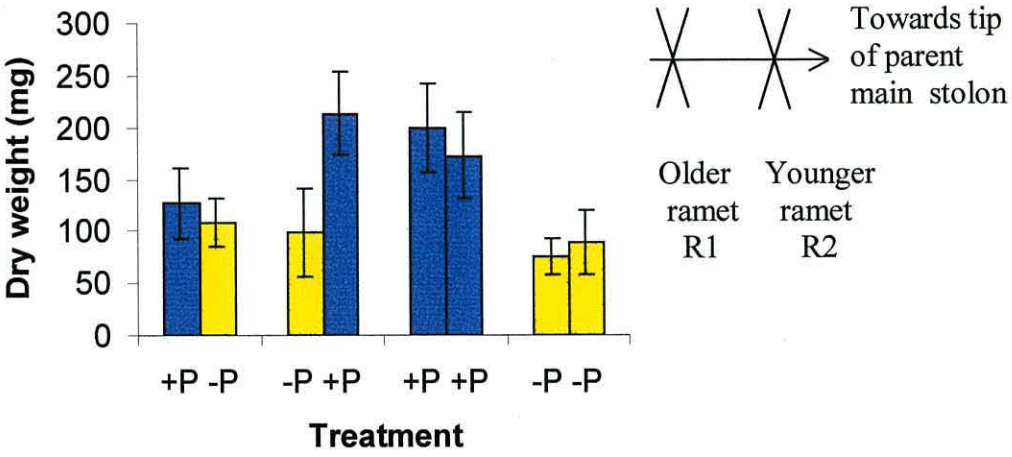


Fig. 2.9 *The mean dry weights of the roots of each of a pair of ramets of A.stolonifera given contrasting phosphate treatments and grown for 29 days.* +P *(blue bars) = 1.33 mmol* dm^{-3} *phosphate;* -P *(yellow bars) = 0.007 mmol* dm^{-3} *phosphate. Error bars show ± 1 standard error of the mean. n = 4. Results from first run of the experiment.*

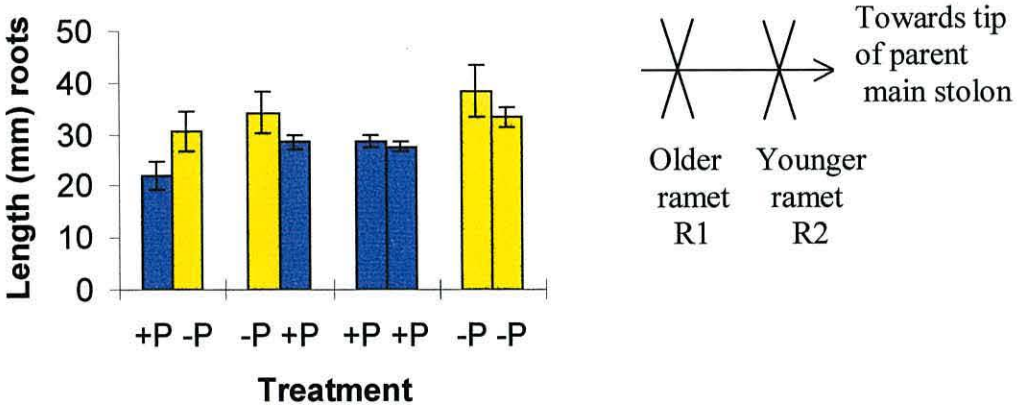


Fig. 2.10 *The mean lengths of the longest root of each of a pair of ramets of A.stolonifera given contrasting phosphate treatments and grown for 29 days.* +P *(blue bars) = 1.33 mmol* dm^{-3} *phosphate;* -P *(yellow bars) = 0.007 mmol* dm^{-3} *phosphate. Error bars show ± 1 standard error of the mean. n = 4. Results from first run of the experiment.*

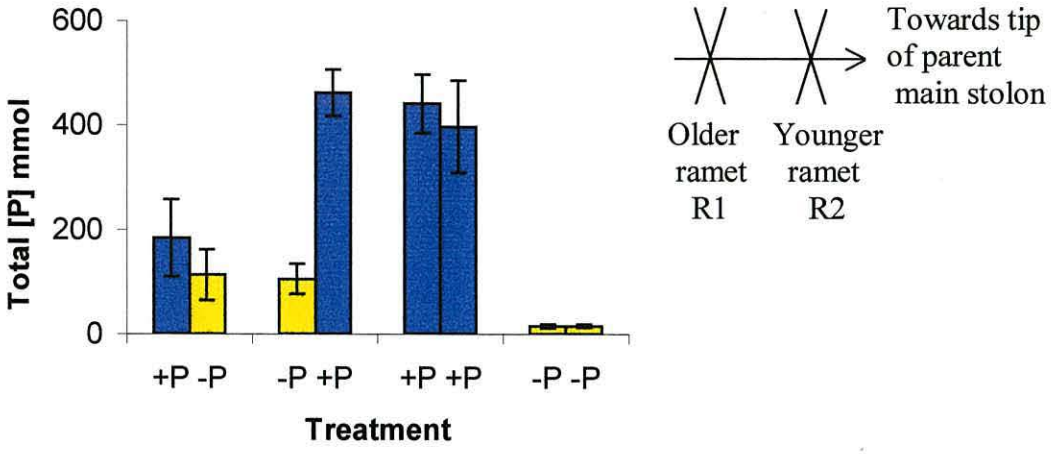


Fig. 2.11 *The mean phosphorus content, expressed in mmol, in the shoots of each a pair of ramets of A.stolonifera given contrasting phosphate treatments and grown for 29 days. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars show ± 1 standard error of the mean. n=4. Results from first run of the experiment.*

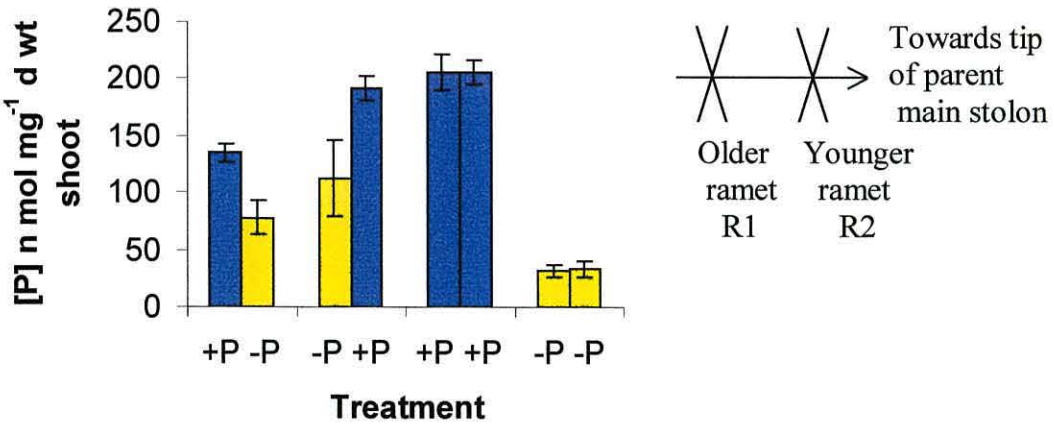


Fig. 2.12 *The mean phosphorus concentration, expressed in nmol mg^{-1}, in the shoots of each a pair of ramets of A.stolonifera given contrasting phosphate treatments and grown for 29 days. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars show ± 1 standard error of the mean. n=4. Results from first run of the experiment.*

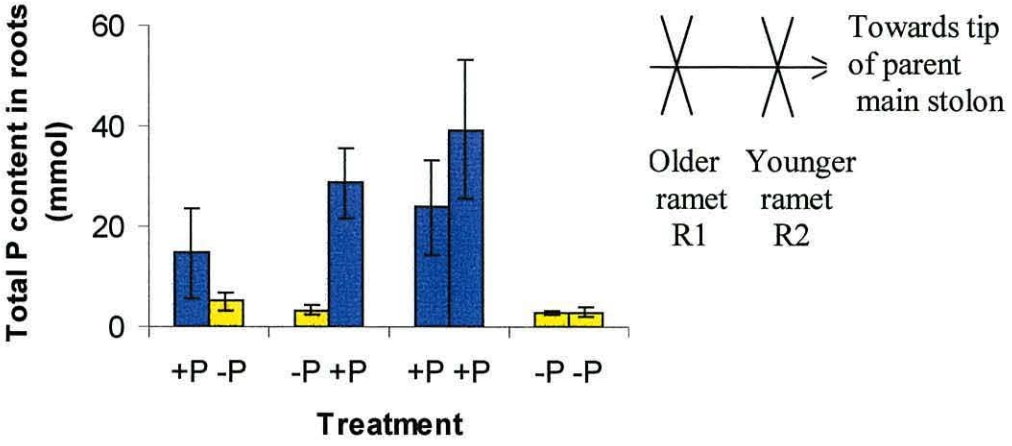


Fig. 2.13 *The mean phosphorus content, expressed in mmol, in the roots of each of a pair of ramets of A. stolonifera given contrasting phosphate treatments and grown for 29 days. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars show ± 1 standard error of the mean. n=4. Results from first run of the experiment.*

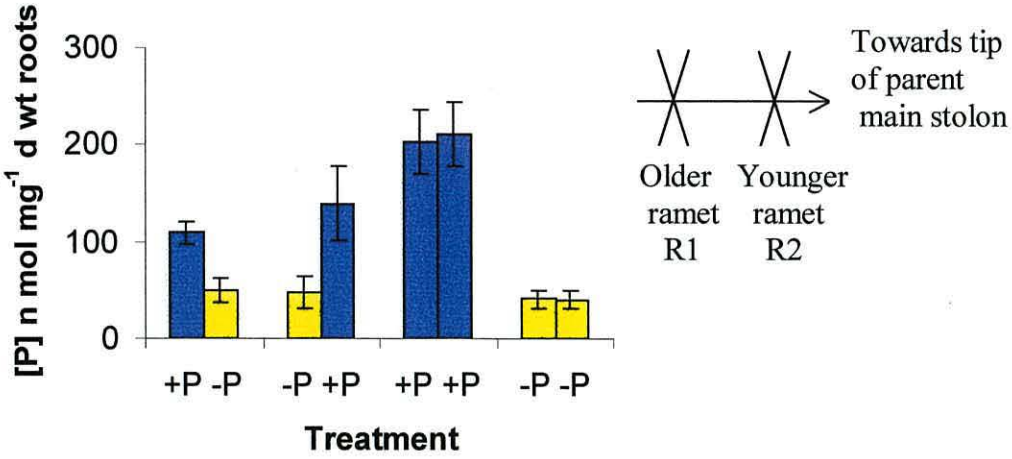


Fig. 2.14 *The mean phosphorus concentration, expressed in nmol mg^{-1}, in the roots of each a pair of ramets of A.stolonifera given contrasting phosphate treatments and grown for 29 days. +P (blue bars) = 1.33 mmol dm^{-3} phosphate e; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars show ± 1 standard error of the mean. n=4. Results from first run of the experiment.*

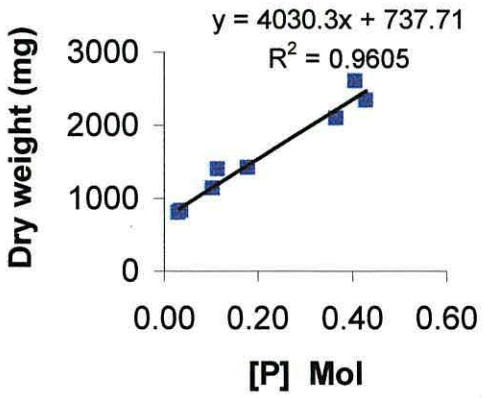


Fig. 2.15 *The relationship between the dry weight (mg) of individual ramets (root + shoot) and the mean phosphorus content of the ramet (mol). Each point represents the mean of four replicates for each ramet given one of the phosphate treatments shown in Table 2.2.*

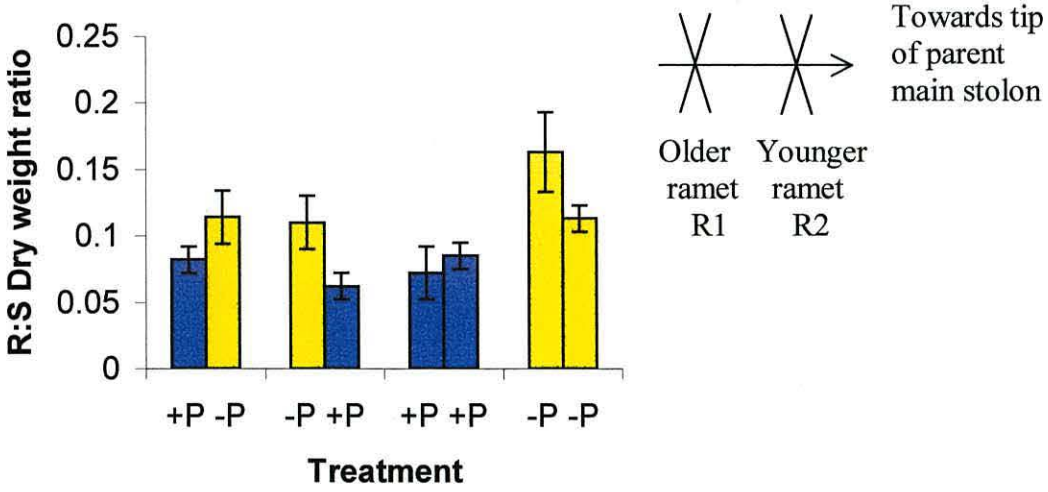


Fig. 2.16 *The root:shoot ratios of dry weight of individual ramets grown for 29 days, for each of the 8 contrasting phosphate treatments shown. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars show 1 standard error of the mean. n = 4.*

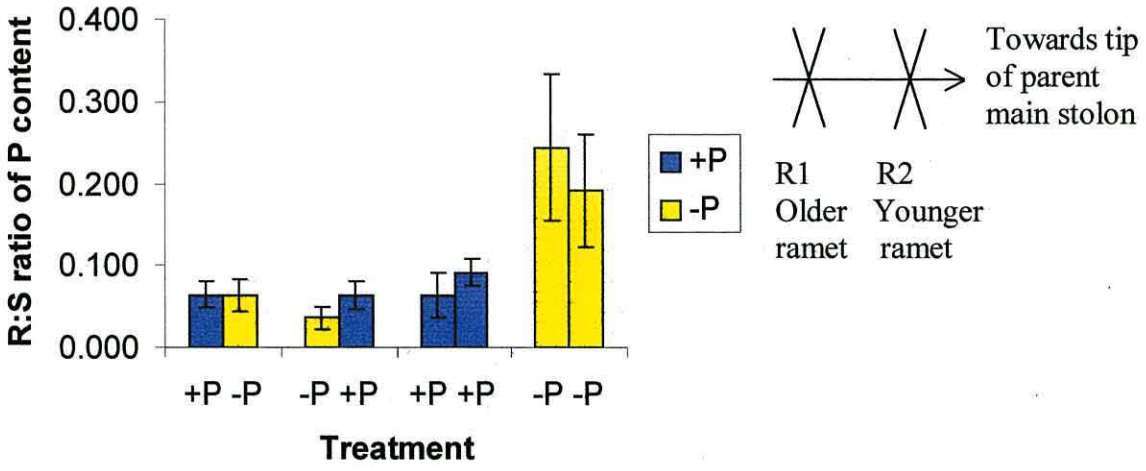


Fig. 2.17 *The root: shoot ratios of phosphorus content of ramets grown for 29 days for each of the phosphate treatments shown. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars show ± 1 standard error of the mean. n = 4. Data from first run of the experiment.*

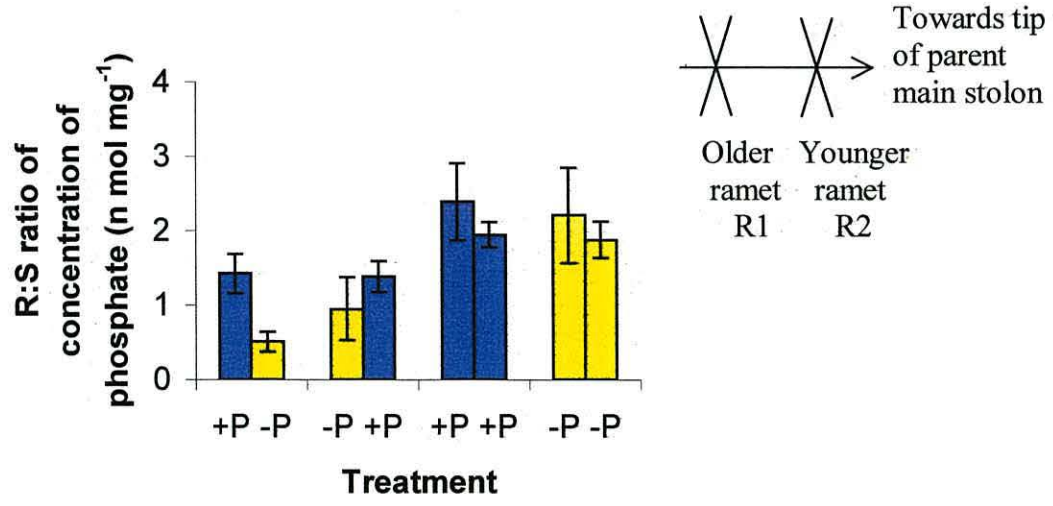


Fig. 2.18 *The root: shoot ratio of phosphorus concentration of ramets grown for 29 days given each of the phosphate treatments. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars show ± 1 standard error of the mean. n = 4. Data from first run of the experiment.*

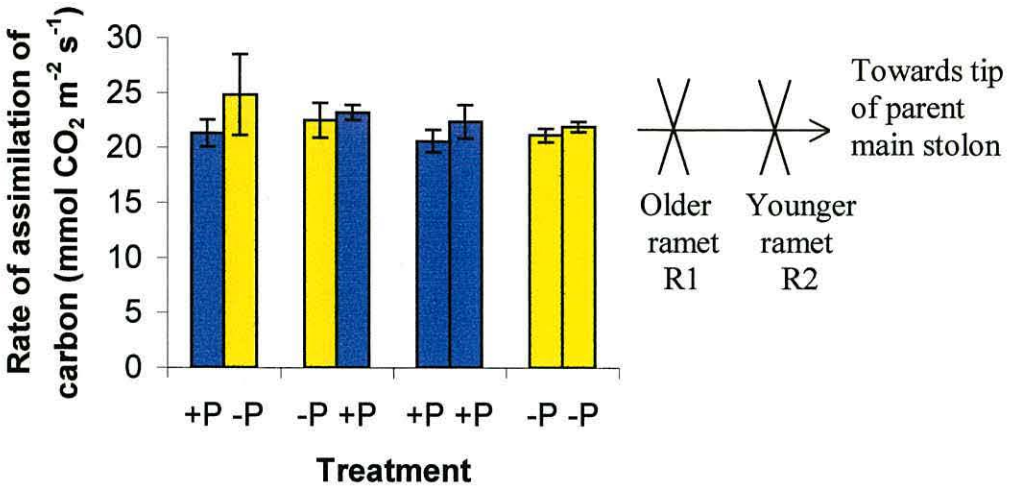


Fig. 2.19 *The rate of carbon assimilation, (mmol CO_2 $m^{-1}s^{-1}$ measured by means of a CIRAS open gas exchange system), in the youngest fully expanded leaf on the main stolon of individual ramets grown as a connected pair for 29 days and given the phosphate treatments shown.* +P *(blue bars) = 1.33 mmol dm^{-3} phosphate;* -P *(yellow bars) = 0.007 mmol dm^{-3} phosphate. N = 5. Error bars show ± 1 standard error of the mean. Data from the second run of the experiment.*

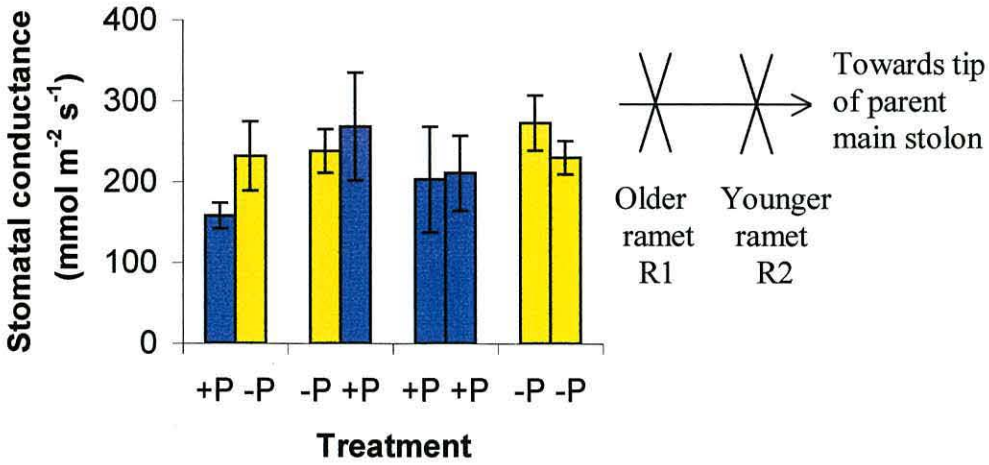


Fig. 2.20 *Stomatal conductance, (mmol m^{-2} s^{-1} measured by means of a CIRAS open gas exchange system), in the youngest fully expanded leaf on the main stolon of individual ramets grown as a connected pair for 29 days and given the phosphate treatments shown.* +P *(blue bars) = 1.33 mmol dm^{-3} phosphate;* -P *(yellow bars) = 0.007 mmol dm^{-3} phosphate. N = 5. Error bars show ± 1 standard error of the mean. Data from the second run of the experiment*

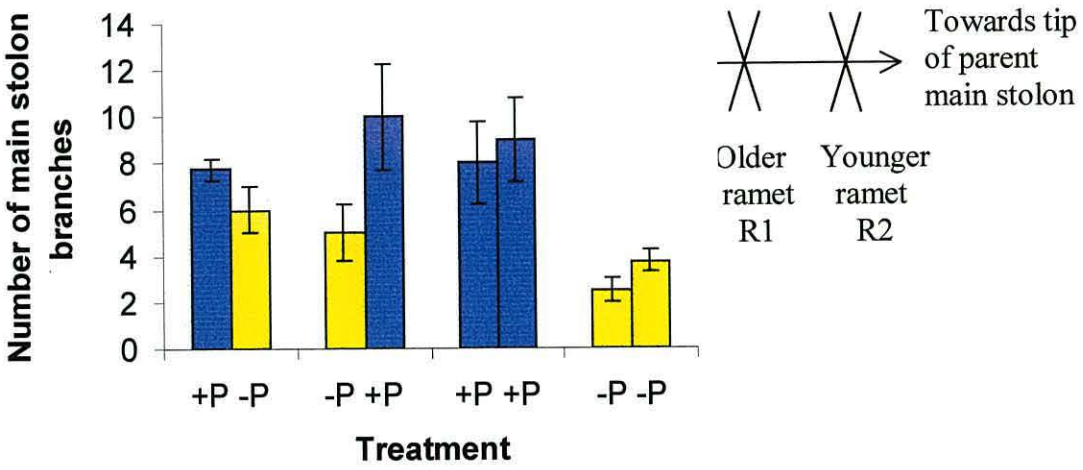


Fig. 2.21 *The mean number of main stolon branches produced by individual ramets given each of the treatments shown. Pairs of ramets were grown for 15 days in a Conviron constant environment cabinet. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mM phosphate. Error bars show ± 1 standard error of the mean. n = 4.*

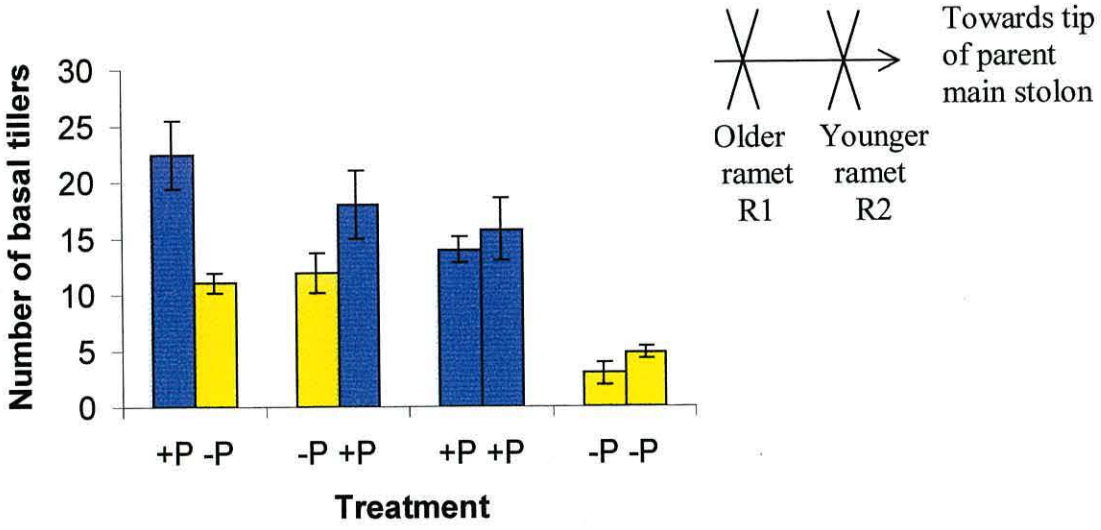


Fig. 2.22 *The mean number of basal tillers produced by individual ramets given each of the treatments shown. Pairs of ramets were grown for 15 days in a Conviron constant environment cabinet. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars show ± 1 standard error of the mean. n = 4.*

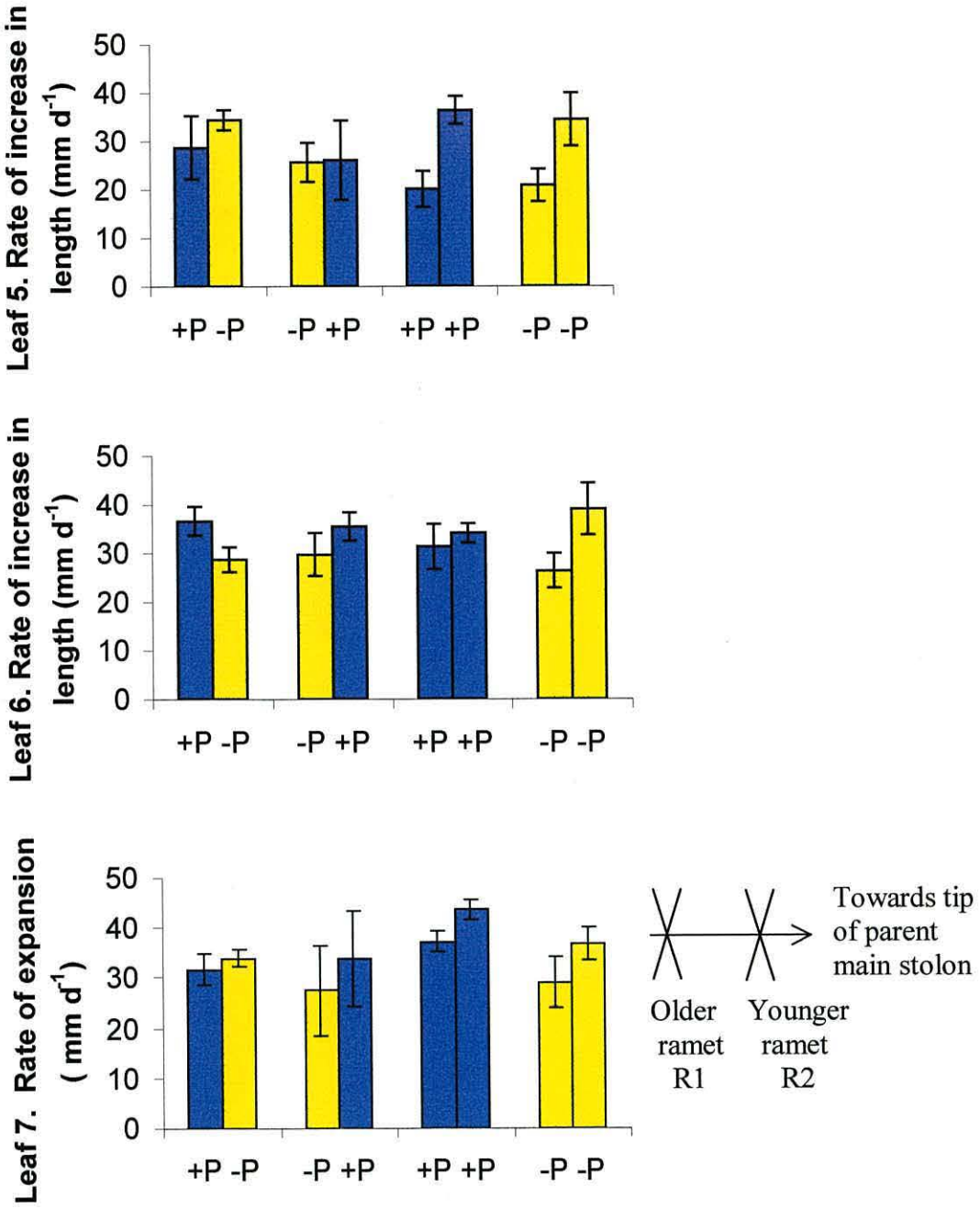


Fig 2.23 *Mean rates of expansion of leaves 5, 6 and 7 on ramets given each of the phosphate treatments shown. Pairs of ramets were grown for 15 days in a growth cabinet. Each leaf was measured daily from its appearance until the lamina was flat and the ligule visible when the leaf was considered to be fully expanded.* +P *(blue bars) = 1.33 mmol* dm^{-3} *phosphate;* -P *(yellow bars) = 0.007 mmol* dm^{-3} *phosphate. n = 4. Error bars are ± 1 standard error of the mean.*

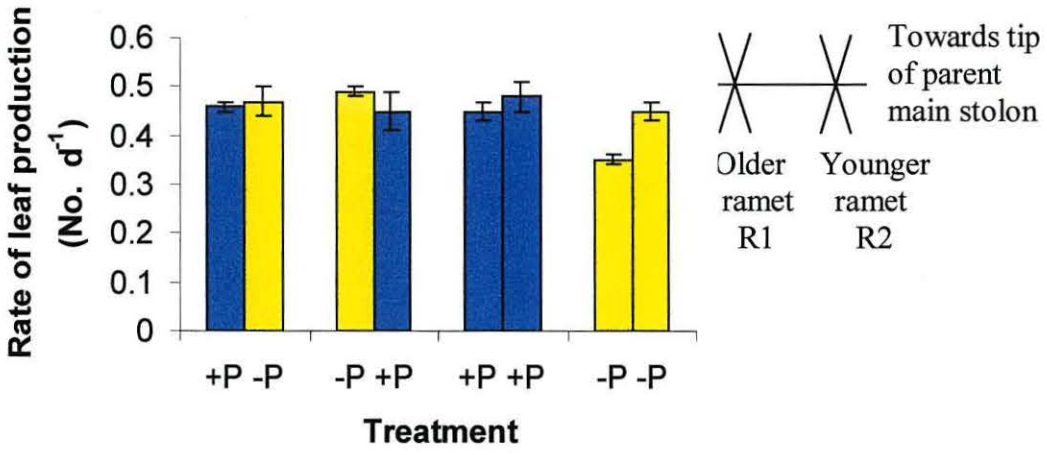


Fig. 2.24 *Mean rate of production (number d^{-1}) of leaves on ramets given each of the phosphate treatments shown. Pairs of ramets were grown for 15 days in a growth cabinet. The rate of production was calculated from the total number of leaves present at the end of 15 days. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars are ± 1 standard error of the mean. n = 4.*

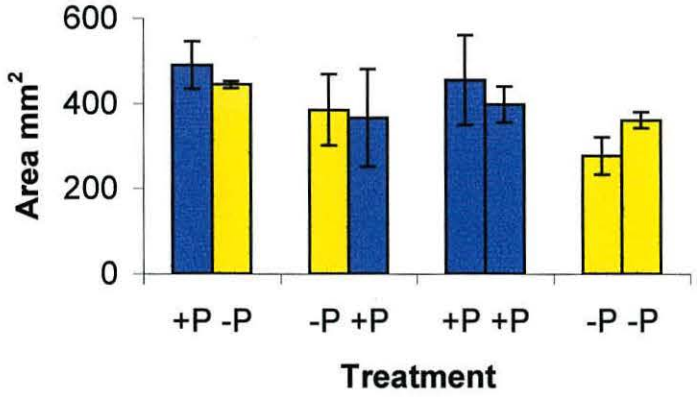


Fig. 2.25 *Mean sum of the areas of leaves 5, 6 and 7 on ramets given each of the phosphate treatments shown. Pairs of ramets were grown for 15 days in a growth cabinet. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars are ± 1 standard error of the mean. n =4*

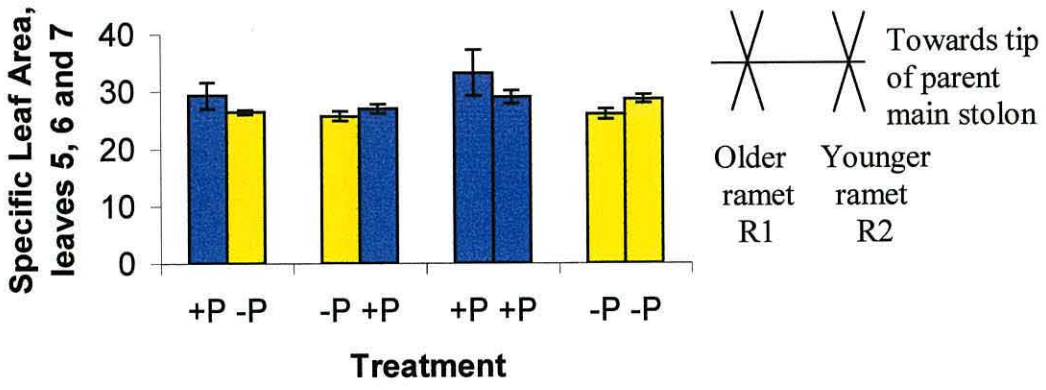


Fig. 2.26 *Specific leaf areas of leaves 5, 6 and 7 on ramets given each of the phosphate treatments shown. Pairs of ramets were grown for 15 days in a growth cabinet. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars are ± 1 standard error of the mean. n=4*

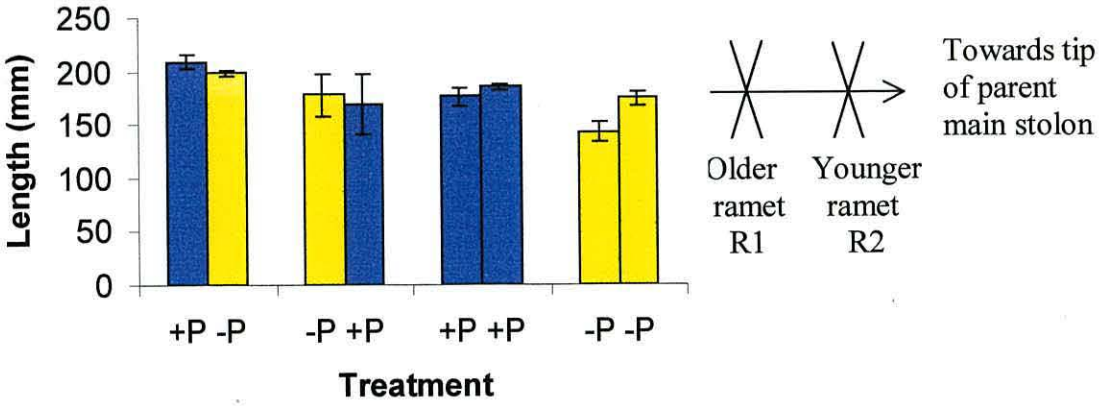


Fig. 2.27 *Mean sum of the lengths of leaves 5, 6 and 7 on ramets given each of the phosphate treatments shown. Pairs of ramets were grown for 15 days in a growth cabinet. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars are ± 1 standard error of the mean. n=4.*

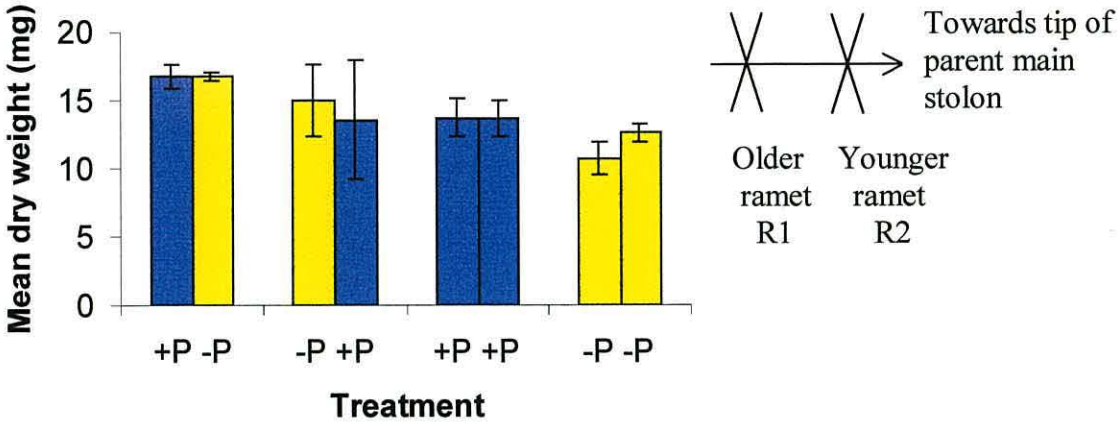


Fig. 2.28 *Mean combined dry weights of leaves 5, 6 and 7 on ramets given each of the phosphate treatments shown. Pairs of ramets were grown for 15 days in a growth cabinet. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars are ± 1 standard error of the mean. n=4.*

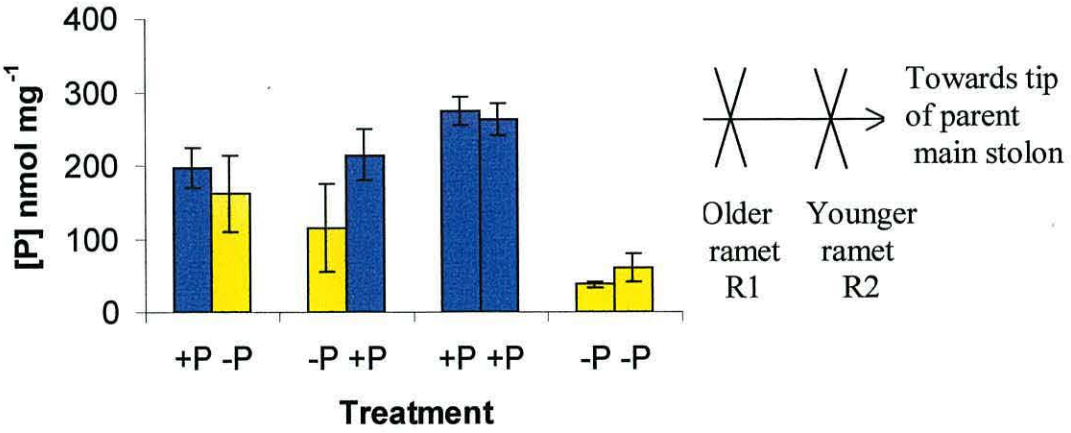


Fig. 2.29 *Mean phosphorus concentration (nmol mg^{-1}) in leaves 5, 6 and 7 on ramets given each of the phosphate treatments shown. Pairs of ramets were grown for 15 days in a growth cabinet. +P (blue bars) = 1.33 mmol dm^{-3} phosphate; -P (yellow bars) = 0.007 mmol dm^{-3} phosphate. Error bars are ± 1 standard error of the mean. n=4*

DISCUSSION

The dose-response experiment (Experiment 2.1) established that a very low supply of phosphate to the roots of *Agrostis stolonifera* L. reduced the growth if the supply was reduced to 1% of the concentration in the high phosphate nutrient solution. Although differences in the number of leaves, between plants given high (1.33 mmol dm^{-3}) and low (0.01 mmol dm^{-3}) phosphate, could not be seen until 14 days treatment, the response of the ramets varied depending on the severity of the shortage. Thus the hypothesis is confirmed that the response to reduced concentrations of phosphate is dose-dependent in *A. stolonifera*.

Both roots and shoots were smaller in the lower concentrations of phosphate. Absence of phosphate significantly reduced the dry weight of shoots compared with shoots grown with phosphate in any concentration, whereas absence of phosphate resulted in roots which had a reduction in dry weight which differed significantly only from the roots of ramets given high phosphate. This is in agreement with the reduction in dry weight found as an effect of phosphate deprivation reported by Burd (1947); Asher and Loneragan (1967); Milthorpe and Moorby (1969); Clarkson *et al.* (1978); Ericsson and Ingestad (1988).

When pairs of ramets were given different phosphate treatments, the total number of leaves diminished as less phosphate was supplied to the roots. There was no significant difference in the rate of elongation of the main stolon for any of the treatments during the 22 days of the experiment. Although there was no significant difference in the lengths of the roots for any of the treatments given, the lowest rate of increase in the lengths of the roots was brought about by the highest concentration of phosphate reported.

In Experiment 2.2 partners in pairs of ramets were given the same or contrasting phosphate treatments. The high phosphate ramets were given 1.33 mmol dm^{-3} P and low phosphate ramets 0.007 mmol dm^{-3} P. The latter is half the lowest concentration supplied in the dose response experiment so greater differences were expected in the growth of +P and -P ramets.

Does attachment to a ramet given high phosphate increase the phosphate content of a ramet given low phosphate?

The phosphorus content and the P concentration in the shoots of ramets given homogeneous low phosphate (-P-P) was lower than for any other treatment. Low phosphate ramets in the pairs given heterogeneous treatments (+P **-P** and **-P** +P) had the same amount of phosphate, indicating that phosphorus had been transported from the high phosphate partners, both acro- and basipetally (Figs 2.11 and 2.12). However the shoots of pairs given homogeneous high phosphate and the younger ramet in the pair given -P **+P** contained more P than the older ramet given high P in the heterogeneous treatment **+P** -P so although acropetal transfer of P might be greater than basipetal transfer there was more P present in the pair given -P+P than in the pair given +P-P (Fig 2.30).

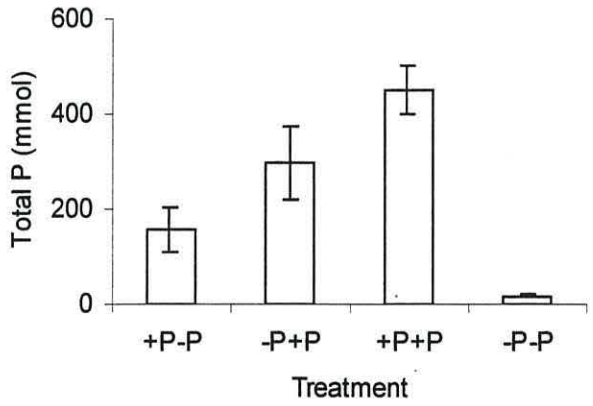


Fig. 2.30 *Total P content (mmol) in roots and shoots of pairs of ramets given contrasting phosphate treatments and grown for 29 days in a constant environment cabinet. Error bars show ± 1 standard error of the mean. n= 4*

The roots of ramets given low phosphate had less P than those given high phosphate. There was no difference in the amount of P in the roots of ramets given low phosphate either in homogeneous (**-P-P**) or heterogeneous (+P **-P** and **-P** +P) treatments (Figs 2.13 and 2.14). There was no significant difference between the P content or P concentration in the roots of ramets given high P in either homogeneous or heterogeneous treatments although the results suggest a trend towards a higher P content in the roots of a younger ramet given high P in the heterogeneous pair given -P **+P** than in the reciprocal treatment, as in the

shoots. These results indicate net movement of phosphorus from the roots of a ramet given high P to the shoot of a low P ramet. Low phosphate ramets attached to high phosphate partners had a significantly greater phosphorus concentration than the low phosphate -P -P ramets. This supports the findings of Pitelka and Ashmun (1985) and Marshall (1990) that clonal plants may transport resources from one ramet to another to support ramets which are deprived of that resource.

Does attachment to a ramet given high phosphate increase the dry weight of a low phosphate ramet?

In Experiment 2.2 the +P +P pair together were significantly heavier than the -P -P pair. The dry weights of the paired shoots of ramets, given contrasting phosphate treatments, were compared with the homogeneous high phosphate and low phosphate pairs. The pair in which the older ramet was given high phosphate and the younger given low phosphate (+P-P) had a combined dry weight not significantly different from the pair in which both were given low phosphate (-P -P). In contrast the pair given the reciprocal heterogeneous treatment (-P +P) had a total dry weight not significantly different from the +P +P pair (Fig. 2.7). This suggests that phosphorus moved from the older, R1 ramet, to the younger if the latter lacked phosphorus, but that basipetal translocation to an older ramet lacking phosphate was less likely to occur. In both replicates of Experiment 2.2 the younger ramet in the pair given -P **+P** was heavier than the younger ramet in the pair given +P **+P**. The greatest combined dry weight was seen, not in the homogeneous pair in which both were given high phosphate, but in the pair given -P +P. However, the dry weights were very variable, even when the results for both runs of the experiment were pooled. Nevertheless the hypothesis is supported that provision of adequate phosphate to one of a pair of attached ramets of *Agrostis stolonifera* L. will increase the dry weight of a phosphate-deprived partner when this is compared with the dry weight of partners in which both are P-deprived.

The increased growth of a daughter ramet must exceed the parent ramet's lost growth if translocation is occurring (Saltzman & Parker, 1985 and Caraco &

Kelly, 1991).

Table 2.10 *Dry weights (mg) of whole ramets from Experiment 2.2 (a) given each of the phosphate treatments shown*

+P	-P	-P	+P	+P	+P	-P	-P
1424 ± 510	1400 ± 388	1008 ± 299	2606 ± 171	2339 ± 239	2092 ± 464	832 ± 123	809 + 81

The dry weight of the R1 ramet of the pair given **+P** -P is 915 mg less (2339 – 1424) than the R1 ramet of the pair given **+P** +P. If this 'lost' dry weight is added to that of the R2 ramet in the pair given -P **-P** the calculated dry weight of the R2 ramet in the pair given +P **-P** should be 1724 mg (809 + 915). Since the recorded dry weight is 1400 ± 388 mg this supports the hypothesis that phosphate will move from R1 to R2 in the +P -P heterogeneous pair to permit an increase in dry weight. However, since the ramets within each pair in these experiments were rooted at the same time they are perhaps to be considered to be siblings rather than parent and daughter.

For plants growing in soil the greater dry weight of the +P ramet in the -P **+P** pair compared with that of the +P ramet in the **+P** -P pair could be explained in terms of the morphology of the roots; the roots of the low phosphate partner extending lengthways but those of the +P ramet branching and extending sideways which would allow uptake of phosphate over a greater volume of soil. For plants grown in stirred, hydroponic solution the explanation is perhaps that the apices of the younger, +P ramets were stronger sinks than those of the -P ramet and phosphorus was therefore translocated to the younger ramet from the older. This effect would not occur in the +P +P pair where the sink strength of the partners would presumably be closer to equality.

Shortage of phosphorus results in the de-repression of high affinity translocators (Bieleski & Lauchli, 1992; Dunlop *et al.*, 1997; Raghothama, 1999). If this occurred in the roots of the R2 ramet of the -P **+P** pair in response to a signal from the P-deprived R1 ramet, the younger ramet could take up phosphate very

rapidly from the high phosphate Long Ashton nutrient solution surrounding its roots and become heavier than the +P +P ramets. This explanation is less likely since the high concentration of phosphate provided is well in excess of that required for normal growth (Epstein, 1972; Delhaize & Randall, 1995; Marschner, 1995; Jeschke *et al.,* 1996).

Is the dry weight directly related to the phosphorus concentrations in roots and shoots?

The total amount of phosphorus in each ramet indicated that a rich supply of phosphate to the roots resulted in an increased amount of phosphorus in the plant tissues and the heaviest ramets had the greatest phosphorus content but not necessarily the greatest phosphorus concentration. The phosphorus concentration in the shoots of ramets given high phosphate was the same but there were differences in their dry weights.

The total phosphorus content of the whole ramets, for both high and low phosphate treatments, correlated closely with the dry weight (Fig 2.15). The closest correlation between phosphorus concentration in either root or shoot and dry weight was found between the phosphorus concentration in the root and the dry weight of the shoot in high phosphate ramets (Table 2.7). There was little correlation between phosphorus concentration and dry weight of shoots for low P ramets. This reflects the greater variability in the dry weights of the low phosphate roots and shoots and probably indicates that they were unable to grow optimally in the phosphate available and that differences in uptake and distribution altered their growth rates.

Does low phosphate result in either heavier or longer root systems?

The roots of ramets grown in high phosphate, as well as their shoots, were heavier than those of low phosphate ramets. The roots of ramets given +P in the -P **+P** pair were heavier than those of +P in the **+P** -P pair, although both were high phosphate ramets attached to low phosphate partners. Shortage of

phosphate affected the length of roots, with most ramets given low phosphate having significantly longer roots than those given high phosphate. Where the older ramet in the heterogeneous treatment was given high phosphate (**+P** -P pairs) the roots were not significantly different from those of low phosphate ramets. This supports the assumption that the older ramet was losing phosphorus to the younger and agrees with the findings of Jackson *et al.* (1990) who reported that root length varied with phosphate concentration.

Does shortage of phosphate increase the root:shoot dry weight ratio of single ramets or pairs?

Low phosphate in the nutrient solution (external P) increased the root:shoot ratio of dry weights. The higher R:S ratio was due to a decrease in the dry weight of the shoots rather than an increase in the dry weight of the roots. The R:S dry weight ratio of single ramets was most closely correlated with the phosphorus concentration in the shoot (internal P) and least closely correlated with the phosphorus content of the roots. Shortage of phosphorus increased the R:S ratio as reported by Minchin *et al.* (1994). Access to high phosphate decreased the root:shoot dry weight ratio. For pairs of ramets in which either partner had access to high phosphate this effect was apparent but not significant. The root:shoot phosphate content ratio was highest in low P ramets without access to high phosphate. The R:S ratio of total phosphorus in low P ramets was greater for the older ramet in the **-P** -P pair than for all other ramets. This agrees with the reports (Bieleski, 1973; Marschner *et al.*, 1996; Jeschke *et al.*, 1997; Raghothama, 1999) that P-deficient plants retain more Pi in their roots. However the R:S ratio of phosphate concentration (nmol P mg^{-1} dry weight) was the same in the low phosphate partner of the **-P** +P pair as in the high phosphate ramet whereas in the +P -P pair the R:S ratio of P concentration was greater in the +P partner showing that these +P ramets contained more phosphorus in their roots than in their shoots. The explanation is perhaps that attachment to a high P partner altered the need of -P ramets to retain Pi. Although the -P -P pair had a high R:S ratio of phosphorus concentration this was also true for +P +P ramets.

As for individual ramets the R:S dry weight ratio of the pairs of ramets was most closely correlated with the phosphorus concentration in the paired shoots and least closely correlated with the phosphorus content of the roots, suggesting that the change in R:S ratio is in response to internal rather than external concentrations of phosphorus.

Does shortage of phosphate bring about a reduction in the rate of photosynthesis or stomatal conductance?

No difference was found in the rate of photosynthesis or in the rate of stomatal conductance of water vapour in ramets of *A. stolonifera* given high or low phosphate. Although shortage of phosphorus often reduces the rate of photosynthesis (Milthorpe & Moorby, 1969; Herold, 1980; Dietz & Foyer, 1986; Heineke, *et al.,* 1989; Rao & Terry 1995), this is not so for all plant species (Fredeen, *et al.,* 1989; Zulu *et al.,* 1991). High phosphate may also increase stomatal conductance. This has been recorded for cotton seedlings (*Gossypium hirsutum)* (Radin & Eidenbock, 1984) and for mulberry seedlings (*Morus nigra)* (Sharma, 1995). In both these cases stomatal conductance was measured on plants grown in soil whereas for *A. stolonifera* in Experiment 2.2 measurements were made on plants grown hydroponically and thus with abundant water which would permit stomata to remain open in both +P and -P ramets.

Does shortage of phosphate reduce the rate of production of leaves, main stolon branches and basal tillers?

Ramets supplied with high phosphate produced more branches on the main stolon and more basal tillers than ramets given low phosphate. The effect of low phosphate in inhibiting branching was apparent for both shoots and roots even though Halstead & Lynch (1996) found that this effect was less marked for monocotyledons. There was little evidence of phosphorus being shared between ramets in sufficient quantity to allow increased branching and tillering in the low P partners in heterogeneous pairs as these did not have any more branches or

basal tillers than the -P -P pair. The greater dry weight of the shoots of the ramets given the higher concentrations of phosphate was due to the greater number of leaves, borne on a greater number of branches and basal tillers.

Chapin *et al.* (1989) found a decrease in the rate of elongation of new leaves in the absence of phosphate, but there was no significant decrease in either the rate of expansion of new leaves in *A. stolonifera* or in the rate of their production. For the R2 ramet in the pair given homogeneous high phosphate the rate of expansion of leaf 5 was greater than for the rest and for R1 of the pair given homogeneous low phosphate the rate of production of new leaves was lower than for all the rest. There was no difference in either the area, SLA or dry weights of the leaves. Only in the high phosphate ramets in the pair given **+P** -P was there an increase in the final length of three leaves together. Fredeen *et al.* (1989) noted a reduction in the total leaf area of soybean, resulting from fewer but also smaller leaves, when plants were given low phosphate. The similarity between the leaves of high- and low-phosphate ramets of *A. stolonifera* may well reflect the lower sensitivity of monocotyledons to phosphate shortage as reported by Ridge (1991). Thus the increase in the dry weights of shoots in ramets given high phosphate is not due to any increase in the size of leaves or in the number of leaves on the main stolon but to the greater number of basal stolons and branches.

CONCLUSIONS

- low phosphate reduces the growth of *A. stolonifera.*
- P is translocated between ramets .
- internal sensing of P is in the shoots.

Chapter 3

The use of dyes to trace the vascular connections in *Agrostis stolonifera* L.

INTRODUCTION

Variation in the pattern of growth of pairs of connected ramets of *A. stolonifera* was seen in the experiments reported in Chapter 2, when high phosphate was supplied to the roots of one ramet but low phosphate given to the other. This variability is possibly attributable to differences in the ability of the pairs of ramets to transport phosphorus between them. Vascular connections need not be between adjacent ramets but between ramets separated more widely along the stolon. Cutting the stolons into pairs may sometimes have resulted in closely-connected pairs and on other occasions not. This may account for the observed variation in patterns of dry weight of adjacent ramets in separate experiments. Milthorpe & Moorby (1969) state that

> "Restrictions of growth attributable to minerals are usually matters of supply to and size of absorbing regions, the circulation in the plant being very efficient."

If this is the case for *A. stolonifera,* the observed differences in growth between the pairs of ramets given contrasting phosphate treatments in several experiments could be due to differences in the vascular connections between the ramets. Birch & Hutchings (1999) found differences in the subsequent growth of fragments of *Glechoma hederacea* depending on whether the fragments were basal or apical cuttings.

Phosphate, and other ions, taken in by roots, moves through the symplast, and through the extra-cellular free space until it reaches the barrier of the endodermis which prevents passive entry of solute into the stele (Marschner, 1995). From the symplasm the ions enter the xylem vessels, by means of carriers, against their concentration gradient. Phosphate is a very mobile ion

inside a plant and an individual phosphorus atom may circulate many times through a plant (Läuchli, 1972; Bieleski, 1973; Marschner, 1995).

Bieleski (1973) also considered that the pattern of redistribution appears to be determined more by the properties of the sources and sinks than by the transport system. Clarkson & Scattergood (1982) however, found that the transport system may be increased or de-repressed in phosphate deficiency and therefore concluded that phosphate transport is controlled by the concentration of a regulatory phosphate pool within the plant. The internal P status of a plant can regulate both the uptake rate by the roots and the distribution of Pi within the plant (Raghothama, 1999). Marschner *et al.* (1996) observed that there is evidence that cycling of phosphorus from shoot to root acts as an important signal in feedback control of nutrient uptake.

Phosphorus moves through the xylem mainly as the inorganic ion PO_4^{3-} (Pi), translocated by mass flow moving at the same velocity as the xylem water. Some of the translocated phosphorus is incorporated into organic compounds and there are significant quantities of organic phosphorus (Po) in the phloem (Lauchli, 1972; Bieleski, 1973; Raghothama, 1999). Hay & Sackville Hamilton (1996) conclude that the allocation of phosphorus from the roots in *Trifolium repens* is determined by the direction of the transpiration stream i.e. phosphorus is carried in the xylem.

The advantage to plants of the clonal habit is the ability for water, minerals and photosynthates to be translocated between ramets (Noble & Marshall, 1983; Pitelka & Ashmun, 1985). Alpert & Mooney (1986) observed that water and photosynthates can move in opposite directions along the same stolons of *Fragaria chiloensis* allowing connected ramets to exchange resources with one another. Reciprocal exchange of nitrogen and carbon has been established in connected ramets of *Fragaria chiloensis* (Friedman & Alpert, 1991) but may occur only if the resources are in abundant supply and may be greater between

adjacent ramets than between more widely separated pairs (Noble & Marshall, 1983). Pitelka & Ashmun (1985) point out that there is variation among clonal species in the extent to which connections remain functional and also in the pattern of movement of materials.

The vascular system of grasses is complex, with numerous leaf trace bundles, with varying orientations, uniting in different ways with other strands. At each node there are abundant anastomoses of bundles. Clonal grasses tend to preserve connections between the parent and daughter ramets (Nyahoza *et al.*, 1973) and generally the parent ramets support the daughters until the latter are well established. It has also been observed that stresses of various kinds can change the pattern of support (Pitelka & Ashmun, 1985) and reciprocal transfer of resources has been observed in *Lolium multiflorum* (Marshall & Sagar, 1968). Ong & Marshall (1979) found that although a parent ramet of *Lolium perenne* may support a daughter ramet initially, eventually support with carbohydrate may be withdrawn from a shaded ramet by the rest of the clone.

Stuefer *et al.* (1998) produced models to demonstrate the crucial role played by the connecting internode in the distribution of resources between ramets. They showed that the extent to which water, and hence dissolved minerals, is conducted along the inter-connecting stolon is determined by the length of the internode, the number of xylem vessels it contains and their diameter. Clonal plants such as *Glechoma hederacea,* grown in nutrient-rich environments, tended to have shorter internodes (Hutchings & de Kroon, 1994). Schmid & Bazzaz (1987) found that for clonal plants with long internodes, severing the internodes increased the density of daughter ramets whereas, given adequate resources, species with short internodes showed little difference in the density of patches in which the plants were left connected and patches in which they were separated. This suggests that shorter internodes may be better for the exchange of resources and that longer ramets may do this to a lesser extent. Apical dominance effects can be reduced if the connections are severed.

Van Kleunen & Stuefer (1999) demonstrated that bi-directional translocation of water and assimilates is possible between connected ramets of *Potentilla anserina* if one partner is provided with water but reduced light and the other partner has the reciprocal treatment. These authors used steam-girdling to destroy the phloem while leaving the xylem intact. Water transport was thus unaffected whilst carbohydrate could no longer be conducted.

Acid fuchsin dye has been used to trace the vascular connections in plants. Pate *et al.* (1985) used acid fuchsin to follow the xylem connections between the developing fruits of cowpea (*Vigna unguiculata)* and the rest of the plant and found that it is possible for the flow of water to be reversed in the xylem supplying the fruits, either partially or totally. Price *et al.* (1996) supplied acid fuchsin to cut petioles of *Glechoma hederacea* and found that all movement of the dye is acropetal - furthermore the movement of the dye is highly sectorial and closely linked to the phyllotaxy of the plant. Sectoriality is the existence within a plant of regions in which the movement of resources is clearly confined (Price *et al.,* (1996). D'Hertefeldt & Jonsdottir (1999) used acid fuchsin to trace the pathway of water through *Carex arenaria* growing on sand dunes and showed that the dye is transported both acropetally and basipetally but that fewer vessels are coloured red proximal to the point of application of the dye than distal to it.

Basic fuchsin is a dye which is taken up by the roots of plants and can be used to study the vascular system of living plants where it stains lignified tissues red (Fuchs, 1963). This dye does not readily cross the endodermis so it is necessary to remove a short section from the tip of the roots to allow entry to the xylem (Bell, 1998, pers. com.). Basic fuchsin is less mobile than acid fuchsin which tends to diffuse out of the xylem into surrounding tissues (Talboys, 1955) but basic fuchsin may induce wilting in some plant species within a few hours. Crystal violet has a similar rate of diffusion to basic fuchsin and contrasts well with it in sections. Talboys (1955) used basic

fuchsin and crystal violet, which had been decolourised by bleaching with sodium metabisulphite, to investigate the presence of tyloses in hop plants infected with *Verticillium*. The colour of the reduced dyes is restored on re-oxidation in the xylem.

This chapter investigates the vascular connections, in whole stolons and in clonal fragments consisting of pairs of ramets of *A. stolonifera,* by means of reduced basic fuchsin and reduced crystal violet dyes. Before treatment with dyes the ramets were grown for 10 – 14 days in Long Ashton nutrient solution containing either 1.33 mmol dm^{-3} or 0.007 mmol dm^{-3} phosphate.

The hypotheses to be tested are that

- movement of dye will be in the direction of the transpiration stream
- dye will be translocated both acropetally and basipetally in the xylem
- the first roots to develop at each node will supply the main stolon whilst roots which develop later at the node will supply the basal tillers
- the rate of transfer of dye is greater in plants grown in high phosphate than in those grown in low phosphate.

MATERIALS AND METHODS

Reduced 1% basic fuchsin was used to trace the xylem connections along which water might travel from the roots into the shoots and to sibling ramets. Basic fuchsin was reduced in the presence of sodium metabisulphite until it was just decolourised (approximately 30–35 g sodium metabisulphite in one dm^3 basic fuchsin). The dye reddens as it re-oxidises in the air or in plant tissues. Since the endodermis may prevent the passage of basic fuchsin into the xylem, the final 2 cm of the root was cut off under the surface of the dye to prevent entry of air bubbles into the xylem.

Experiment 3.1 To test the hypothesis that the rate of movement of dye along the main stolon of *A. stolonifera* is greater with the unrooted distal end of the main stolon left *in situ* than if this is removed

(a) With unrooted distal end of the main stolon left in situ

The youngest node at the tip of each of the basal tillers of parent plants of *A. stolonifera* rooted in compost, was pinned down into compost by means of plastic-covered wire. When these nodes had rooted, the cuttings were detached from the parent and their main stolon allowed to grow. As each produced its own stolon this was in turn pinned down in compost at the second and at each alternate node until three rooted, successively-younger daughter tiller-ramets had been established, with three unrooted ramets developed from the nodes between. The new plants were given full Long Ashton nutrient solution weekly and watered between feeding. The roots of parent and daughter ramets were then carefully excavated from the compost and the whole plant transferred to a light bank at a temperature of 26 °C and a light intensity (Skye Quantum sensor, Model SKP 200) of 145 μmol m^{-2} s^{-1} supplied by five 1.5m 65/80W white fluorescent tubes. The length of the main stolon of each was measured and the roots of the parent ramet placed in a test tube containing 1% reduced basic fuchsin. The roots of the three daughter ramets were placed in water (again with the terminal 2 cm removed under the water). Fig. 3.1.1 shows the arrangement of the rooted parent, three rooted daughter ramets and six unrooted ramets.

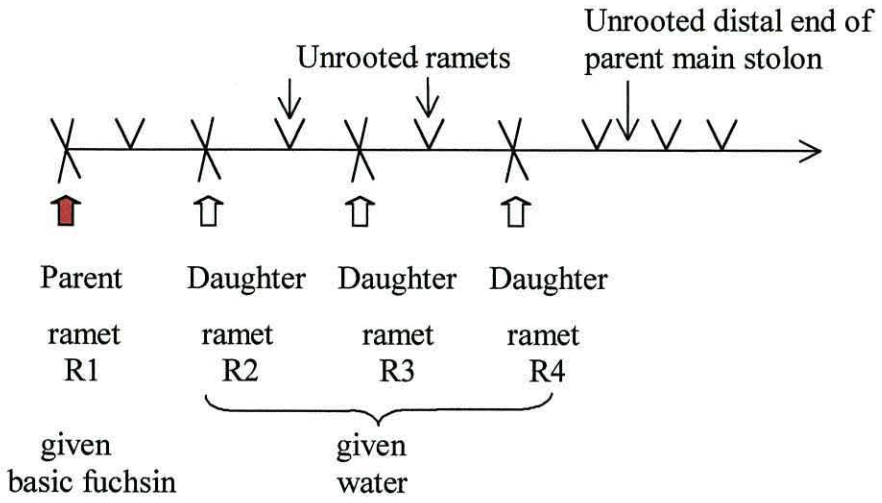


Fig. 3.1.1 *Diagrammatic representation of a stolon of A. stolonifera with a parent and three rooted successively-younger daughter ramets excavated from compost and with the parent main stolon left intact.* ⇧ *= reduced basic fuchsin supplied to the roots of the parent plant.* ⇧ *= water supplied to the roots of rooted daughter ramets.*

The progress of the basic fuchsin along the main stolon and into the daughter ramets was observed by means of a hand lens. When, after 90 minutes, red colour could be seen in the veins of the leaves of the third daughter ramet (R4), the plants were placed in lactic acid and held at a temperature of 80 °C overnight to clear the tissues when the red colour in the vascular tissues could be clearly seen. The plants were then washed free of acid and stored in 40% v/v ethanol. Three replicates were used.

(b) With the unrooted distal end of the main stolon removed just distal to R4

Experiment 3.1(a) was repeated with the parent main stolon cut off, just prior to application of the basic fuchsin, beyond R4 (Fig. 3.1.2). The progress of the basic fuchsin into the daughter ramets was observed by means of a hand lens. When, after 90 minutes, red colour could be seen in the veins of the leaves of the third daughter ramet (R4), the plants were cleared, washed and stored as before. Three replicates were used.

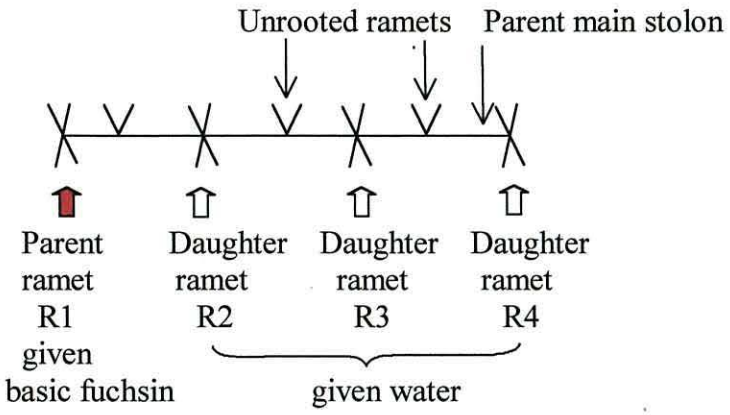


Fig. 3.1.2 *Diagrammatic representation of the A. stolonifera plants with the parent main stolon cut off beyond the last rooted ramet (R4).* ⇧ *= reduced basic fuchsin supplied to the roots of the parent plant.* ⇧ *= water supplied to the roots of daughter ramets.*

Experiment 3.2 To test the hypothesis that the rate of movement of basic fuchsin is faster acropetally than basipetally from the roots of the 2nd daughter ramet midway along a parent main stolon, if the unrooted distal end of the main stolon is left *in situ* than if the unrooted distal end of the main stolon is cut off immediately distal to the 3rd and last, rooted daughter ramet, R4

(a) With the unrooted distal end of the main stolon left in situ

Main stolons of *A. stolonifer*a were grown as before and transferred to a light bank where they were treated with basic fuchsin. The length of the main stolon was measured and the stolon left intact beyond R4. The basic fuchsin was applied to the roots of the middle daughter ramet (R3) (Fig. 3.2.1) and the movement of the basic fuchsin, both acropetal and basipetal, was followed by inspecting the stolon by means of a hand-lens.

The experiment was left to allow movement of the basic fuchsin for 60 minutes. The plants were placed in lactic acid and held at 80°C overnight to clear the tissues as before then washed free of acid and stored in 40% v/v ethanol.

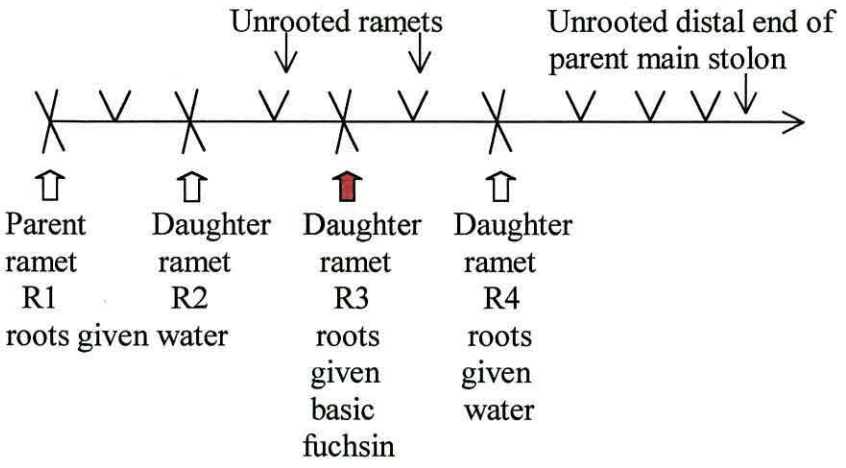


Fig. 3.2.1 *Diagrammatic representation of a stolon of A. stolonifera with three successively-younger, rooted, daughter ramets. The parent main stolon was left intact beyond R4, the last rooted daughter ramet.* ⇧ *= reduced basic fuchsin supplied to the roots of the middle, rooted, daughter ramet (R3).* ⇧ *= water supplied to the roots of other ramets.*

(b) With the with the unrooted distal end of the main stolon cut off distal to daughter ramet, R4

Experiment 3.2(a) was repeated, using three replicates, with the unrooted distal end of the main stolon cut off distal to R4 just before application of the basic fuchsin (Fig. 3.2.2).

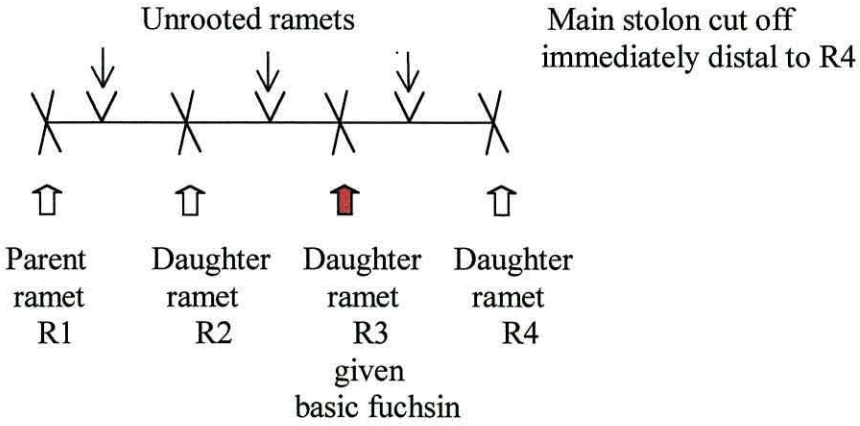


Fig. 3.2.2 *Diagrammatic representation of a stolon of A. stolonifera with three successively-younger, rooted, daughter ramets. The parent main stolon was cut off beyond R4, the last rooted daughter ramet.* ⇧ *= reduced basic fuchsin supplied to the roots of the middle, rooted, daughter ramet (R3).* ⇧ *= water supplied to the roots of other ramets.*

In each case the experiment was left to allow movement of the basic fuchsin for 60 minutes. The plants were cleared, washed and stored as before.

Experiment 3. 3 To test the hypothesis that both acropetal and basipetal translocation of basic fuchsin from the roots of a ramet is in the xylem

In experiments 3.3 to 3.7 reduced crystal violet, decolourised with sodium metabisulphite, was used to show the flow from the roots of a second ramet.

A. stolonifera plants consisting of a parent and three daughter ramets were grown and transferred to a light bank. The length of the main stolons was measured and the unrooted distal end of the main stolon left intact beyond R4. The terminal 2 cm of the roots was cut off to allow entry of the dyes. The roots of the parent ramet (R1) were immersed in reduced basic fuchsin in a test tube and the roots of the 1st daughter ramet (R2) were immersed in reduced crystal violet. Daughter ramets R3 and R4 had their roots in water (Fig. 3.3).

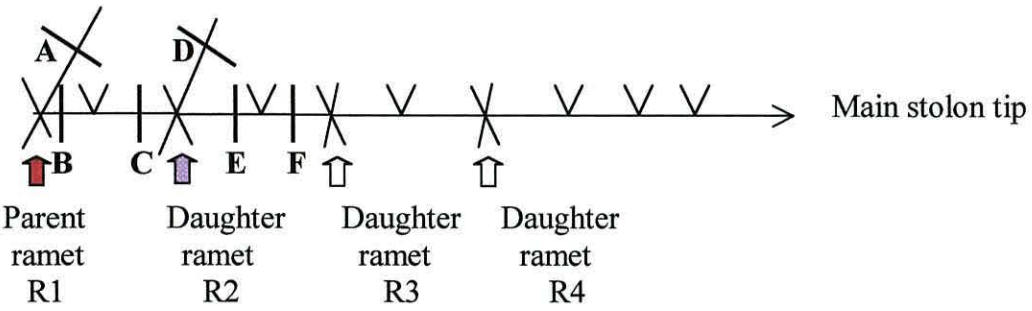


Fig. 3.3 *Diagramatic representation of a stolon of A. stolonifera with three successively-younger, rooted, daughter ramets.* ⇧ *= Basic fuchsin supplied to root of parent ramet.* ⇧ *= crystal violet supplied to root of R2.* ⇧ *= water supplied to roots of R3 and R4. A, B, C, D, E and F are points where transverse sections were made. The unrooted distal end of the main stolon was left intact beyond the youngest daughter ramet, R4. Cut A was across the oldest basal stolon of R1 and D was across the main stolon of daughter ramet R2.*

The plants were left for 60 minutes, sectioned (cuts B and C) on either side of an unrooted ramet between the R1 and R2 ramets and similarly on either side of an unrooted ramet between the R2 and R3 ramets (cuts E and F). The main stolons of each of the two treated ramets, R1 (cut A) and R2 (cut D), were also sectioned. The sections were examined and photographed.

Experiment 3.4 To test the hypothesis that darkening the shoot of the parent ramet from the start of the experiment will limit the influx of basic fuchsin to the shoot

Plants of *A. stolonifera* were grown and transferred to a light bank as before. The main stolon was measured and the unrooted distal end of the main stolon left intact beyond R4. The roots of the parent ramet, R1, were immersed in reduced basic fuchsin in a test tube and the roots of the daughter ramet, R2, were immersed in reduced crystal violet. The R3 and R4 daughter ramets had their roots in water. The whole shoot of the parent ramet was darkened with an aluminium foil cover for 1 hour before dyes were supplied to the roots of either ramet and during the experiment. This was to ensure that the stomata had an adequate chance of closing and thus that transpiration from the parent ramet shoot was reduced to a minimum. After 60 minutes the plants were sectioned at the points marked A to F (Fig. 3.4).

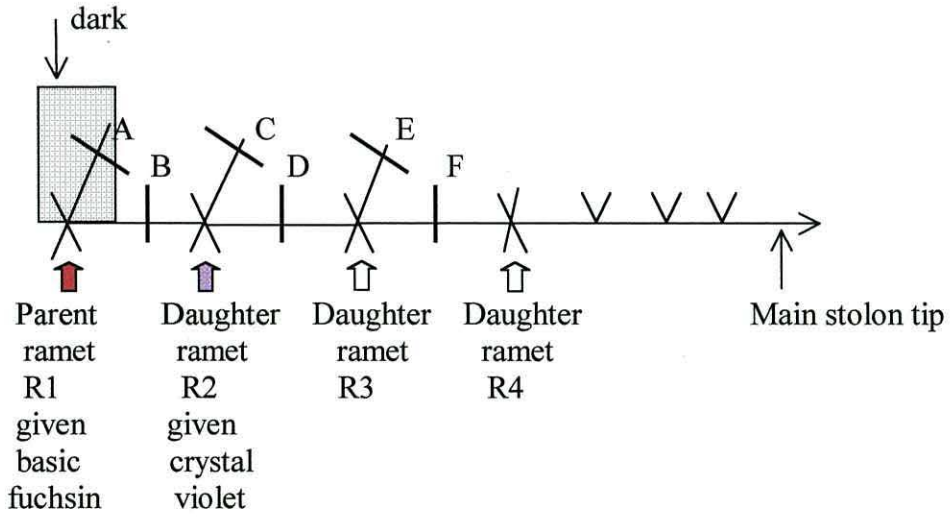


Fig. 3.4 *Diagramatic representation of a parental main stolon of A. stolonifera with three successively-younger, rooted, daughter ramets. showing where basic fuchsin and crystal violet were applied. Aluminium foil was placed over the parent shoot at the start of the experiment to darken the shoot and reduce transpiration. A – F indicate where transverse sections were made after 60 minutes.*

In experiments 3.5 to 3.8 single ramets and pairs of connected ramets of *A. stolonifera* were prepared from plants grown in John Innes No 1 potting compost

in a greenhouse until they had produced a main stolon and basal tillers. The nodes of the main stolon and tillers were gently scarified with fine-grade wet-and-dry paper to encourage rooting. The stolons and tillers were then laid on wet paper and the nodes covered with strips of paper. The paper was kept damp until small roots and shoots about 5 cm long had developed at the nodes and thus the required daughter ramets had developed. A small collar of Blu-tack™ was wrapped around the roots of ramets just below the node (Fig. 3.5). This was to allow the initial root to grow and branch below the collar but subsequent roots, produced as the basal tillers developed, would grow laterally above the collar and could thus be separated from the original roots. The roots of the ramets were then inserted through split discs of plastic foam into 1cm holes drilled in the snap-on lids of paired, 0.5 dm^3 plastic containers as described in Chapter 2, Experiment 2. All ramets were given Long Ashton nutrient solution with either 1.33 mmol dm^{-3} phosphate or 0.007 mmol dm^{-3} phosphate and grown for 10 days until a main stolon and several basal tillers had developed from each.

Experiment 3.5 To test the hypothesis that the initial roots of a ramet supply the main stolon acropetally whilst the basal tillers are supplied by roots which develop later

Single ramets, grown as described in Long Ashton nutrient solution containing 1.33 mmol dm^{-3} phosphate, were transferred to a light bank. The terminal 2 cm of the roots of the ramets was cut off to ensure that the dye could enter the roots of the ramet. The initial root, enclosed in its collar, was inserted into a test tube containing reduced basic fuchsin whilst the roots which developed later were immersed in reduced crystal violet. The progress of the dyes was followed by examining the plant tissue, especially the leaves, with a hand lens. Sections were cut across the main stolon and across the oldest basal tillers at the points marked A, B, C and D (Fig. 3.5).

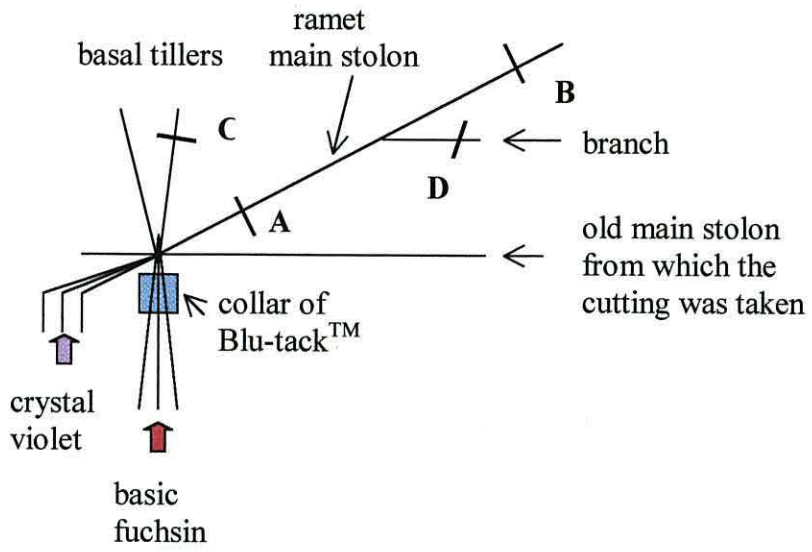


Fig. 3.5 *Diagramatic representation of the separation of initial and later developed roots by means of a collar of Blu-tack™ and the provision of basic fuchsin to the initial roots with crystal violet provided to the later-developed roots. Transverse sections were cut at points A, B, C and D. Plants were grown in either high phosphate or low phosphate for 10 days before the experiment.*

Experiment 3.6 To test the hypothesis that the initial roots of a younger ramet supply the main stolon through which basipetal transfer of dye occurs.

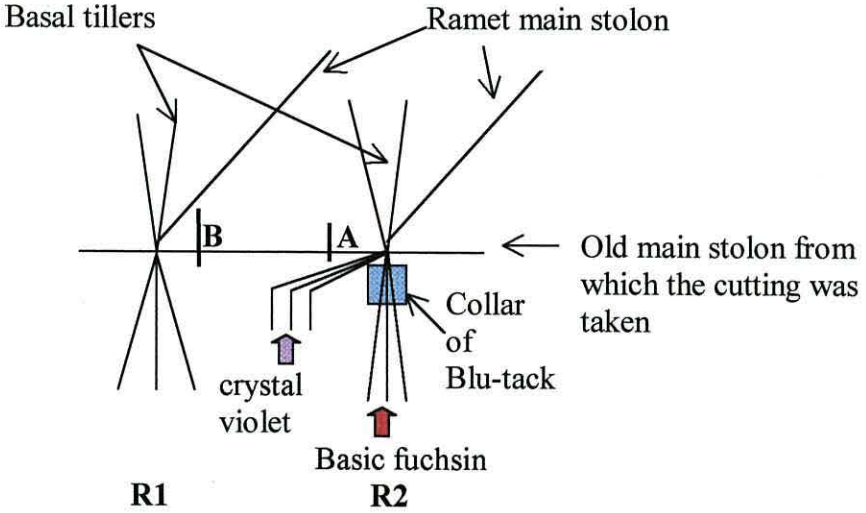


Fig. 3.6 *Diagramatic representation of the separation of initial and later developed roots by means of a collar of Blu-tack™ and the provision of basic fuchsin to the initial roots with crystal violet provided to the later-developed roots. Transverse sections were cut at points A and B. Plants were grown in either high phosphate or low phosphate for 10 days before the experiment.*

Connected pairs of ramets were grown in Long Ashton nutrient solution containing 1.33 mmol dm^{-3} phosphate. The initial roots of the younger ramet (R2) were enclosed in a collar of Blu-tack™ and inserted into a test tube containing reduced basic fuchsin (Fig. 3.6). Later-developed roots emerging above the collar were immersed in reduced crystal violet. When basic fuchsin could be seen by means of a hand lens in the leaves the main stolons of both ramets, transverse sections of the main stolon were cut at A and B.

Experiment 3.7 To test the hypothesis that pre-treatment with high or low concentrations of phosphate will alter the pattern of distribution of basic fuchsin and crystal violet in connected paired ramets of *A. stolonifera*.

Connected pairs of ramets were grown as described, with the roots of the R1 ramet in Long Ashton nutrient solution containing 1.33 mmol dm^{-3} phosphate and the roots of the R2 ramet in Long Ashton nutrient solution containing 0.007 mmol dm^{-3} phosphate. The terminal 2 cm of the roots of the ramets was cut off to ensure that reduced basic fuchsin could enter the roots of the older ramet. The initial roots of the older ramet were separated from later developing roots by means of a collar of Blu-tack™. The initial roots were given reduced basic fuchsin and the later-developed roots were given reduced crystal violet (or *vice versa*). The roots of the younger ramet were given water (Fig.3.7).

The progress of the basic fuchsin and crystal violet was followed by examining the plant tissue with a hand lens. The main stolon and basal tillers of both partners were sectioned at the points marked A, B, C, D and E (Fig.3.7).

The experiment was repeated with the R1 grown in Long Ashton nutrient solution containing 0.007 mmol dm^{-3} phosphate and the R2 grown in Long Ashton nutrient solution containing 1.33 mmol dm^{-3} phosphate

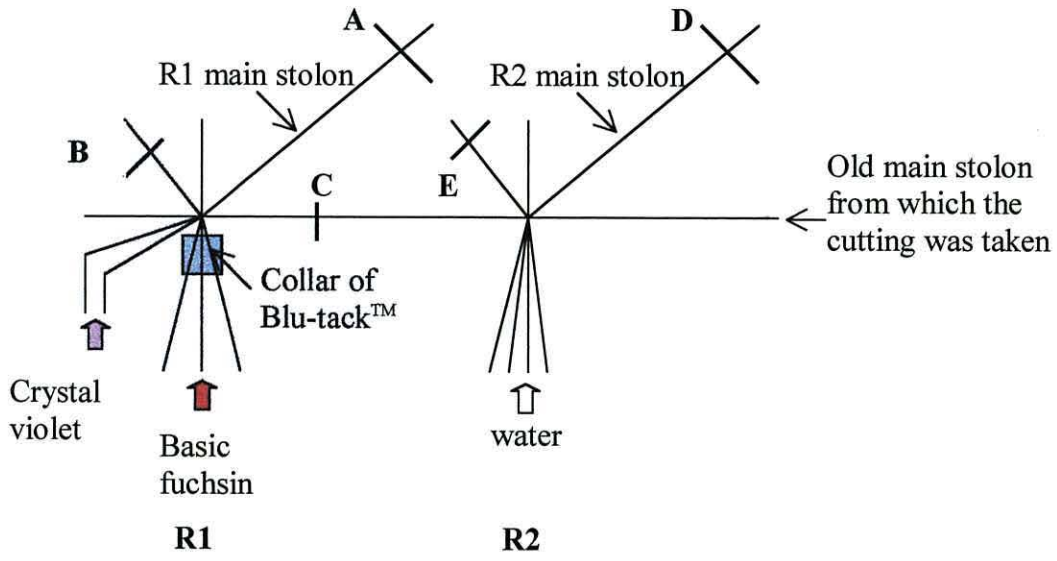


Fig. 3.7 *Diagramatic representation of the separation of initial and later developed roots by means of a collar of Blu-tack™ and the provision of red basic fuchsin to the initial roots with crystal violet to the later developed roots. R2 had water supplied to the roots. Transverse sections were cut at points A – E. Plants were grown in Long Ashton nutrient solution with either high phosphate (1.33 mmol dm^{-3}) or low phosphate (0.007 mmol dm^{-3}) for 10 days before the experiment.*

Experiment 3.8 To test the hypothesis that pre-treatment with high or low concentrations of phosphate will alter the rate of translocation of basic fuchsin along a whole stolon of *A. stolonifera*.

Pairs of ramets were grown for 15 days with two heterogeneous and two homogeneous phosphate treatments as described in Chapter 2, Experiment 2.1. The paired ramets were placed under the light bank. The total length of the main stolon, all its branches and all the basal tillers were measured for both ramets. The terminal 2 cm of the roots of the ramets was cut off to ensure that reduced basic fuchsin could enter the roots of the older ramet. The roots of the younger ramet (R2) were immersed in water. When all the shoot tissues of both ramets contained red basic fuchsin the rate of transport throughout the pair of ramets was calculated.

RESULTS

Experiment 3.1 When reduced basic fuchsin was supplied to the roots of a parent ramet of *Agrostis stolonifera,* dye was taken up and the pattern and rate of flow of basic fuchsin through the three experimental plants was very similar. Basic fuchsin passed acropetally into the shoot of the parent ramet and also into the shoots of rooted and unrooted daughter ramets (Fig. 3.8.1). When Experiment 3.1 was repeated with the roots of the parent ramet given basic fuchsin but with the parent main stolon cut off distal to the youngest, rooted daughter ramet (R4), the general pattern of distribution of the dye was unchanged (Fig 3.8.2) whilst the rate of flow of basic fuchsin was significantly slower. In no case was dye observed in the roots of ramets unless they were immersed in it. Table 3.1 summarises the lengths of the parts of the stolons used and shows that the presence of the unrooted portion of the main stolon increases the rate of acropetal flow of dye.

Table 3.1 *Summary of the rate of passage of the dye when basic fuchsin was fed to the roots of the parent ramet (a) with the parent main stolon left intact beyond the youngest, rooted daughter-ramet and (b) with the parent main stolon cut off distal to the last rooted ramet. The rates without the parent main stolon tip in place were significantly slower (p = 0.011). The lengths of parts of a stolon of A. stolonifera are shown.*

	(a) Rate of flow (cm min^{-1}) with main stolon intact	(b) Rate of flow (cm min^{-1}) with main stolon cut off distal to R4
Mean rate of flow of basic fuchsin along the stolon from the roots of the parent ramet towards the tip of the main stolon. (n = 3)	0.26 ± 0.02	0.09 ± 0.03**
Mean length of longest roots of parent ramets given basic fuchsin. (n = 3)	14.8 ± 0.7	14.7 ± 0.9
Mean length of part of stolon bearing rooted daughter ramets. (n = 3)	46.3 ± 4.7	52.7 ±0.7
Mean total length of main stolon from R1 to the tip of its main stolon. (n = 3)	94.7 ± 5.9	90.3 ± 1.9

Experiment 3.2 When basic fuchsin was supplied to the roots of the middle of three rooted daughter ramets, R3, in the presence and in the absence of the unrooted part of the main stolon, in both experiments basic fuchsin was observed after one hour in the ramets distal to the ramet which had dye supplied to its roots (Figs. 3.9.1 and 3.9.2). Where the unrooted portion of the main stolon was left intact, basic fuchsin passed for a short distance basipetally along the parent main stolon into the next older unrooted ramet. The dye was also seen in all the leaves on the parent main stolon distal to R4 (Fig. 3.9.1). When the parent main stolon was cut off immediately distal to the last rooted daughter ramet, R4, the pattern of distribution and the rate of movement of dye changed (Fig. 3.9.2). The dye again moved acropetally to the cut end of the main stolon and after 60 minutes was observed in the lower leaves of the shoot of R4 and it was assumed that this was the limit of the flow of dye even though the main stolon was cut off just beyond this ramet. There was more basipetal movement of dye when the main stolon was cut off and a faster rate of acropetal movement with the main stolon intact (Table 3.2).

Table 3.2 *Comparison of the rates of flow of basic fuchsin, acropetally and basipetally, along a stolon of A. stolonifera with three, successively-younger, rooted, daughter-ramets. Basic fuchsin dye was fed to the roots of the 2nd daughter ramet and the parent main stolon was removed immediately distal to the youngest, rooted daughter-ramet. The difference between the acropetal rates of flow was not statistically significant. The difference in the rate of basipetal flow with and without the main stolon is slightly significant (p=0.09). n = 3.*

	rate (cm min^{-1}) of basipetal movement	rate (cm min^{-1}) of acropetal movement
With main stolon intact	0.13 ± 0.05	0.45 ± 0.17
With main stolon cut off	0.35 ± 0.09	0.25 ± 0.02

Experiment 3.3 In cross section the stolons of *A. stolonifera* show a peripheral ring of large discrete vascular bundles with smaller trace bundles towards the outside. This structure is shown in Fig. 3.10.

The xylem in any vascular bundle contained either red basic fuchsin or violet crystal violet. Passage of dye was therefore possible both acropetally and basipetally in separate bundles. Dye was confined to the xylem until the tissue became saturated with dye in which case the phloem and the surrounding sclerenchyma were also stained. (Fig. 3.10).

The distribution of the two dyes is shown in Fig. 3.11. In all replicates of this experiment only the basic fuchsin, supplied to the roots of the parent ramet, appeared in the branch tiller of the parent (R1) (transverse section A). It was also present, with crystal violet, in the 1st internode of the parent main stolon (transverse section B). (The parent main stolon is the axis of the plant material). Transverse section C, across the 2nd internode, had mainly the crystal violet supplied to the roots of R2, whilst the main stolon of R2 (transverse section D) frequently had no dye in its vascular bundles or all crystal violet. The parent main stolon distal to the ramet fed crystal violet had only that dye in its xylem (transverse sections E and F) although a trace of basic fuchsin appeared in section E in one plant. The main stolon the unrooted ramet between R1 and R2 was also sectioned (not shown in the diagram) and this had either red-stained xylem or no colour at all.

Experiment 3.4 Basic fuchsin was applied to the roots of the parent, and crystal violet to the roots of the 1st daughter ramet (R2), with the shoot of the parent ramet darkened for one hour before the start and during the course of experiment. The branch tiller of the parent ramet, fed basic fuchsin to its roots, (T.S A) had vascular bundles in which the xylem contained only red basic fuchsin dye (Fig. 3.12). The first internode distal to the parent ramet contained some bundles containing red basic fuchsin and others containing crystal violet. In each case the 1st rooted daughter ramet contained many vascular bundles in which the xylem showed no traces of dye – in two of the sections no dye was visible at all, whereas one plant showed some bundles with crystal violet present. The 2nd internode contained both red- and violet-stained xylem in two plants whilst the third had no dye. The pattern of distribution of dyes in the main

stolon of the 2nd rooted daughter ramet was different in each of the three plants studied (T.S E) with a mixture in one, all basic fuchsin in the second and no dye at all in the third. Basic fuchsin was found in 3rd internodes of the parent main stolon in two of the three replicates but by the 3rd internode of the parent main stolon most of the basic fuchsin had disappeared and mainly crystal violet was present. Crystal violet was present in all sections of the 3rd internode of Plant 3 even though it had been apparent in only trace quantity in the second internode. It is possible that the sections for internodes 2 and 3 were out of order.

Experiment 3.5 The main stolon of the ramet of which the initial roots were separated from later developing roots by a collar of Blu-tack™ and the two groups of roots given contrasting dyes, contained both basic fuchsin from the initial roots and crystal violet from the later-developed roots, irrespective of the previous treatment of the ramet with high or low phosphate (Fig. 3.13). In the two plants given high phosphate pre-treatment the basal tillers contained only crystal violet whereas in the single plant given low phosphate beforehand the basal tillers contained both red and violet dye. In all three plants the branch from the main stolon (T.S D) contained only the red basic fuchsin supplied to the initial roots and none of the crystal violet supplied to the later-developed roots even though the ramet main stolon, proximal and distal to the section, contained both dyes. The vascular bundles at both proximal and distal ends of the main stolon had bundles containing basic fuchsin confined to one side and crystal violet on the other side.

Experiment 3.6 Sections of the internode of the main stolon and the ramet main stolon proximal to the roots of the ramet given basic fuchsin had red stained large vascular bundles in all four plants. Only a few small peripheral bundles contained crystal violet.

Experiment 3.7 Whereas the previous experiments with basic fuchsin and crystal violet dyes gave results for separate plants which were comparable, in the experiments where pairs of ramets were grown with contrasting pre-treatment

with high or low phosphate the results were very variable. Table 3.3 shows the results when the older ramet, R1, was given high phosphate and the younger ramet, R2, was given low phosphate

Table 3.3 *The distribution of basic fuchsin and crystal violet dyes applied separately to the initial and later developing roots of the older R1 ramet when this was given 10 days pre-treatment with Long Ashton nutrient solution containing high phosphate (1.33 mmol dm^{-3}). The R1 ramet was attached to a younger sibling (R2) given 10 days pre-treatment with Long Ashton nutrient solution containing low phosphate (0.007 mmol dm^{-3}). Transverse sections A – E were cut at the places shown in Fig. 3.7.*

Plant T.S	1	2	3
A T.S. R1 main stolon	Most bundles red. 2 violet bundles on one side	All bundles red	All bundles red at the tip. Proximal bundles all violet.
B T.S. R1 basal tiller	Main bundles violet. Small, peripheral bundles red.	Equal numbers of red and violet bundles.	Red bundles at tip. Violet bundles at base.
C T.S. Connecting internode	Main bundles red. Small, peripheral bundles violet.	8 main bundles red. 6 main bundles violet. Small, peripheral bundles red.	Equal numbers of red and violet bundles.
D T.S. R2 main stolon	All bundles red.	Red bundles at tip Bundles at base mainly violet with 4 red bundles.	Equal numbers of red and violet bundles.
E T.S. R2 basal tiller	All but 2 bundles red. 2 violet bundles.	All bundles red.	All bundles red.

Irrespective of pre-treatment the tips of stolons tended to show red basic fuchsin whereas the bases showed crystal violet. This was so whether the initial or later-developing roots were given basic fuchsin dye. In these plants there was a clear difference in many sections of older (R1) ramets given high phosphate, between

the large, main vascular bundles in which one colour occurred, and the small, peripheral leaf-trace bundles in which the second dye was seen. The experiment was repeated with R1 (the older ramet) given Long Ashton nutrient solution with low phosphate (0.007 mmol dm^{-3}) and R2 (the younger ramet) given Long Ashton nutrient solution with high phosphate (1.33 mmol dm^{-3}) for 10 days before the start of the experiment. The results are shown in Table 3.4 (over page).

Experiment 3.8 The time taken for basic fuchsin to penetrate throughout the total length of main stolon, branches and basal stolons of pairs of adjacent ramets linked by the original parent internode and given contrasting phosphate treatments are shown in are shown in Table 3.5. There was no significant difference in the lengths of the roots after 15 days of contrasting phosphate treatment but the shoots of plants given homogeneous high phosphate, (+P +P), were significantly longer than those given all other treatments (except the low phosphate, R1, ramet in the **-P** +P pair). The shoots of the +P +P pair had a significantly greater rate of flow of basic fuchsin through them than ramets given other phosphate treatments (Fig 3.14).

Table 3.4 *The distribution of basic fuchsin and crystal violet applied separately to the initial and later-developing roots of the older R1 ramet when this was given 10 days pre-treatment with Long Ashton nutrient solution containing low phosphate (0.007 mmol dm^{-3}). This ramet was attached to a younger, R2, ramet given 10 days pre-treatment with Long Ashton nutrient solution containing high phosphate (1.33 mmol dm^{-3}). Sections A – E were cut at the places shown in Fig. 3.7*

Plant T.S	1	2	3	4 **This plant**: basic fuchsin to later-developing roots; crystal violet to initial roots
A R1 main stolon	Red bundles at tip. Violet bundles at base.	Equal numbers of red and violet bundles.	Red bundles at tip. Violet bundles at base.	Most bundles violet. 3 red bundles.
B R1 basal tiller	Large bundles violet. Small peripheral bundles red.	Red bundles at tip. Violet bundles at base.	Red bundles at tip. Violet bundles at base.	All bundles red at tip. 11 red and 4 violet bundles at base.
C Connecting internode	Equal numbers of red and violet bundles.	Bundles on one side all red. Bundles on opposite sides all violet.	Equal numbers of red and violet bundles.	All bundles violet at R2 end. Equal numbers of red and violet bundles at R1 end.
D R2 main stolon	All bundles red	Red bundles at tip. Violet bundles at base.	Equal numbers of red and violet bundles.	All bundles violet
E R2 basal tiller	All bundles violet	Some violet bundles. Most bundles colourless	All bundles red	All bundles red

Table 3.5 *The mean lengths of the longest root and total shoot (main stolon, main stolon branches and basal tillers) for older and younger ramets, in pairs connected by the parent main stolon internode. The paired ramets were given contrasting phosphate treatment. Treatment with high phosphate (+P) was full Long Ashton nutrient solution with 1.33 mmol dm^{-3} phosphate, low phosphate treatment (-P) was Long Ashton nutrient solution with 0.007 mmol dm^{-3} phosphate. The rate of movement of basic fuchsin dye was calculated from the time taken for the dye to appear at the most distal point of the younger ramet from the roots of the older ramet to which the dye was supplied*

	Mean length longest root (cm). (n = 3)		Mean total length shoot (cm). (n = 3)		Mean time taken for dye to appear throughout plant (min)		Mean rate of flow (cm. min^{-1})	
Treatment	R1	R2	R1	R2	R1	R2	R1	R2
+P -P	12.3 ± 0.6	8.5 ± 2.2	29.2 ± 10.3	28.0 ± 1.9	101.7 ± 31.8	125.0 ± 21.8	0.28 ± 0.3	0.24 ± 0.03
-P +P	13.8 ± 1.2	10.3 ± 3.1	32.2 ± 15.9	44.7 ± 21.4	111.7 ± 68.3	181.7 ± 89.8	0.35 ± 0.3	0.25 ± 0.04
+P +P	12.7 ± 1.6	11.3 ± 1.7	62.3 ± 4.8	62.2 ± 12.0	93.3 ± 24.6	113.3 ± 11.7	0.81 ± 0.3	0.55 ± 0.11
-P -P	10.0± 4.9	9.0 ± 2.3	34.7 ± 14.9	30.8 ± 11.9	176.7 ± 59.7	156.8 ± 14.2	0.19 ± 0.04	0.14 ± 0.11

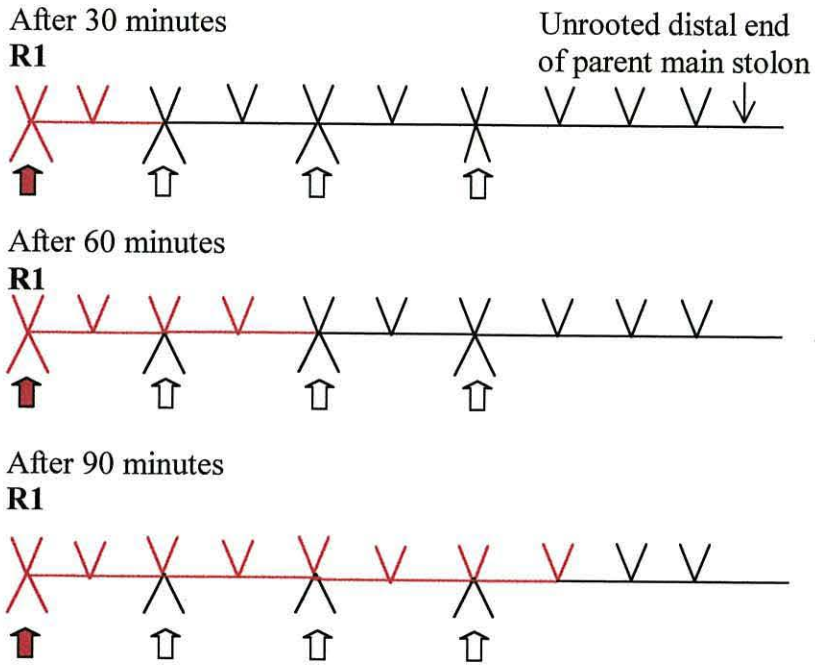


Fig. 3.8.1 *Progress of basic fuchsin through a stolon of A. stolonifera with three successively-younger, rooted, daughter ramets. during 90 minutes when the parent main stolon was left intact beyond R4, the last rooted daughter ramet.* ⇧ *= basic fuchsin dye provided to roots of parent ramet.* — *= presence of basic fuchsin in plant tissues.* ⇧ *= water supplied to roots of daughter ramets.*

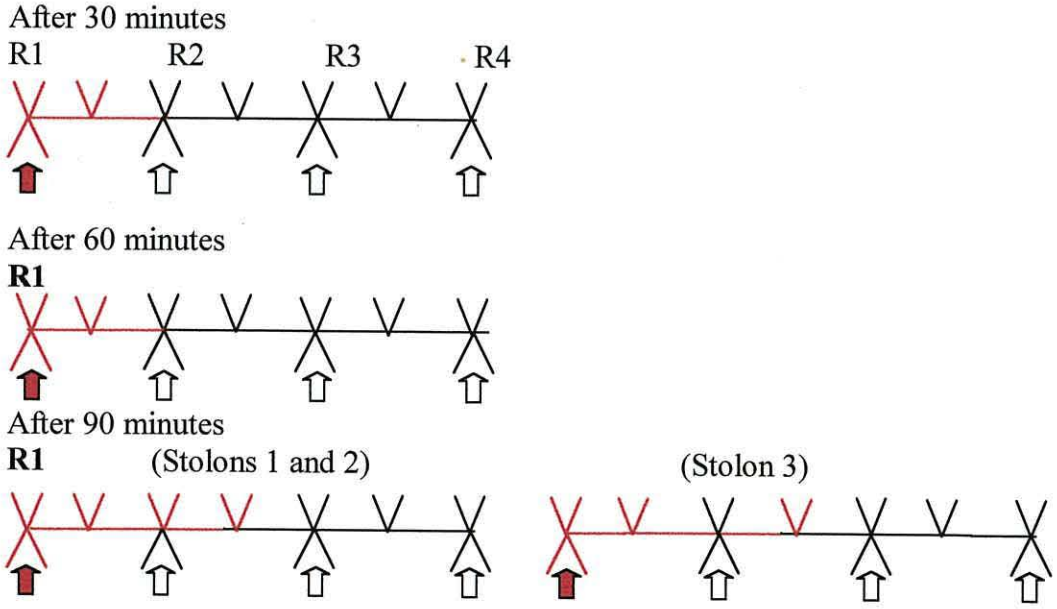


Fig. 3.8.2 *Passage of basic fuchsin through A. stolonifera with three successively-younger, rooted, daughter ramets and the parent main stolon was cut off beyond R4, the last rooted daughter ramet.* ⇧ *= basic fuchsin dye provided to roots of parent ramet.* — *= passage of basic fuchsin in plant.* ⇧ *= water supplied to the roots of the daughter ramets.*

After 60 minutes

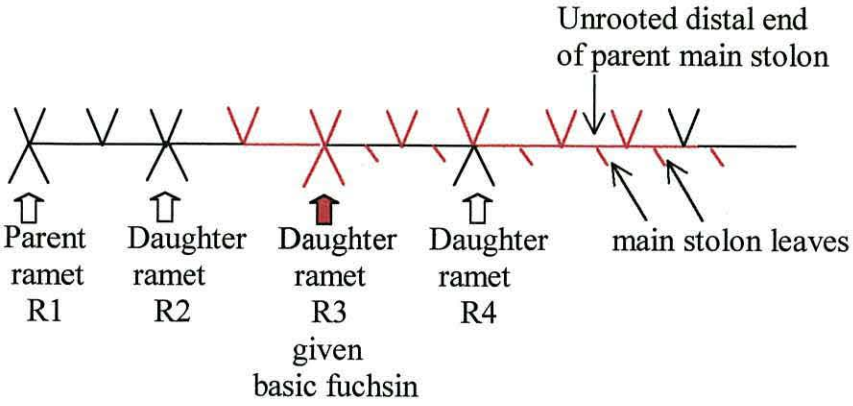


Fig. 3.9.1 *Diagrammatic representation of a stolon of A. stolonifera with three successively-younger, rooted, daughter ramets with the parent main stolon was left intact beyond R4, the last rooted daughter ramet, showing the presence of basic fuchsin dye in the plant tissues after 60 minutes shown by the red line —— ⇧ = basic fuchsin supplied to the roots of R3. ⇧ = water supplied to roots of other ramets. The parent main stolon was left intact beyond R4.*

After 60 minutes

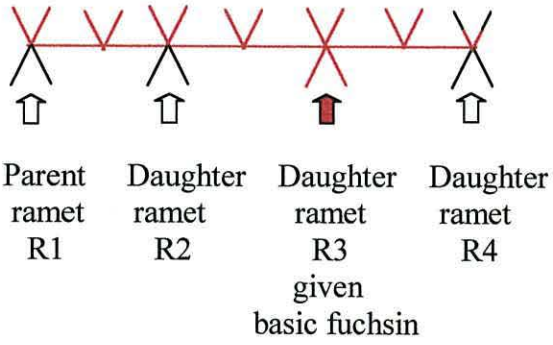
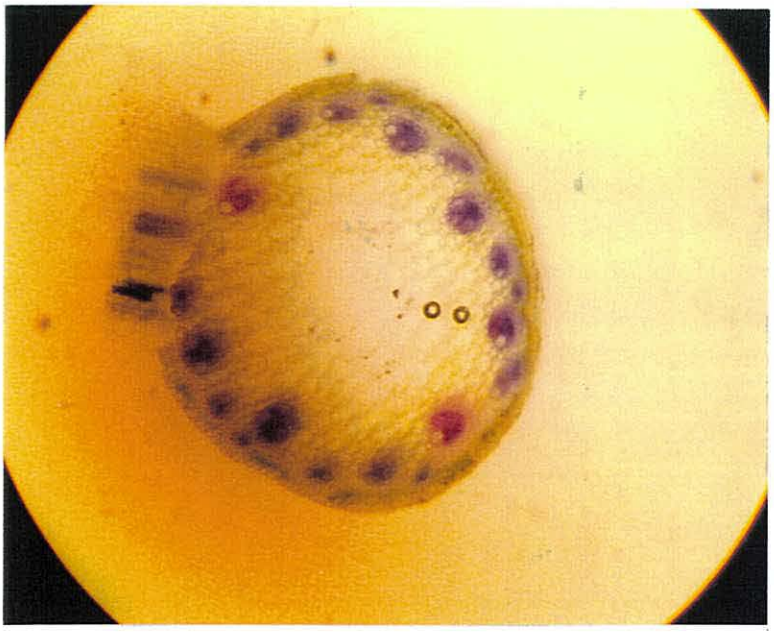


Fig. 3.9.2 *Diagrammatic representation of a stolon of A. stolonifera with three successively-younger, rooted, daughter ramets and the parent main stolon cut off beyond R4, the last rooted daughter ramet, showing the presence of basic fuchsin dye in the plant tissues after 60 minutes shown by the red line ——— . ⇧ = basic fuchsin supplied to the roots of R3. ⇧ = water supplied to roots of other ramets.*

(a)



(b)

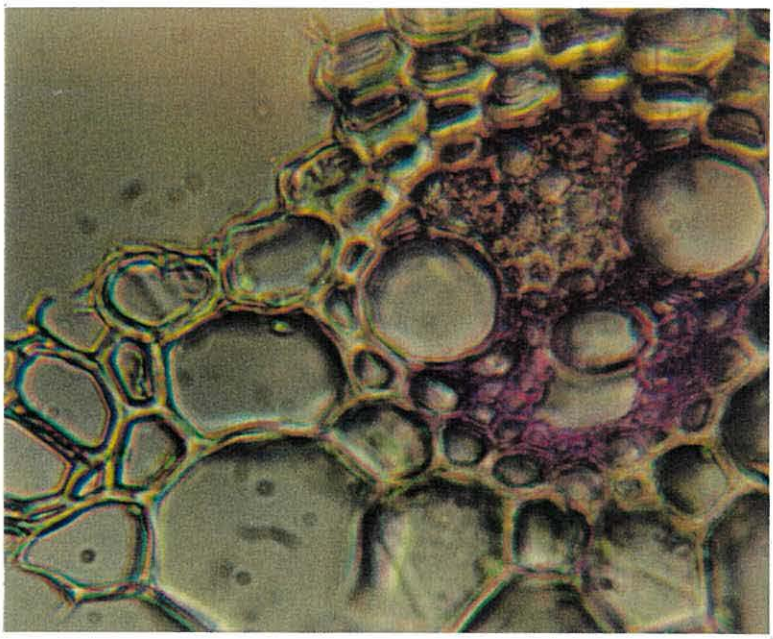


Fig. 3.10 *Cross section at C in Fig. 3.3. The stolon consisted of a parent ramet with three rooted daughter ramets. The roots of the parent ramet were supplied with basic fuchsin dye and the roots of the oldest rooted daughter ramet (R2) supplied with crystal violet dye. The section was cut distal to the parent but proximal to the daughter.*

(a) The section shows two vascular bundles with the xylem stained red with basic fuchsin and the remaining vascular bundles stained violet with crystal violet. (Mag. x40)

(b) The dye was confined to the xylem; the phloem sieve tubes remained unstained. (Mag. x 400)

Fig. 3.11 *Diagrams to show the distribution of basic fuchsin and crystal violet dyes in vascular bundles in cross sections of 3 stolons of A. stolonifera when roots of parent ramet given basic fuchsin and roots of R1 given crystal violet. The sections were cut at the points in Fig. 3.3 marked A, B, C, D, E and F. Red spots represent vascular bundles with the xylem stained with basic fuchsin; violet spots represent vascular bundles with the xylem stained with crystal violet; colourless spots represent vascular bundles in which no dye was visible.*

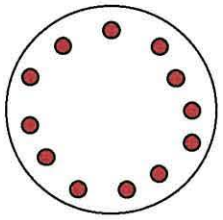
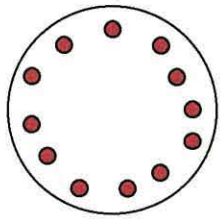
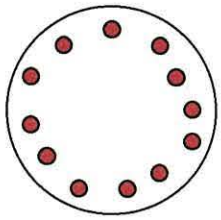
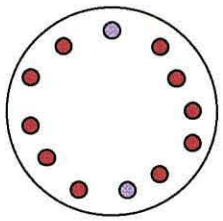
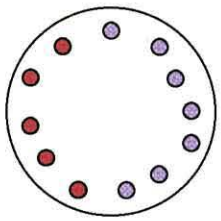
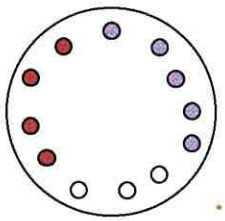
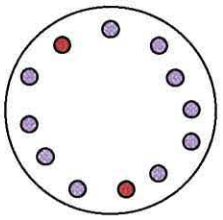
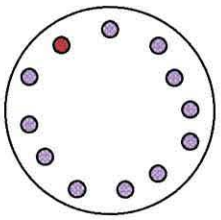
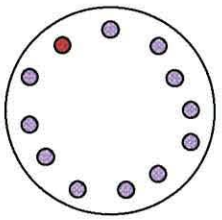
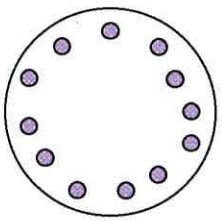
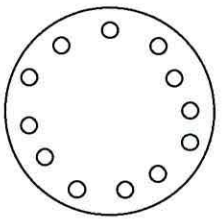
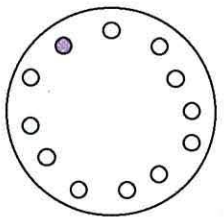
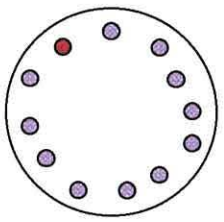
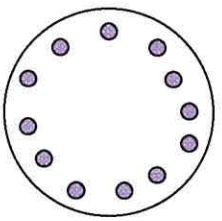
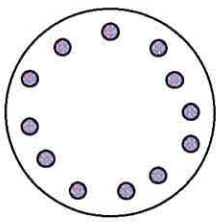
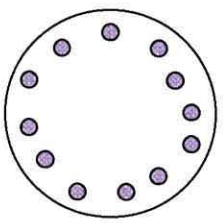
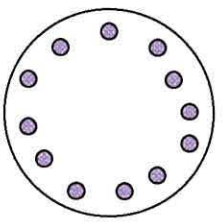
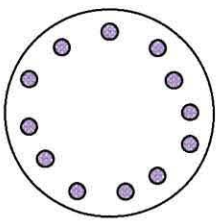
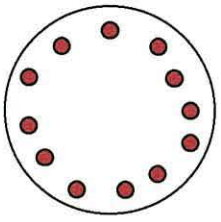
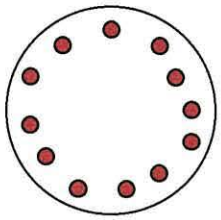
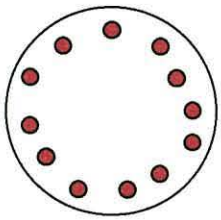
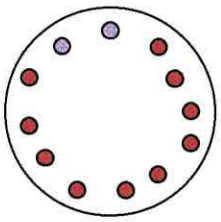
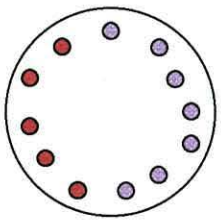
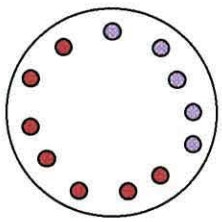
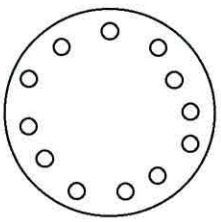
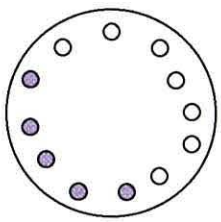
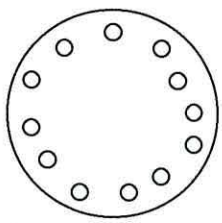
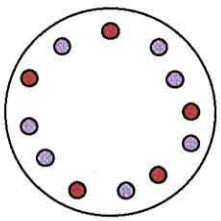
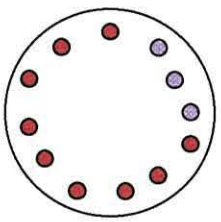
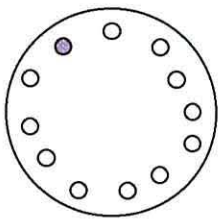
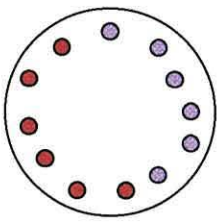
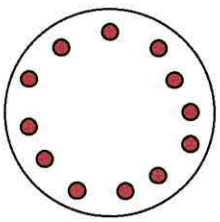
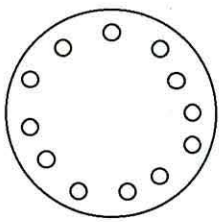
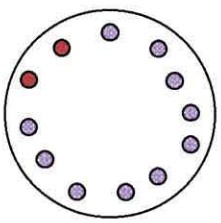
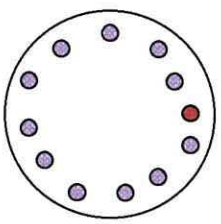
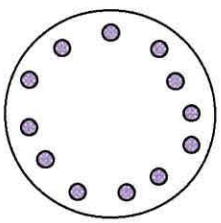
Experiment 3.3	Plant 1	Plant 2	Plant 3
T.S A Parent ramet branch tiller			
T.S. B 1st internode of parent main stolon			
T.S C 2nd internode of parent main stolon			
T.S. D 1st daughter ramet (R2) main stolon			
T.S E 3rd internode of parent main stolon			
T.S F 4th internode of parent main stolon			

Fig. 3.12 *Diagrams to show the distribution of basic fuchsin and crystal violet dyes in vascular bundles in cross sections of 3 stolons of A. stolonifera when roots of parent ramet given basic fuchsin and roots of R1 given crystal violet. The parent ramet was darkened with aluminium foil for 1 h before the start and throughout the experiment. The sections were cut at the points in Fig. 3.4 marked A, B, C, D, E and F. Red spots represent vascular bundles with the xylem stained with basic fuchsin; violet spots represent vascular bundles with the xylem stained with crystal violet; colourless spots represent vascular bundles in which no dye was visible.*

Experiment 3.4	Plant 1	Plant 2	Plant 3
T.S A Parent ramet branch tiller			
T.S. B 1st internode of parent main stolon			
T.S C Main stolon of 1st rooted ramet			
T.S. D 2nd internode of parent main stolon			
T.S E Main stolon of (R3) 2nd rooted ramet			
T.S F 3rd internode of parent main stolon			

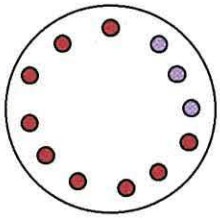
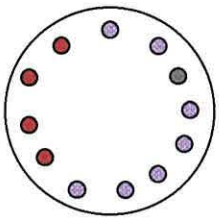
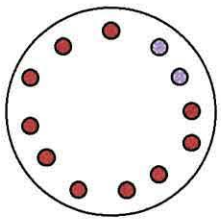
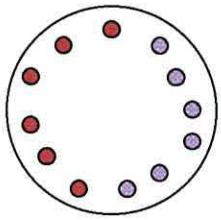
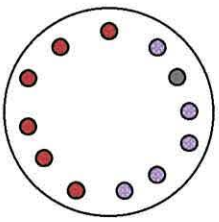
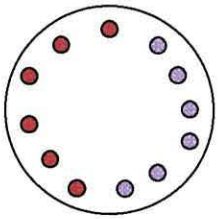
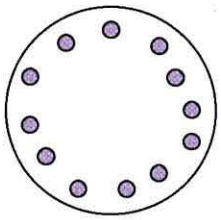
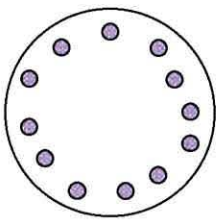
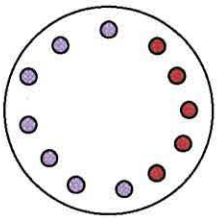
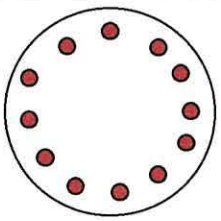
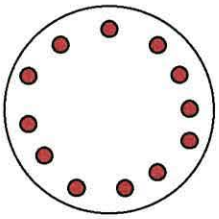
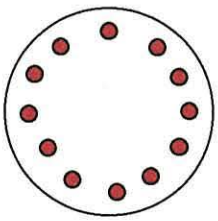
Expt 3.5	1 (+P plant)	2 (+P plant)	3 -P plant)
T.S A Ramet main stolon: proximal			
T.S B Ramet main stolon: distal			
T.S C Ramet basal tiller			
T.S D Ramet main stolon branch			

Fig. 3.13 *Diagrams to show the distribution of basic fuchsin and crystal violet dyes in vascular bundles in cross sections of 3 stolons of A. stolonifera set up as shown in Fig. 3.8. with the initial nodal roots and the later-developing roots separated and supplied with contrasting dyes. Sections were cut at the points marked A, B, C and D in Fig 3.5. Plants 1 and 2 were grown for 10 days in Long Ashton nutrient solution with high phosphate (1.33 mmol dm^{-3}) and plant 3 was grown for 10 days in Long Ashton nutrient solution with low phosphate (0.007 mmol dm^{-3}). Red spots represent vascular bundles with the xylem stained with basic fuchsin; violet spots represent vascular bundles with the xylem stained with crystal violet. In sections A and B (and 3C) the vascular bundles containing basic fuchsin were all on one side, those with crystal violet were on the opposite side.*

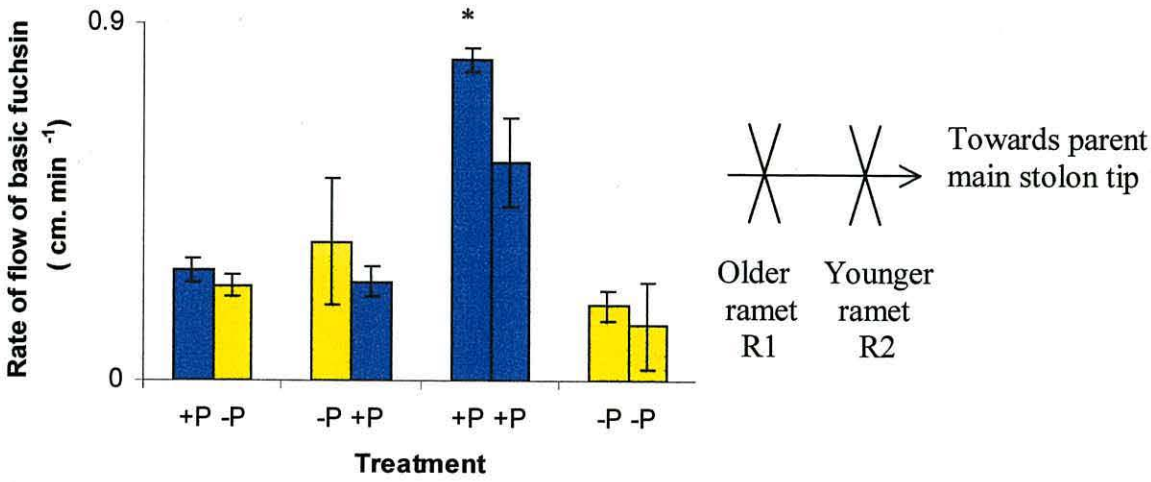


Fig. 3.14 *The rate of flow of basic fuchsin paired ramets of* <u>*Agrostis stolonifera*</u> L. *joined by the original parent internode. The arrangement of the ramets is shown in the logo. The rate of flux through the older ramet (R1) is represented by the left-hand bar of each pair, the rate of flux through the younger ramet (R2) by the right hand bar. Blue bars show ramets given high phosphate pre-treatment(1.33 mmol* dm^{-3}*), the yellow bars represent ramets given low phosphate pre-treatment (0.007 mmol* dm^{-3}*). Error bars =* ± 1 *standard error of the mean. n = 3. * =*$p<0.05$.

DISCUSSION

The pattern of distribution of dyes through the stolons of *Agrostis stolonifera* shows the xylem connections between ramets. When basic fuchsin was supplied to the roots of a parent ramet of *A.stolonifera* (Experiment 3.1) flow of the dye was seen towards the tip of the main stolon. Dye entered the shoots of all the daughter ramets, rooted and unrooted, as far as the dye penetrated along the main stolon. Dye did not enter the roots of any ramet except the root to which it was supplied. When the unrooted distal portion of the main stolon was removed the rate of acropetal flow was significantly diminished. The unrooted portion made up almost half the length of the stolon, with three or more unrooted ramets as well as the stolon tip. Since there was an abundant supply of dye to the parent roots, the reduced size of the transpiring region could account for the reduced rate of flow (Milthorpe & Moorby, 1969). The distribution of dye was therefore determined by the direction of the transpiration stream, as found in *Trifolium repens* by Hay & Sackville Hamilton (1996) for phosphorus. If the transpiration stream were responsible for the flow of dye through the stolon then the large size of the older parent ramet with its greater number of leaves can also explain the basipetal flow of basic fuchsin from a daughter ramet back to the parent (Fig. 3.12). This is further supported by the increase in the basipetal rate of flow when the part of the main stolon with its unrooted ramets distal to the last rooted ramet was cut off. Transpiration would now be greater from the parent ramet than from the sum of the small, youngest daughter ramets.

Application of contrasting dyes to two adjacent ramets showed that the internode between them carried both dyes in opposite directions in the xylem of separate vascular bundles. The hypothesis is supported that dye may be transported both acropetally and basipetally in the xylem. D'Hertefeldt & Jonsdottir (1999), using acid fuchsin, found dye in xylem vessels both basipetal and acropetal to the point of application in *Carex arenaria*. Dye could be carried in the xylem transpiration stream from several sources and the overall flow would then be a balance between the strength of the shoots as sinks for water and solutes in the

xylem. Pate *et al.* (1985) reported basipetal flow of water from the fruits of cowpea (*Vigna unguiculata*), which did not transpire, into proximal leaves. Movement of dye between ramets shows a connection between them. The presence of crystal violet in xylem vessels on opposite sides of the stolon suggests that certain vessels supply particular sinks. Had movement of crystal violet in a basipetal direction been due to only to diffusion then it would be expected that all the xylem vessels in that part of the stolon would contain the same dye.

Several authors have noted the bi-directional transfer of resources in plants. Alpert & Mooney (1986) demonstrated the bi-directional movement of water and photoassimilates within the same stolon of *Fragaria chiloensis*. Reciprocal exchange of water and nitrogen was demonstrated in the same species (Friedman & Alpert, 1991) and in *Carex flacca* (de Kroon *et al.,* 1998). Bi-directional transport in *Potentilla anserina* was investigated by steam-girdling (Van Kleunen & Stuefer, 1999) who showed that water moved acropetally and photoassimilates basipetally, the water carried in the xylem, the photoassimilates in the phloem. Transfer of PO_4^{3-} and other ions occurs from xylem to phloem in stems (Lauchli, 1972; Bieleski, 1973; Marschner, 1995). There was no evidence of the transport of either basic fuchsin or crystal violet from the xylem to the phloem. Thus the movement of dyes may or may not reflect the passage of phosphate between ramets.

Darkening the shoot of parent ramets of *A. stolonifera* has the effect of reducing the rate of transpiration. This would be expected to alter the pattern of distribution of the dye along the stolon and in the daughter ramets. There was, however, little difference between the patterns of distribution of dye when the parent shoot was undarkened (Experiment 3.3) and when it was darkened for an hour beforehand and throughout the experiment (Experiment 3.4). When the parent shoot was darkened, basic fuchsin was found in both the 2nd and 3rd internodes of the parent main stolon in two out of three cases. If the stomata

were partly closed, a reduced transpiration stream could carry basic fuchsin into the parent shoot but not be adequate to bring crystal violet from the roots of the next rooted ramet. Similarly, by keeping the parent shoot dark before and during the experiment, the greater transpiration of the ramets distal to the parent could ensure that both basic fuchsin and crystal violet moved acropetally. If movement of basic fuchsin relied only on transpiration, the rate of flow into the darkened parent would be expected to slow down or stop. However a number of reports indicate that the transport of ions through the xylem does not depend on transpiration, although this may accelerate the rate of flow through the xylem (Tanner & Beevers, 1990; Canny, 1995). Van Bel (1995) noted the evidence that parenchyma cells at the ends of the xylem and phloem release solutes into these channels and it is the activities of these cells rather than mass flow of water which determines the bulk movement of solutes in the xylem and phloem.

The hypothesis was tested that the first roots to develop at each node supply the main stolon whilst the later-developing roots supply the basal tillers. There was evidence that for cuttings consisting of a single ramet (Experiment 3.5), the initial nodal roots supply the main stolon and that the later-developing roots supply the basal tillers. Since these later-developing roots contain some nodal roots as well as basal tiller roots it is to be expected that the main stolon would have both basic fuchsin from the earliest roots and crystal violet from the later-developing roots. The majority of the basal tillers had only crystal violet in their vascular bundles. The single cutting which had been grown in low phosphate did have some basic fuchsin too but this could be due to failure to separate the two generations of roots adequately. In these cuttings the basic fuchsin was confined to the xylem vessels in the bundles on one side of the stolon only with crystal violet on the other side. The sections were hand cut so it was not possible to determine the orientation of each section but since the branches on the stolons contained only basic fuchsin it is likely that the sectoriality of the main stolon was such that the branch was supplied only by xylem vessels from the initial nodal roots containing basic fuchsin. Movement of dye injected into

the petioles of *Glechoma hederacea* was acropetal and sectorial in the xylem whereas basipetal and acropetal translocation of photoassimilates in the phloem was less markedly sectorial (Price *et al.*, 1996). Marshall (1996) commented on the non-sectorial nature of the nodal plexus of a young grass plant, at least in the seedling stage, but the cuttings used in Experiment 3.6 were from well-established stolons in which the initial vascular connections may well have been altered as older leaves senesced, new leaves developed and the original connections between ramets were disrupted by cutting the stolon into pairs of ramets.

When contrasting dyes were supplied to the separated roots of the older ramet of a pair (Experiment 3.7) in which the partners had been given contrasting phosphate treatments, the resulting patterns of distribution were more variable than had been seen in the earlier experiments. The variability in the pattern of distribution of the two dyes in pairs of ramets is an interesting reflection of the variability in dry weight of paired ramets grown with contrasting concentrations of phosphate. Separation of stolons of *Glechoma hederacea* into clonal fragments affects the subsequent growth of the fragments depending on the number of ramets proximal and distal to the point of cutting and hence on the age of the fragments (Birch & Hutchings, 1999). Variation in the subsequent growth of clonal fragments of *A. stolonifera* could similarly be the result of differences in the extent to which the ramets had developed physiological independence before being cut from the parent stolon, and on the pattern of vascular connections between the ramets.

Basic fuchsin was supplied to the initial roots and since these were older roots they could also be longer and more branched; thus it is probable that their rate of uptake of dye would be greater. This would account for the presence of red dye at the distal ends of the structures sectioned. However where the treatments were reversed so that the initial roots were given crystal violet dye and the later-developing roots basic fuchsin, the red basic fuchsin still appeared at the tips

with crystal violet near the base. This suggests that basic fuchsin is more mobile in the xylem than is crystal violet. The variability may, on the other hand, reflect the rate of production of later roots. If different roots feed different sectors of the pair of ramets and if, in the absence of a stolon tip, the rate of translocation of dye is dependent on the rates of transpiration in the two ramets, then the size of the shoot and the root and thus the root:shoot ratio would have an effect on the rate of uptake of materials from the xylem into parts of the plant. Thus differences in sizes of ramets given the same phosphate treatment to their roots, could be due, not to availability of resources such as phosphate to the roots, but to differences in uptake and distribution of the resource within the plant i.e to properties of the source and sink as considered by Bieleski (1973).

The evidence that differences between the patterns of distribution of dyes could be related to previous treatment with high or low phosphate is equivocal. Basic fuchsin is said not to cross the endodermis readily so a short section was removed from the tip of the longest roots to allow entry to the xylem. However, the tips of the shorter roots were not cut off and all the roots immersed in basic fuchsin were red throughout so it may be that in this grass the dye moved through the symplasm of the root and its movement into the xylem was not impeded by the endodermis (Canny, 1995). Roots of both high and low phosphate ramets were stained red throughout so although the increased branching of the high phosphate ramets may have allowed the entry of dye from the apoplast where the branch roots pierced the endodermis, the fewer branches on the low phosphate ramets make this less likely to explain the presence of basic fuchsin throughout the root system of low phosphate ramets.

Phosphate can be carried in both xylem and phloem whereas the dyes were seen to be confined to the xylem until they damaged the plant to the extent that diffusion of the dyes occurred into other tissues. Although reduction in the rate of transport of phosphate may result from previous treatment with low phosphate (Clarkson & Scattergood, 1982; Jeschke *et al.*, 1997), there was no difference

between the patterns of distribution of basic fuchsin for ramets grown in high or low phosphate (Experiment 3.7). Such treatment takes at least 14 days for differences to become apparent and these plants were grown for 10 days in contrasting phosphate concentrations before the start of the experiments. However Bowen (1970) concluded that the activity of phosphate transport in roots increased before significant effects on growth were visible so if contrasting phosphate treatments before the experiment were to have an effect on the rate of transfer of dyes, the 10 days of treatment should have been sufficient to allow this to occur. Experiment 3.8 showed faster flow of basic fuchsin dye through the shoots of connected pairs of ramets if both partners were given homogeneous high phosphate treatment for 15 days before the experiment. However, these ramets had significantly longer shoots than ramets given other phosphate pre-treatment and again the faster rate of flow can be attributed to a greater surface area over which transpiration could occur and hence a greater rate of flow.

For pairs of ramets the variable distribution of the two dyes, reduced basic fuchsin and reduced crystal violet, would appear to be attributable to differences in size of the roots altering the rate of uptake, to differences in the size of the shoots altering the rate of transpiration and thus to differences in the rate of mass flow of dye towards the transpiring shoot in the transpiration stream and to the vascular architecture within the clone allowing the transfer of different amounts of Pi to different parts of the clone.

CONCLUSIONS

- dye is transported both acropetally and basipetally in the xylem with contrasting dyes from adjacent roots moving in opposite directions in different vascular bundles in the same stolon
- earliest-developing roots and later-developing roots supply different parts of the stolon
- darkening the shoot of the parent ramet to reduce its rate of transpiration did not alter the pattern of distribution of the dyes
- previous treatment with high or low phosphate did not significantly alter the rate of flow of dyes.

Chapter 4

What effect does transpiration have on the transport of phosphate in *Agrostis stolonifera* L. grown in sand when given adequate water or subjected to drought?

INTRODUCTION

Experiments described in Chapter 3 using dyes to trace the passage of water in the xylem, in both complete stolons and in clonal fragments of *A. stolonifera,* suggest that water and solutes in the xylem move along a water potential gradient from the roots to transpiring shoots. Experiments with dyes show the movement of water only within the xylem; there is no circulation of the dye in the phloem. Movement of water and minerals in xylem occurs in response to water potential gradients that develop as a result of transpiration from leaf surfaces (Pitelka & Ashmun, 1985). In non-clonal plants this movement is from the roots *via* stems to mesophyll cells and ultimately to the transpiring surfaces of the leaves. In clonal plants each ramet within the clone not only has all the components of this system but also the connections between ramets allow water potential gradients to be established both within and between ramets (Pitelka & Ashmun, 1985).

When ^{32}P is supplied to the roots of a ramet of *A. stolonifera,* it is transported distally along a stolon but the flow can be reversed if ramets basipetal to the fed one are deprived of water (Anderson-Taylor, 1982; Marshall, 1990). In *Carex arenaria* radiophosphorus supplied to a root is transported acropetally to young roots and shoots and to the rhizome apex, with limited basipetal transport (Noble & Marshall, 1983). Similarly, water and nutrients pass acropetally from a well-watered ramet to a water-deprived sister ramet. In *Trifolium repens* ^{32}P is transported from source roots to nearby sinks such as the apex of the stolon and young branches (Chapman & Hay, 1993). An unrooted ramet of *A. stolonifera,* probably by its transpiration, creates a water potential difference between itself and a well-watered ramet, so water and mineral nutrients are transported into the unrooted ramet (Marshall & Anderson-Taylor, 1992). In contrast a ramet rooted in sand with water but without nutrients fares less well as it takes up water but

has no access to minerals (Marshall & Anderson-Taylor, 1992). In *Trifolium repens* the flow of ^{32}P is acropetal and ^{32}P is carried in the transpiration stream (Hay & Sackville Hamilton, 1996).

Cotton seedlings (*Gossypium hirsutum)* without phosphate have a transpiration rate 26% lower than seedlings grown with 0.5 mmol dm^{-3} phosphate (Radin, 1984). Mulberry seedlings (*Morus nigra)* grown without phosphate have a stomatal conductance less than half that of seedlings supplied with phosphorus (Sharma, 1995). More abscisic acid accumulates in the leaves of phosphorus-deficient cotton plants subjected to drought stress than in phosphorus-sufficient (Marschner, 1995).

Plants compensate for environmental heterogeneity by proliferating in rich patches and avoiding poor patches (Caldwell *et al.,* 1991; Robinson, 1996). *Glechoma hederacea* grown in a patchy environment produces more than twice the dry weight of plants grown with the same amount of nutrients distributed uniformly (Birch & Hutchings, 1994). Friedman & Alpert (1991) provided paired *Fragaria chiloensis* ramets with a heterogeneous system in which one was provided with 50 mg dm^{-3} nitrogen but was shaded to allow it only 10% of the light reaching its partner which had no nitrogen. The shaded plant partitioned its growth to leaves and shoots while the ramet with no nitrogen partitioned growth to roots - the classical partitioning of plants deprived of resources. However the allocation altered when the shaded partner was provided with only 20% of the nitrogen supplied in the first experiment. In the second arrangement the ramet grown in light but no nitrogen allocated resources to leaf growth while the shaded partner "specialised" in root growth. These plants were showing division of labour. Stuefer *et al.* (1996) showed similar division of labour in *Trifolium repens* when connected ramets were provided with a heterogeneous environment with either light but no water, or water but no light. Classical partitioning theory suggests that the ramets deprived of water should grow bigger roots whereas the ramets provided with low light should partition growth to leaves if the resources are uniformly distributed (Grime, 1979; Stuefer *et al.,*

1996). The converse was seen - the ramets grown in low light grew large root systems to maximise their uptake of water while the connected ramets deprived of water grew large leaves to maximise their uptake of light energy. Control plants, grown with the whole group in low light or in water-stress conditions, partitioned growth in accordance with the classical theory - but the final dry weight of these plants was less that that of plants grown in the heterogeneous system. Thus clonal plants have the potential to respond plastically to environmental heterogeneity by spatial division of labour among potentially independent units by partitioning biomass to structures capturing locally abundant resources. However non-clonal plants, grown with some roots in a rich source of nutrients and the others in poor, show increased proliferation of roots in the rich source (Drew, 1975; Drew & Saker; 1975: Barta; 1977).

In summary, the ramets of clonal plants can exchange resources, particularly from an older ramet to a younger one. The direction may be reversed if the environment imposes a stress on an older ramet which can be relieved by movement of resources from a younger ramet but support is not inevitable if the cost to the clone is greater than the gain derived by supporting a stressed ramet.

This chapter investigates the transport of phosphate between pairs of ramets, cut from a parent stolon of *A. stolonifera* and still connected by the internode of the parent plant, when they were grown in sand with one partner supplied with phosphate and the other given water. The effect of high phosphate on stomatal conductance in water-stressed ramets is also investigated both in clonal fragments consisting of pairs of ramets and in whole stolons.

The hypotheses tested are that

- phosphate will be translocated from a well-watered ramet to a water-stressed ramet i.e. in the direction of the transpiration stream
- ramets given no phosphate and low water will acquire more water and hence also more phosphate from a high P partner than those given no phosphate but high water

- ramets given high phosphate will have a higher rate of stomatal conductance of water vapour than those given no phosphate
- ramets given high phosphate but low water will reduce their rate of stomatal conductance in order to conserve water, but ramets given low phosphate will not do this.

MATERIALS AND METHODS

Experiment 4. 1 To test the hypothesis that the movement of phosphate through *Agrostis stolonifera* L., rooted in sand, occurs in the direction of the transpiration stream

Parent plants from the stock of *A. stolonifera* maintained for several years at Pen y Ffridd field station, University of Wales, Bangor, were grown in John Innes No. 1 compost. Their stolons were pegged into sand in separate 9 cm diameter plastic pots, and watered so each node developed a small root and shoot. The parent plants were then discarded and the rooted stolons cut to give paired ramets. Pairs of adjacent ramets, connected by the internode of the original stolon, were selected for their similarity in size and development. Within each pair of ramets one partner was given one of three water treatments whilst the other was given nutrient solution with one of two phosphate concentrations. Some single ramets were also prepared. At the onset of the experiment three single ramets were watered to field-capacity and the pots weighed. These field-capacity control plants were weighed on alternate days and the volume of water required to restore them to their recorded weight was noted.

In the first run of this experiment three water treatments were used – (i) high water (in which water was provided to restore the field-capacity control plants to their recorded field-capacity weight), (ii) low water in which 25% of this 'field-capacity volume' was provided, and (iii) no water, in which the pots were allowed to dry out. Two phosphate treatments were used; high phosphate treatment was full Long Ashton nutrient solution containing 1.33 mmol dm^{-3} phosphate and low phosphate was Long Ashton solution with 0.133 mmol dm^{-3} phosphate. The field-capacity volume of nutrient solution was added in each case. Single plants were set up with one of these five treatments. Paired ramets were set up so that in each case within a pair the older ramet (R1) was given a phosphate treatment and the younger ramet (R2) was given a water treatment. The reciprocal arrangement was also set up with the younger sibling given the phosphate treatment and the older sibling the water treatment. Ramets given a water treatment were given no nutrients. Ramets supplied with nutrient solution

were fed once each week and all ramets were watered every two days. Four replicates of the treatments summarised in Table 4.1 were used.

Table 4.1 *Phosphate and water treatments given to one partner in a pair of adjacent ramets linked by the parent internode and to single ramets which acted as controls.*
Water treatments were $+H_2O$ *= field-capacity;* $-H_2O$ *= 25% this volume; dry = no water. Phosphate treatments were* +P = *1.33 mmol* dm^{-3}; -P = *0.133 mmol* dm^{-3} *(field-capacity volume of Long Ashton nutrient solution supplied in each case).* R1 = *The older ramet;* R2 = *the younger ramet*

Paired ramets in which one partner was given high phosphate		Paired ramets in which one partner was given low phosphate		Single ramets
R1	R2	R1	R2	
+P	$+H_2O$	-P	$+H_2O$	$+H_2O$
$+H_2O$	+P	$+H_2O$	-P	$-H_2O$
+P	$-H_2O$	-P	$-H_2O$	+P
$-H_2O$	+P	$-H_2O$	-P	-P
+P	dry	-P	dry	dry
dry	+P	dry	- P	

The ramets, either in pairs or singly, were grown for 30 days in a glasshouse with temperatures maintained between 16 °C and 25 °C. Ambient light was supplemented with sodium lighting to give a range of photon flux density of 225 - 550 μmol m^{-2} s^{-1}. Before harvest the stomatal conductance for both the upper (adaxial) and the lower (abaxial) epidermis of the youngest fully expanded leaves on the main stolon of each ramet was measured using a Mark 4 ΔT porometer (Delta Devices, Cambridge, UK). Recordings of stomatal conductance were made on a sunny day two days after the last watering. Measurements were made throughout the day between 09. 30 and 17. 30.

At harvest, for each ramet, the length of its main stolon was measured and the number of main stolon leaves, main stolon branches and basal stolons were

counted. The plants were then dried at 80 °C for 48 h and weighed. The phosphorus concentration was determined in the terminal three nodes, their internodes and leaves, of the main stolon of each ramet and also in the root.

The experiment was repeated, but in the repeat experiment the only water treatments given were field-capacity and 25% of this, as in the first experiment some of the ramets totally deprived of water did not survive. In the repeated experiment stomatal conductance was measured after 30 days, on the upper epidermis of the youngest fully expanded leaf on three consecutive days. On the first day readings were made 16 h after watering, on the second day immediately after watering, and on the third day readings were made 24 h later. In each case readings were made between 09.00. and 13.00. The results of these stomatal conductance measurements were compared with the measurements made 48 h after watering. In the repeat of the experiment, at harvest the shoots were removed leaving a stump 1cm long to which plastic pipette-tips were attached by Blu-tack ™. The plants were left overnight in the dark. Each cut shoot, either main stolon or basal tiller, yielded between 0.1 and 0.2 cm^3 xylem sap, more from -P than from +P ramets, which collected in the pipette tips. The pooled xylem sap from each ramet, approximately 0.5 cm^3, was transferred to a microscope slide and the pH measured using a Cole Parmer flat bulb pH electrode.

Experiment 4.2 To test the hypothesis that if a gradient of phosphorus concentration is established in daughter ramets along a stolon of *A. stolonifera*, where daughter ramets have high phosphate status and are water-stressed their stomatal conductance will be reduced

Three methods were used to try to establish a gradient of water and phosphorus in a series of daughter ramets along the length of a main stolon. For all three methods plants of *A. stolonifera* were grown in John Innes No. 1 potting compost until each had produced a main stolon. Along the length of the stolon, nodes were pegged down in 9 cm diameter pots containing sand and the nodes watered to encourage rooting. After 8 days a daughter ramet had developed at each node.

The parent plant was discarded and the daughter ramets were numbered ramet 1, (R1), the oldest to R5, the youngest.

Method 1

Four replicates of each of the following four treatments were used

1. R1 given high phosphate; younger daughter ramets given field-capacity water
2. R1 given high phosphate; younger daughter ramets given 25% field-capacity water
3. R1 given low phosphate; younger daughter ramets given field-capacity water
4. R1 given low phosphate; younger daughter ramets given 25% field-capacity water.

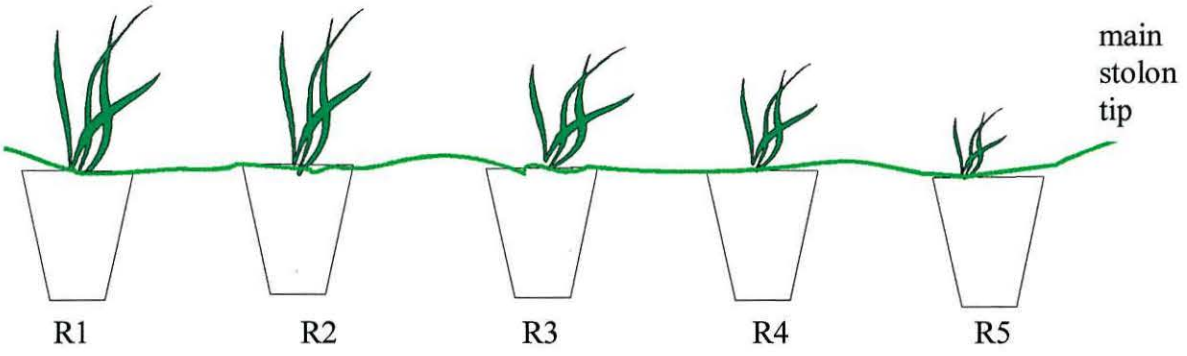


Fig. 4.1. *The arrangement of rooted ramets along the length of a parent stolon of A. stolonifera. The R1 ramet was given Long Ashton nutrient solution with either high or low phosphate and ramets R2 – R5 were given either field-capacity water or 25% of that volume.*

A second set of stolons was also set up in which the nodes were not pegged down so only R1 was rooted. Branches developed into unrooted ramets at each node. Two treatments were used; the parents were provided with Long Ashton nutrient solution with either high (1.33 mmol dm^{-3}) or low (0.133 mmol dm^{-3}) phosphate. Four replicates of each were set up as shown in Fig. 4.2.

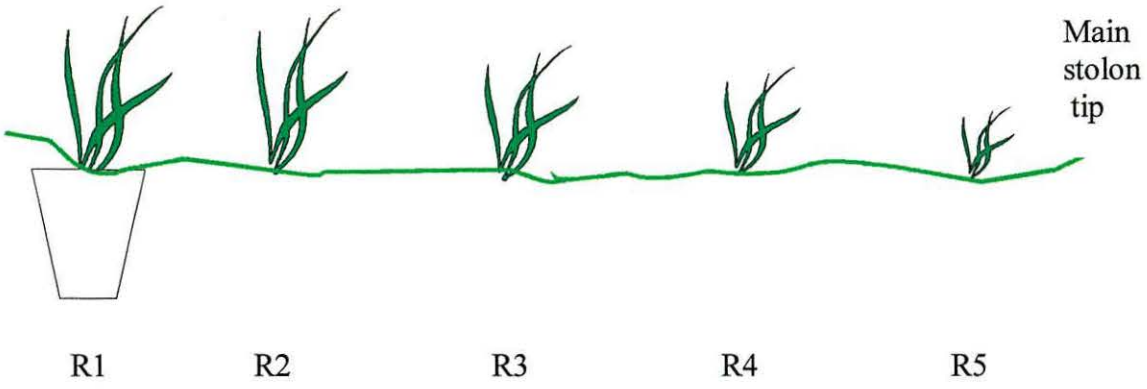


Fig. 4.2. *Diagram to show the arrangement of unrooted ramets along the length of a stolon of A. stolonifera. The R1 ramet was given Long Ashton nutrient solution with either high (1.33 mmol dm^{-3}) or low (0.133 mmol dm^{-3}) phosphate.*

The plants were grown for 40 days and the stomatal conductance of the upper epidermis was then measured on the youngest fully expanded leaves. The plants were harvested, weighed fresh, then they were dried at 80 °C and reweighed. The apices of the shoots, consisting of the terminal three nodes, internodes and leaves were then analysed for their phosphorus content.

Method 2

Stolons of rooted ramets were grown as in Fig. 4.1. Using this method each younger rooted daughter-ramet was given less water than older ramets. Beyond R5 all younger ramets were given 2 cm^3 water. The treatments given are summarised in Table 4.2.

Table 4.2. *Summary of high and low phosphate and graded water treatments provided to ramets R1 to R6 on a stolon of A. stolonifera*

	High phosphate	R1	R2	R3	R4	R5	R6
Weekly	1.33 mmol dm^{-3} phosphate	50 cm^3					
Daily	water	25 cm^3	15 cm^3	10 cm^3	5 cm^3	2 cm^3	2 cm^3
	Low phosphate	R1	R2	R3	R4	R5	R6
Weekly	0.133 mmol dm^{-3} phosphate	50 cm^3					
Daily	water	25 cm^3	15 cm^3	10 cm^3	5 cm^3	2 cm^3	2 cm^3

The plants were grown for 45 days, after which time the stomatal conductance of the upper epidermis was measured on the youngest and second youngest fully expanded leaves. The plants were harvested, weighed fresh, then dried and re-weighed to find their water content. The apices of the shoots were analysed for their phosphorus content.

Method 3

Stolons of rooted ramets were grown as in Fig. 4.1. Two phosphate and two water treatments were used.

1. High phosphate treatment

On alternate days the R1 ramets were given 15 cm^3 Long Ashton solution containing 1.33 mmol dm^{-3} phosphate. R2 ramets were given 15 cm^3 Long Ashton solution with 0.013 mmol dm^{-3} phosphate (1/100). R3 had 15 cm^3 of Long Ashton solution with 0.007 mmol dm^{-3} phosphate (1/200). R4 was given 15 cm^3 Long Ashton without phosphate. Two sets of four replicates of the phosphate treatment were set up. The first set consisted of ramets given high water so at the same time as receiving nutrient solution all the connected ramets were given 50 cm^3 water to restore the water-content of the sand to field-capacity whilst in the second, low water set, ramets were given 15cm^3 water.

2. Low phosphate treatment

In a second set of plants, grown in the same way, the ramets were given the same treatment as the first set except that in the second set the oldest ramet, R1, was given 15cm^3 Long Ashton nutrient solution with 0.013 mmol dm^{-3} phosphate, the same phosphate concentration as the second ramet, R2. High or low water was provided as for the high phosphate treatment.

These treatments are summarised in Table 4.3

Table 4.3 *Summary of graded phosphate and high and low water treatments provided to ramets R1 to R4 on a stolon of A. stolonifera*

High phosphate	R1	R2	R3	R4
15cm^3 phosphate on alternate days	1.33 mmol dm^{-3}	0.013 mmol dm^{-3} ($\frac{1}{100}$)	0.007 mmol dm^{-3} ($\frac{1}{200}$)	No phosphate
High water	50 cm^3	50 cm^3	50 cm^3	50 cm^3
Low water	15cm^3	15cm^3	15cm^3	15cm^3
Low phosphate	**R1**	**R2**	**R3**	**R4**
15cm^3 phosphate on alternate days	0.013 mmol dm^{-3}	0.013 mmol dm^{-3} ($\frac{1}{100}$)	0.007 mmol dm^{-3} ($\frac{1}{200}$)	No phosphate
High water	50 cm^3	50 cm^3	50 cm^3	50 cm^3
Low water	15cm^3	15cm^3	15cm^3	15cm^3

The plants were grown for 40 days and the stomatal conductance of the upper epidermis was then measured on the youngest fully expanded leaf on the main stolon of each ramet. The fresh weights of the shoots were found and the shoots were dried and weighed to find their water content, then analysed for their phosphorus content. The shoots were removed at the end of method 3 and xylem sap collected from the cut stumps for determination of pH. The leaf area of the youngest fully expanded leaf was found by scanning each leaf with a Hewlett Packard deskscan 2, v. 2.3 and measuring the scanned image using Delta-T Logger Analysis software, v.2 (Delta-T Devices, Cambridge, UK).

STATISTICAL ANALYSIS

Results were tested for homogeneity using Cochran's Test for Equality of Variance then analysed statistically by SPSS 8.0 for Windows 1998 using ANOVA followed by the Least Significant Difference *post hoc* test. Where data were not homogeneous they were converted to natural logarithms before statistical analysis. The mean difference was accepted as significant at the 0.05 level unless otherwise stated.

RESULTS

Experiment 4.1

a) Growth of the shoot

(*i*) *Ramets grown singly* Single ramets given no water died. There was no significant difference between the lengths of the main stolons of those given nutrient solution containing high phosphate and those given low phosphate but for both phosphate treatments the main stolons were significantly longer than for those ramets given water only (Fig 4.3). Single ramets given high water had shorter main stolons than those given low water. Single ramets given either high or low phosphate had the same numbers of main stolon leaves (Fig. 4.4), main stolon branches (Fig.4.5) and basal tillers (Fig. 4.6). Single ramets given high or low water had the same number of main stolon leaves, significantly fewer than ramets given either of the phosphate treatments. Ramets given no phosphate produced neither main stolon branches nor basal tillers with the exception of one ramet given low water which produced a single tiller.

(ii) Ramets grown in pairs Ramets given no water but attached to a partner given nutrient solution did not die. Older, R1, ramets given either high or low phosphate (shown in bold) had shorter main stolons if they were attached to a ramet given no water (**+P** dry and **-P** dry) (Fig. 4.3). For other R1 ramets the length of the main stolon was the same for those given 1.33 mmol dm^{-3} phosphate as for ramets given 0.133 mmol dm^{-3} phosphate. The lengths of the main stolons of R1 ramets given water treatments were more variable. Those R1 ramets given high water and attached to a high P younger ramet (**+H_2O** +P) were significantly shorter than those attached to a low P ramet (**+H_2O** -P). Those given low water or allowed to dry were the same for **-H_2O** +P, **dry** +P and **-H_2O** -P, but **dry** -P had a shorter main stolon, the same length as for **+H_2O** +P.

There was no significant difference between the lengths of the main stolons for any treatment of R2 ramets.

There was no significant difference between the number of leaves on the main stolons of R1 ramets of those grown in pairs (Fig 4.4) but where R2 ramets, attached to R1 ramets given high phosphate, were allowed to dry out, there were fewer leaves than the rest. The number of leaves produced on the main stolons of ramets given high and low phosphate was the same, whether in pairs or grown singly. Single ramets given no phosphate had fewer leaves than those given either high or low phosphate.

The number of branches from the main stolons of R1 ramets was not significantly different for any treatment (Fig 4.5) but within the R2 ramets those given high phosphate or attached to a partner given high phosphate had significantly more branches than those given low phosphate or attached to a low phosphate partner.

Amongst the R1 ramets those given high phosphate had a significantly greater number of basal tillers than all other R1 ramets (Fig. 4.6) whilst in the R2 ramets those given high phosphate had significantly more basal tillers than those given a water treatment. There was no difference between the numbers of basal tillers on R2 ramets given high phosphate compared with those given low phosphate.

In summary, single ramets given no water died but ramets given no water and attached to a partner survived. Single ramets given no phosphate produced fewer leaves, main stolon branches and basal tillers than those given phosphate. Attachment to either an older or younger partner given phosphate increased the number of leaves, branches and tillers produced by a ramet given water. Amongst R1 ramets there was no difference in the number of branches produced for any treatment, whereas for R2 ramets those given high phosphate produced more branches than any given low phosphate with the exception of the R2 given low phosphate in pairs where the R1 had high water ($+H_2O$ **-P).** The combined number of leaves for both ramets in a pair was the same for all treatments whereas the combined number of branches and basal stolons was greatest for those pairs in which one partner was given high phosphate.

b) Dry weights

Shoots

Ramets grown singly, without phosphate, had a highly significantly ($p<0.001$) lower dry weight than those provided with phosphate (Fig 4.7). Those given high water were no heavier than those given low water. A reduction in phosphate supply from 1.33 mmol dm^{-3} to 0.133 mmol dm^{-3} had no effect on the dry weight of single ramets.

R1 shoots Shoots of ramets given high water and attached to an R2 partner given high phosphate were significantly heavier than those grown singly with only a water treatment whereas a high water ramet attached to a low phosphate R2 partner was heavier than low water or dry attached to low P. These latter were heavier than the wet and damp singles. There was no significant difference between the shoots of +P ramets or of -P ramets. A reduction in phosphate supply from 1.33 mmol dm^{-3} to 0.133 mmol dm^{-3} had no effect on the dry weight of shoots of R1 ramets of pairs.

R2 shoots The high phosphate R2 ramets attached to an R1 given high water were significantly heavier than all other R2 ramets whereas there was no significant difference in the dry weight of any of the low P R2 shoots. There was no difference between high water, low water or dry shoots attached to high or low P partners.

Roots

The roots were removed from the sand but root material was lost in uprooting the ramets and also in washing sand from them. The roots of the ramets totally deprived of water were white and fleshy and similar in appearance to those of ramets given water, even for the single plants grown without water, where the shoot had dried out and died. The dry weights of the roots are shown in Fig. 4.8. In the paired ramets, amongst the R1 ramets there was no significant difference between the dry weights of the roots given high and those given low phosphate, but both had a significantly greater dry weight than those given water treatments. Amongst the R2 ramets, the roots of those given high phosphate were

significantly heavier than of those given low phosphate and the roots of both sets given phosphate were heavier than those lacking phosphate.

The dry weight of the shoots, roots and whole single ramets grown without phosphate (i.e. with high or low water or allowed to dry) was compared with the dry weights of ramets grown in pairs in which the partner given high, low or no water was attached to a high phosphate or low phosphate partner. The significance of the increase in dry weight resulting from attachment to a ramet provided with phosphate is shown in Table 4.4.

Table 4.4 *The significance of the increase in dry weight resulting from attachment to a ramet provided with either high or low phosphate compared with the dry weights of roots, shoots and whole ramets grown singly and given only a water treatment. The data were not homogeneous so were converted to natural logarithms before analysis by ANOVA followed by the post hoc test for Least Significant Difference. + H_2O = field capacity water; -H_2O = 25% field capacity water; "dry" = no water.*

Part of ramet	Attached to	Single ramets with which dry weight compared		
		+H_2O	-H_2O	dry
R2 Shoot	R1 +P	**	*	dead
	R1 -P	**	nsd	dead
R2 Root	R1 +P	*	nsd	*
	R1 -P	*	nsd	nsd
R2 Ramet	R1 +P	**	*	**
	R1 -P	*	nsd	*
R1 Shoot	R2+P	**	*	dead
	R2-P	**	*	dead
R1 Root	R2 +P	*	nsd	nsd
	R2 -P	*	nsd	nsd
R1 Ramet	R2 +P	**	*	*
	R2 -P	**	nsd	nsd

* $p < 0.05$; ** $p < 0.005$. nsd = no significant difference

The combined dry weights of ramets, that of roots and shoots for both members of each pair, are shown in Fig. 4.9. There was no difference between the total dry weights of pairs given reciprocal treatments. Shortage of either phosphate or of water reduced the dry weight, particularly of roots, and the lowest dry weight was in the pairs given low phosphate and no water. There was no significant

difference in the total combined dry weights for both ramets for any treatment. There was no interaction between ramet age and water treatment or phosphate treatment (Table 4.5)

Table 4.5 *The results of ANOVAs of the treatment effects on the dry weights of shoots, roots and whole ramets of joined pairs of ramets of A. stolonifera in which one partner was given either 1.33 mmol dm^{-3} or 0.133 mmol dm^{-3} phosphate and the other given field capacity water, 25% field capacity water or allowed to dry.*

		Dry weights		
Factors	df	shoot	root	whole ramet
Ramet	1	nsd	nsd	nsd
phosphate	1	*	**	**
water	2	nsd	**	nsd
ramet x phosphate	1	nsd	nsd	nsd
ramet x water	2	nsd	nsd	nsd
phosphate x water	2	nsd	nsd	nsd

* $p<0.05$; **$p<0.005$. nsd = no significant difference

The dry weight of the shoot correlated most closely with the number of basal tillers and branches and least with the number of leaves on the main stolon (Table 4.6)

Table 4.6 *Correlation between the dry weight of the shoot (mg) and the number of leaves, branches and basal tillers and the length of the main stolon of A. stolonifera in which one partner was given either 1.33 mmol dm^{-3} or 0.133 mmol dm^{-3} phosphate and the other given field capacity water, 25% field capacity water or allowed to dry. N=24*

Correlation of dry weight of shoot with	R^2
Main stolon leaves	0.44
Main stolon branches	0.63
Main stolon length	0.57
Basal tillers	0.65

c) Stomatal conductance

Stomatal conductance for the upper (adaxial) surface of the leaf was greater than that of the lower (abaxial) surface (Figs. 4.10 and 4.11). Stomatal conductance on both surfaces was slightly greater in the ramets given low phosphate than in those given high phosphate but this difference was not significant. There was no significant difference between the stomatal conductance of ramets with access to water or phosphate when grown singly but most of the single ramets given no water died. There was no difference between the stomatal conductance of the ramets grown singly in high phosphate and those given high phosphate and attached to a well-watered ramet. For high phosphate ramets attached to a water-stressed sibling there was a significant reduction in stomatal conductance in both the water-stressed ramet and in the partner although the latter had access to adequate water through its own root system. This reduction in stomatal conductance did not occur in those pairs where one ramet was water-stressed but attached to a partner given low phosphate. When the stomatal conductance was measured at different intervals after watering it was seen that the reduction in stomatal conductance in high phosphate ramets attached to water-stressed partners did not occur until 24 hours after watering and the effect increased as the interval between watering and measuring increased (Fig 4.12). The same reduction in stomatal conductance in water-stressed ramets and their attached high phosphate siblings was seen in the second leaf from the apex as well as in the youngest fully expanded leaf.

c) Phosphorus

Phosphorus content of shoots

There was a significant difference in the phosphorus content of the shoots of R1 ramets given high phosphate (1.33 mmol dm^{-3}) compared with those given low phosphate (0.133 mmol dm^{-3}) (Fig 4.13). In the R2 ramets the phosphorus content was also significantly greater ($p<0.001$) in ramets given high phosphate than in those given low phosphate. Ramets grown without water (dry) were included in an initial analysis of phosphorus content but contained too little to

give an acceptable result and there was insufficient material to repeat the analysis. The results for these ramets are not included in Fig. 4.13.

P concentration in shoots

This reflected the P content of the shoots. There was a significantly higher concentration in the shoot tissues of high P shoots compared with low P shoots in both R1 and R2 ramets.

P content of roots

The phosphorus content in the roots is shown in Fig. 4.15 and is greatest in the high phosphate R1 ramet attached to a low water R2 ramet.

P concentration in roots

The phosphorus concentration in the roots of ramets grown singly was highest in those supplied with 1.33 mmol dm^{-3} phosphate. The highest concentration of P occurred in the roots of ramets provided with nutrient solution containing high phosphate (Fig. 4.16).

The correlation between the concentration of phosphorus and the number of leaves, branches and tillers, and with the length of the main stolons is shown in Table 4.7 and shows that the only significant correlation is between the P concentration in the shoot and the number of branches produced.

Table 4.7 *Correlation between the phosphorus concentration (nmol mg^{-1} dry weight) in the shoot and the number of leaves, branches and basal tillers and the length of the main stolon of A. stolonifera in which one partner was given either 1.33 mmol dm^{-3} or 0.133 mmol dm^{-3} phosphate and the other given field capacity water or 25% field capacity water. n = 20.*

Correlation of [P] nmol mg^{-1} with	R^2	p
Main stolon leaves	0.17	>0.1
Main stolon branches	0.61	<0.01
Main stolon length	0.23	>0.1
Basal tillers	0.29	>0.1

Insufficient xylem sap was collected from the pairs of ramets given high phosphate to allow comparison of the pH values with those of sap collected from low phosphate ramets.

Experiment 4.2

Method 1

There was no significant difference in the stomatal conductance of the youngest fully expanded leaf of any of the rooted ramets along the stolons suggesting that method 1 established no gradient of water or phosphate between the oldest and youngest ramets (Fig. 4.17). For the unrooted ramets the stolon on which the oldest ramet was given high phosphate had slightly higher stomatal conductances than the ramets on the low phosphate stolon (Fig. 4.18). In the unrooted ramets the low P parent had a higher stomatal conductance than the +P parent but the difference between the stomatal conductances of +P and -P ramets increased along the stolon.

Method 2

a) Stomatal conductance

Although there was a trend towards reduction in stomatal conductance along the length of the stolon there was no significant difference between the stomatal conductances of ramets on the stolon in which R1 was given high phosphate. On the stolon in which the R1 was given low phosphate the youngest ramets had a lower stomatal conductance than the oldest (Fig. 4.19). In both the +P and -P stolons there was an increase in the stomatal conductance of the youngest fully expanded leaf of the youngest ramet compared with R7.

b) Water content

Although there was a trend to increased water content along the length of both +P and -P stolons there was no significant difference in the water content expressed as percentage of the fresh weight between the ramets given the same volume of water (Fig. 4.20). The difference between R2 ramets given +P and is

significant only at p = 0.1, and for the difference between R6 +P and -P ramets p=0.07.

c) Phosphorus content.

Although the R1 ramets were given different concentrations of phosphate there was no significant difference in the phosphorus concentration in ramets along the high phosphate stolons compared with the low phosphate stolons (Fig. 4.21). In both stolons the concentration of phosphorus increased in the two youngest ramets and was highest at the tip.

Method 3

a) Stomatal conductance

Ramets 1 and 2 on the stolon given high phosphate and low water had a significantly lower stomatal conductance than the equivalent ramets on stolons given the three other treatments (Fig. 4.22). There was no significant difference in the stomatal conductance of ramets 3, 4 and 5 for any of the four treatments but those given high P and low water had the lowest stomatal conductance.

b) Water content

Although there was a significantly lower water-content in the oldest ramet, R1, given low water and low phosphate, all the other ramets had the same water content (Fig. 4.23).

c) Phosphorus concentration

The highest phosphorus concentration was in plants given high phosphate and low water (Fig 4.24). Ramets R1 and R4 on the stolon given this treatment had a statistically greater phosphate concentration than the equivalent ramets on the stolons given the three other treatments.

d) Xylem sap pH

Approximately 0.05 – 0.15 cm^3 xylem sap was collected from the cut stems of each ramet. Although the first ramet (R1) on each stolon was given the same amount of water as the younger ramets on the same stolon there was no difference in the pH of the xylem sap collected from the cut stems of ramets R1

for any treatment (Fig 4.25). For the younger ramets, R2 to R4, those given high water had a significantly lower pH than those given low water irrespective of the phosphate treatment they were given. The pH of fresh Long Ashton nutrient solution containing 1.33 mmol dm^{-3} phosphate was 6.00 ± 0.03 and that of Long Ashton nutrient solution containing 0.133 mmol dm^{-3} phosphate was 6.88 ± 0.02.

e) Area of leaves

There was no difference in the area of the youngest fully expanded leaf of any of the ramets (Fig 4.26). Although those given low phosphate and low water were slightly smaller this difference was not significant. The area of older leaves was not measured.

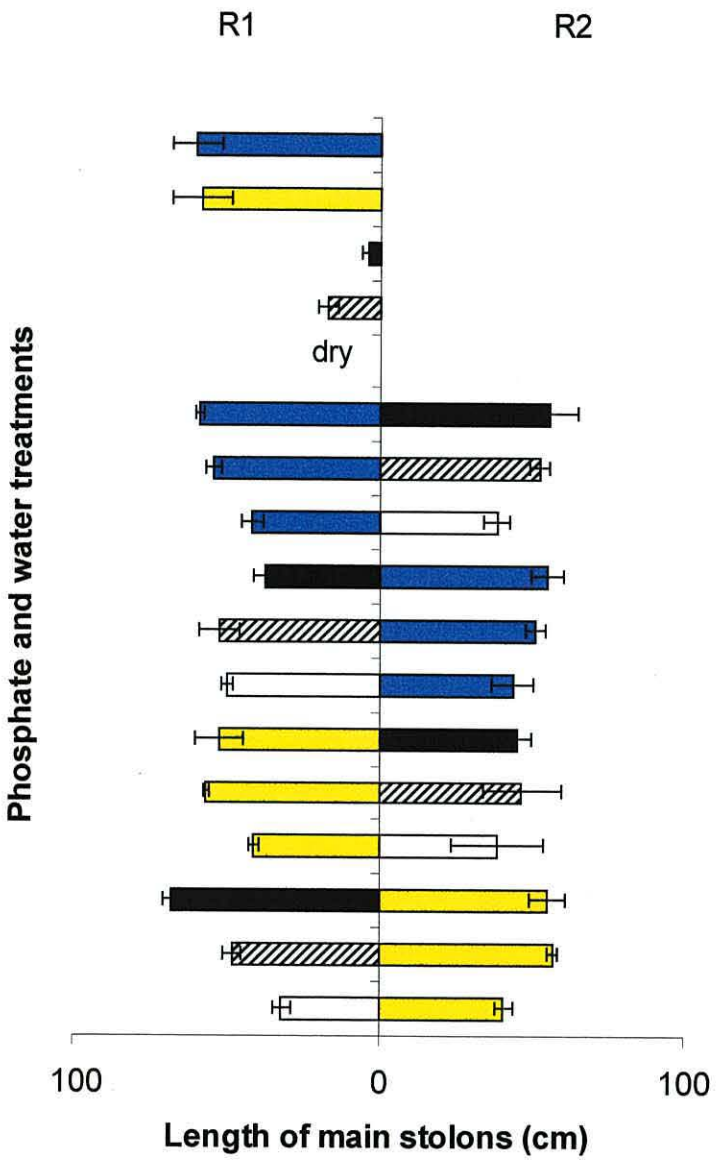


Fig. 4.3 *Mean length of main stolons of single ramets and of each of a pair of ramets of A. stolonifera after 30 days in which one partner was given a phosphate treatment and the other was given a water treatment. The bars on the left hand side show the length of the main stolon of the older ramet (R1) and those on the right hand side show length of the main stolon of the younger ramet R2. +P (Blue bars) = high phosphate (1.33 mmol dm^{-3}); -P (yellow bars) = low phosphate treatment (0.133 mmol dm^{-3}); $+H_2O$ (black bars) = field capacity water; $-H_2O$ (cross-hatched bars) = 25% field capacity water; 'dry' (open bars) = no water. Error bars are ± one standard error of the mean. N = 4.*

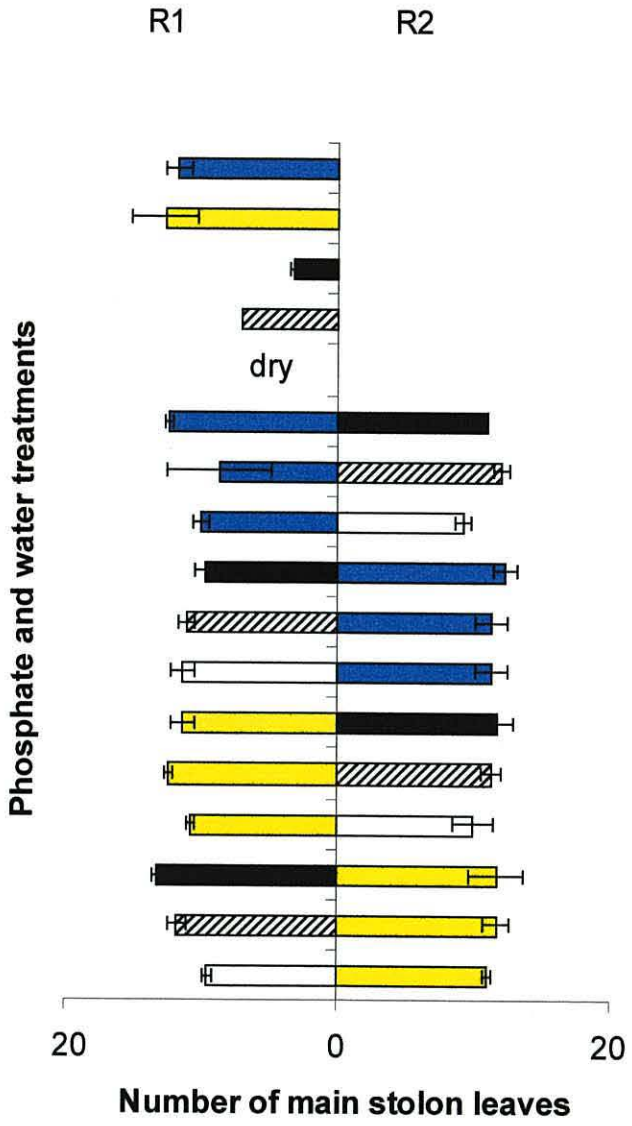


Fig. 4.4 *Mean number of leaves on the main stolon of single control ramets and of each of a pair of ramets of A. stolonifera after 30 days in which one partner was given a phosphate treatment and the other was given a water treatment. The bars on the left hand side show the number of leaves on the main stolon of the older ramet (R1) and those on the right hand side the number of leaves on the main stolon of the younger ramet R2.* +P *= high phosphate (1.33 mmol* dm^{-3}*);* -P *= low phosphate treatment (0.133 mmol* dm^{-3}*).* $+H_2O$ *(black bars) = field capacity water;* $-H_2O$ *(cross-hatched bars) = 25% field capacity water; 'dry' (open bars) = no water. Error bars are ± one standard error of the mean. N = 4.*

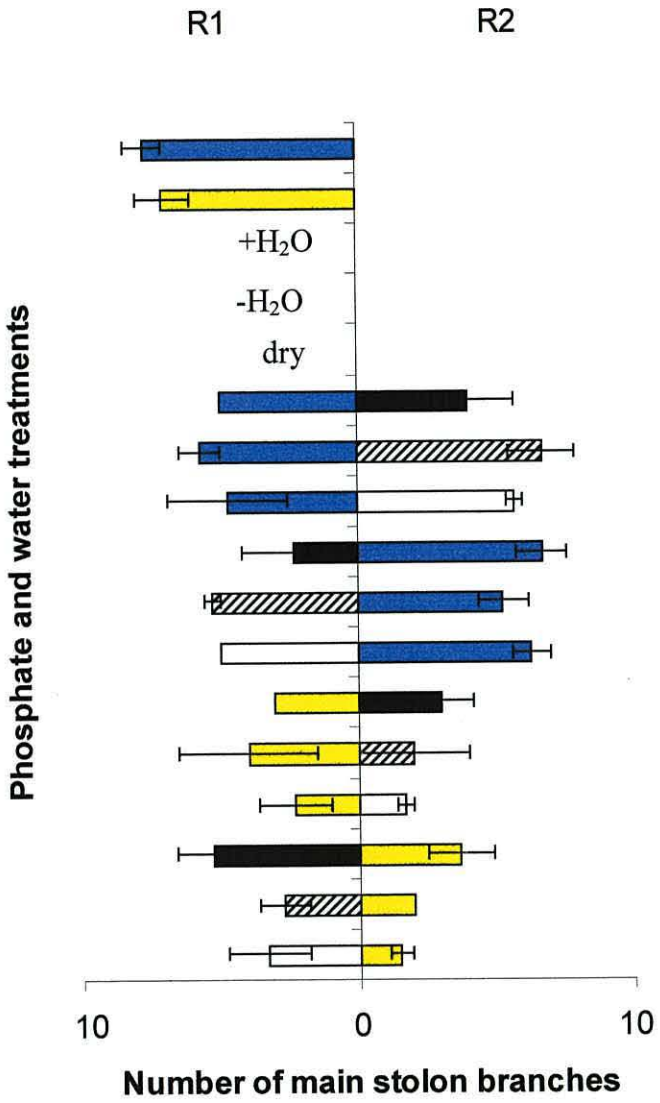


Fig. 4.5 *Mean number of main stolon branches for single ramets and for each of a pair of ramets of A. stolonifera after 30 days in which one partner was given a phosphate treatment and the other was given a water treatment. The bars on the left hand side show the number of branches on the main stolon of the older ramet R1 and those on the right hand side show the number of branches on the main stolon of the younger ramet R2.* +P *(Blue bars) = high phosphate (1.33 mmol* dm^{-3}*);* -P *(yellow bars) = low phosphate treatment (0.133 mmol* dm^{-3}*);* $+H_2O$ *(black bars) = field capacity water;* $-H_2O$ *(cross-hatched bars) = 25% field capacity water;* 'dry' *(open bars) = no water. Error bars are ± one standard error of the mean. N = 4.*

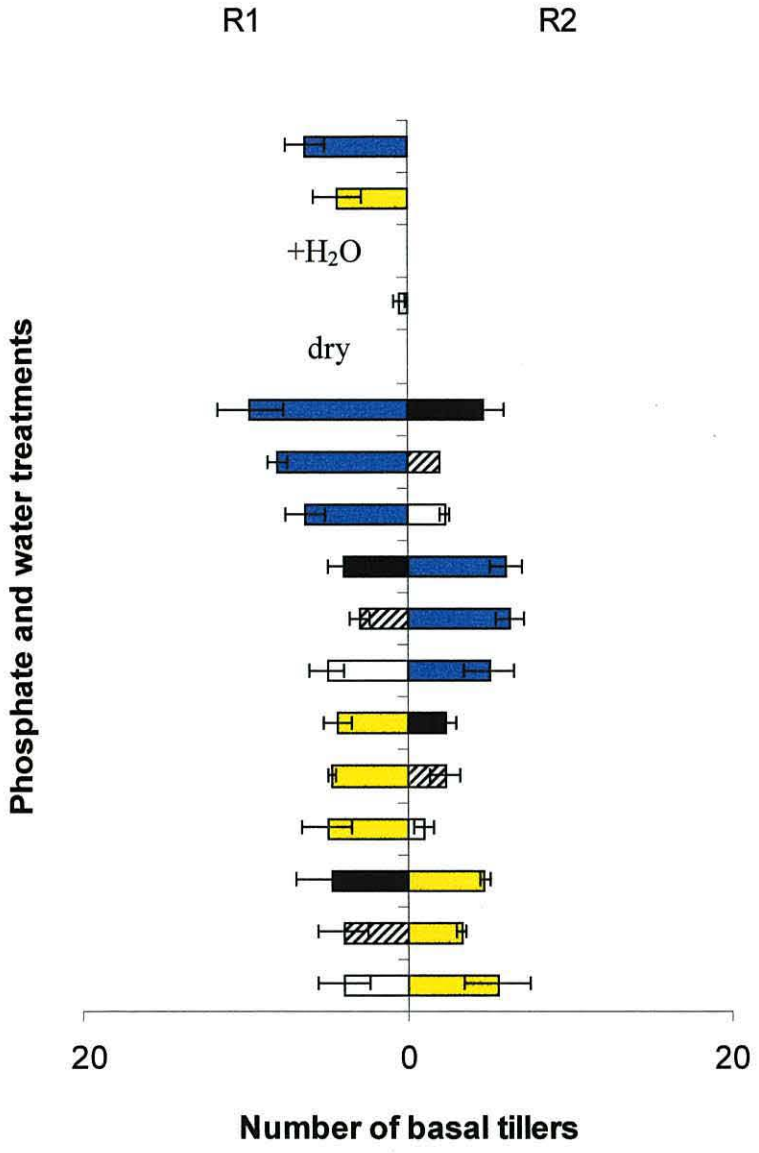


Fig 4.6 *Mean number of basal tillers of single control ramets and of each of a pair of ramets of A. stolonifera after 30 days in which one partner was given a phosphate treatment and the other was given a water treatment. The bars on the left hand side show the number of basal tillers on the main stolon of the older ramet R1 and those on the right hand side show the number of basal tillers on the main stolon of the younger ramet R2.* +P *(Blue bars) = high phosphate (1.33 mmol* dm^{-3}*);* -P *(yellow bars) = low phosphate treatment (0.133 mmol* dm^{-3}*);* $+H_2O$ *(black bars) = field capacity water;* $-H_2O$ *(cross-hatched bars) = 25% field capacity water;* 'dry' *(open bars) = no water. Error bars are ± one standard error of the mean. N = 4.*

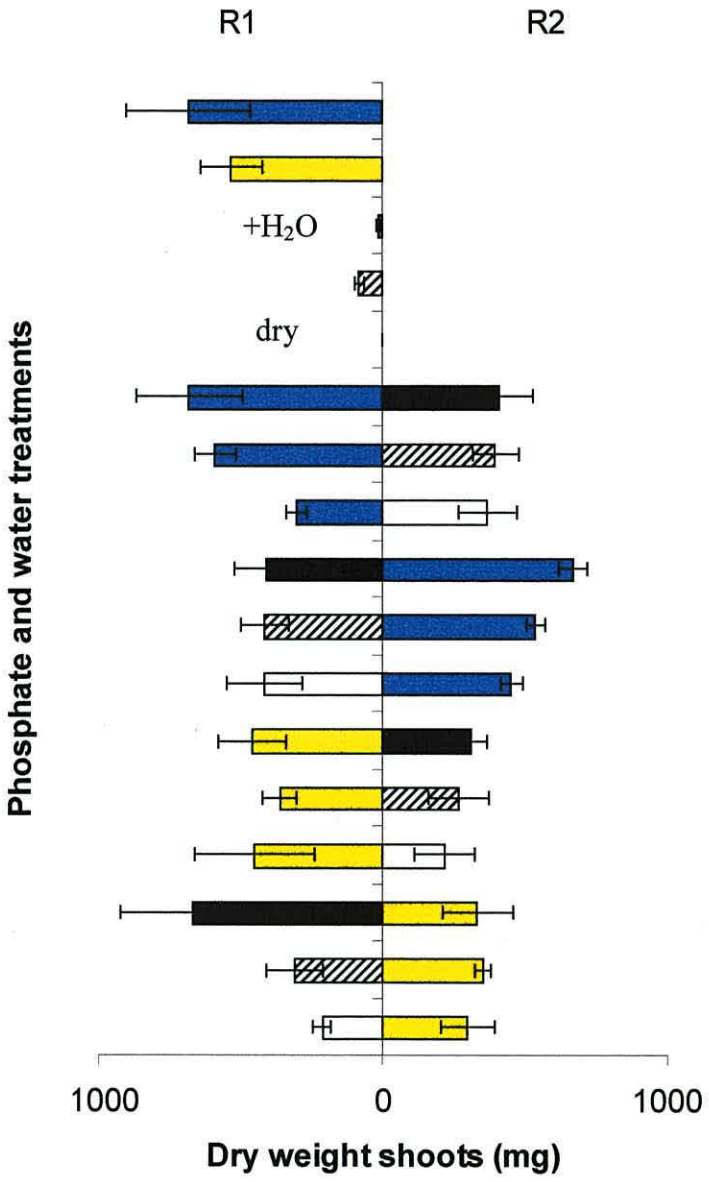


Fig 4.7 *Mean dry weights of shoots of single ramets and of each of a pair of ramets of A. stolonifera after 30 days in which one partner was given a phosphate treatment and the other was given a water treatment. The bars on the left hand side show the mean dry weight of shoots of the older ramet R1 and the bars on the right hand side show the mean dry weight of the shoots of the younger ramets R2. +P (Blue bars) = high phosphate (1.33 mM); -P (yellow bars) = low phosphate treatment (0.133 mM); +H_2O (black bars) = field capacity water; -H_2O (cross-hatched bars) = 25% field capacity water;* 'dry' *(open bars) = no water. Error bars are ± one standard error of the mean. N = 4.*

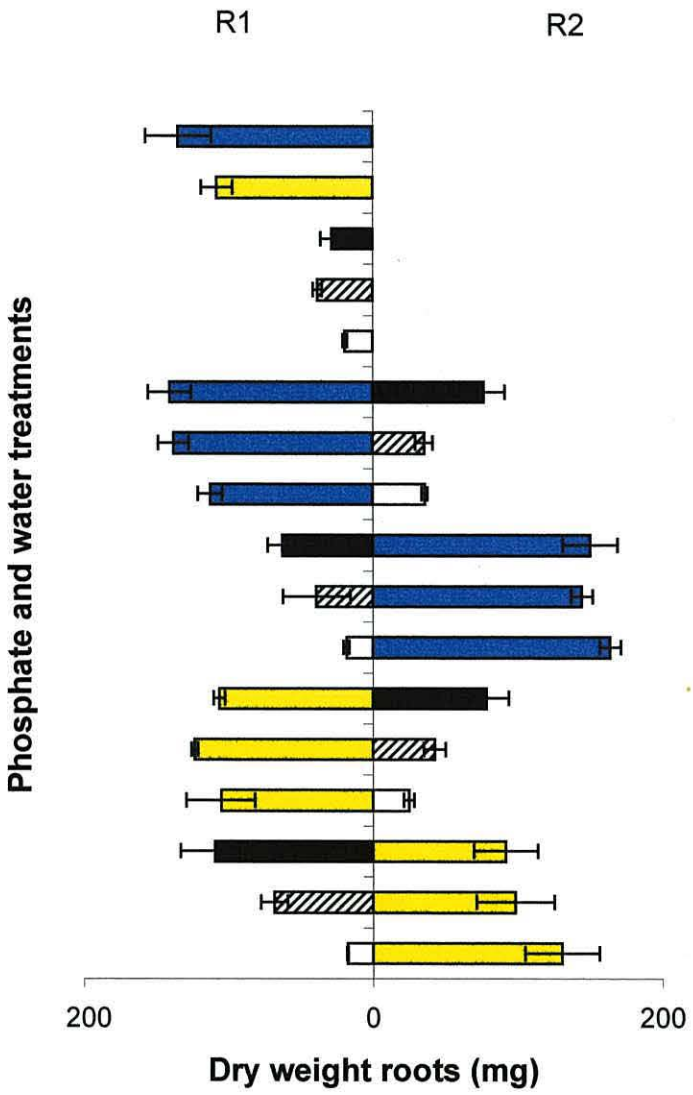


Fig. 4.8 *Mean dry weights of roots of single ramets and of each of a pair of ramets of A. stolonifera after 30 days in which one partner was given a phosphate treatment and the other was given a water treatment. The bars on the left hand side show the mean dry weight of roots of the older ramet R1 and the bars on the right hand side show the mean dry weight of roots of the younger ramets R2.* +P *(Blue bars) = high phosphate (1.33 mM);* -P *(yellow bars) = low phosphate treatment (0.133 mM);* $+H_2O$ *(black bars) = field capacity water;* $-H_2O$ *(cross-hatched bars) = 25% field capacity water;* 'dry' *(open bars) = no water. Error bars are ± one standard error of the mean. N = 4.*

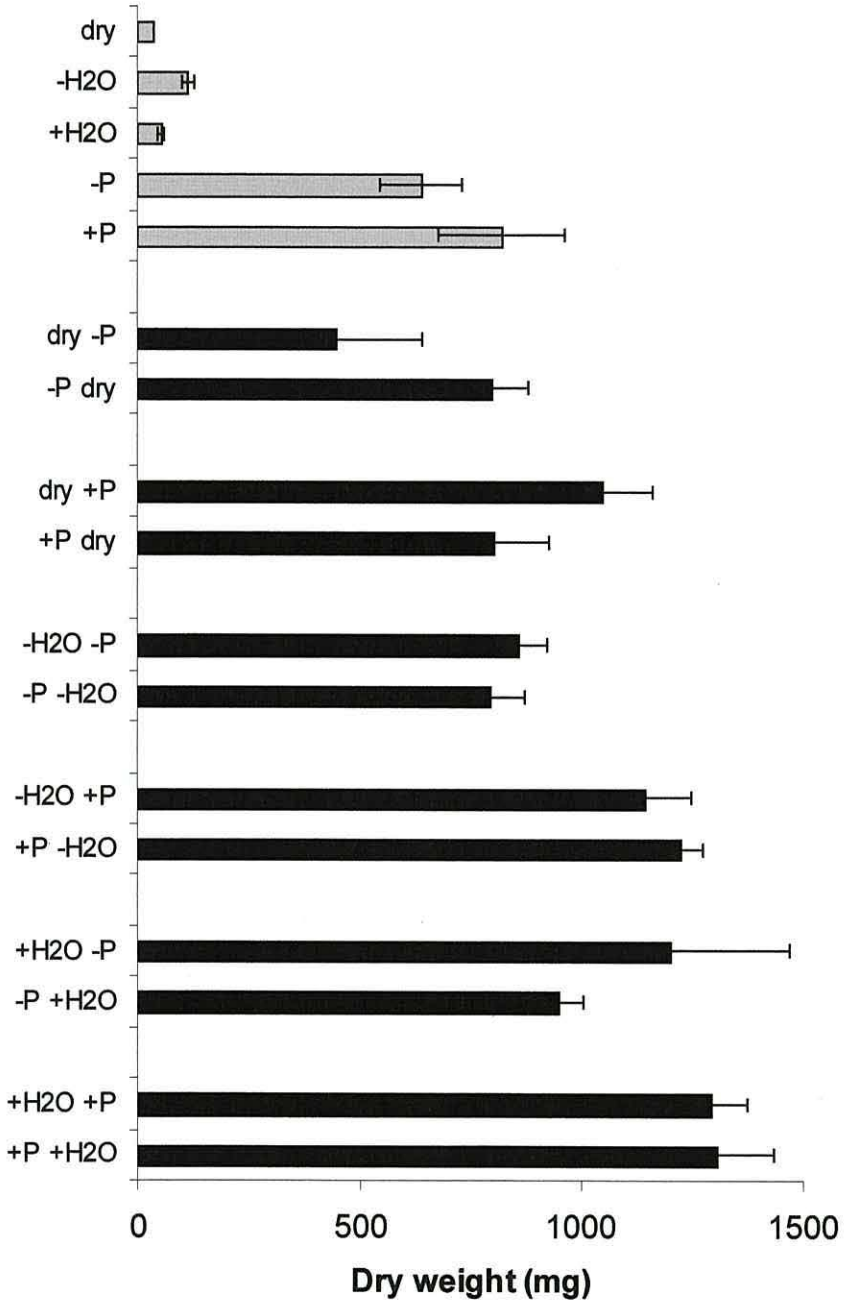


Fig. 4.9 *Mean total dry weight of both ramets in the pair of A. stolonifera in which one partner was given a phosphate treatment and the other was given a water treatment. The first 5 bars show the dry weight of the roots and shoot of single ramets given the treatment shown. Error bars are ± one standard error of the mean. N = 4.*

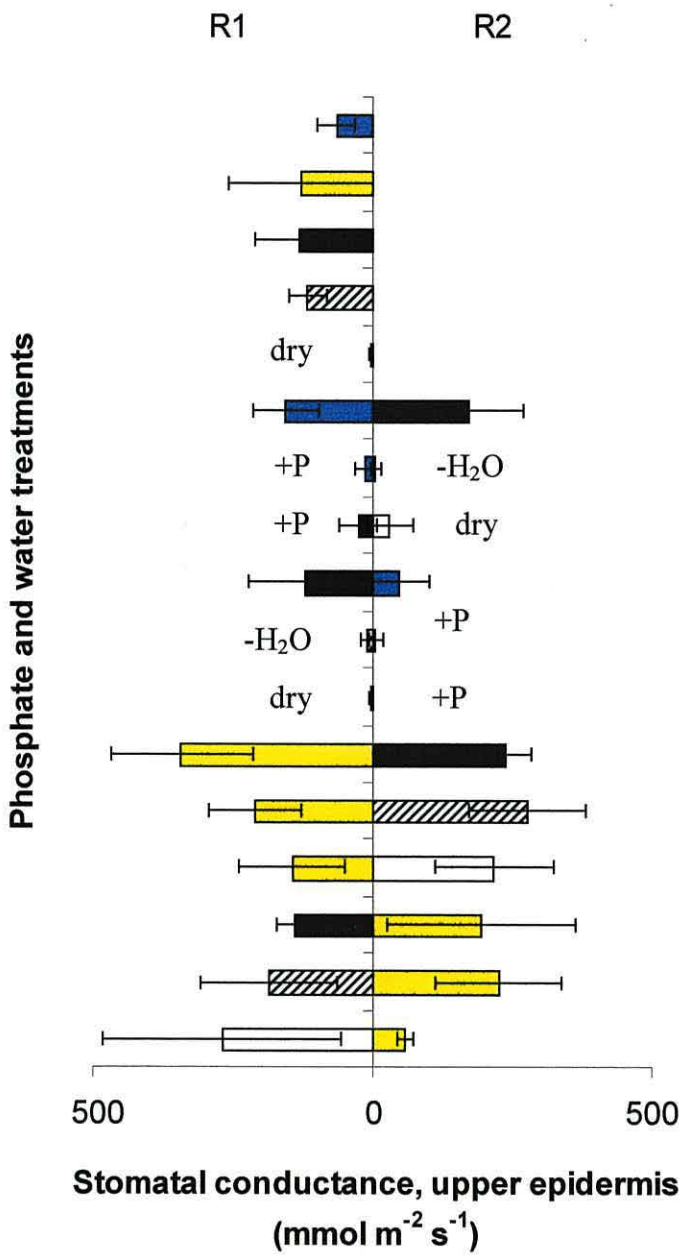


Fig. 4.10 *Mean stomatal conductance (mmol m^{-2} s^{-1}) of the upper epidermis of the youngest fully expanded leaves of single ramets and each of pairs of ramets of A. stolonifera after 30 days in which one partner was given a phosphate treatment and the other was given a water treatment. The bars on the left hand side show the stomatal conductance of the older ramet (R1) and those on the right hand side show the stomatal conductance for the younger ramet R2. +P (Blue bars) = high phosphate (1.33 mmol dm^{-3}); -P (yellow bars) = low phosphate treatment (0.133 mmol dm^{-3}); +H_2O (black bars) = field capacity water; -H_2O (cross-hatched bars) = 25% field capacity water;* 'dry' *(open bars) = no water. Error bars are ± one standard error of the mean. N = 4.*

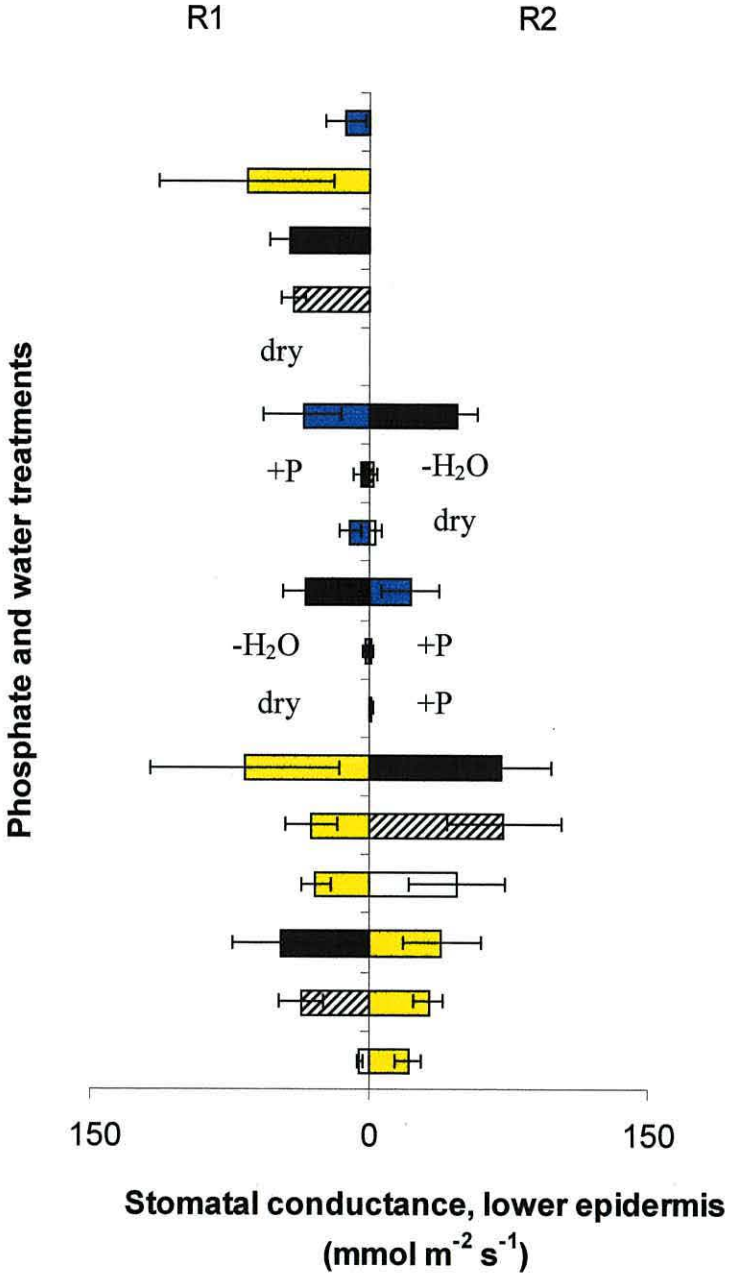


Fig 4.11 *Mean stomatal conductance (mmol m^{-2} s^{-1}) of the lower epidermis of the youngest fully expanded leaves of single ramets and each of a pair of ramets of A. stolonifera after 30 days in which one partner was given a phosphate treatment and the other was given a water treatment. The bars on the left hand side show the stomatal conductance of the older ramet (R1) and those on the right hand side show the stomatal conductance for the younger ramet R2. +P (Blue bars) = high phosphate (1.33 mmol dm^{-3}); -P (yellow bars) = low phosphate treatment (0.133 mmol dm^{-3}); +H_2O (black bars) = field capacity water; -H_2O (cross-hatched bars) = 25% field capacity water;* 'dry' *(open bars) = no water. Error bars are ± one standard error of the mean. N = 4.*

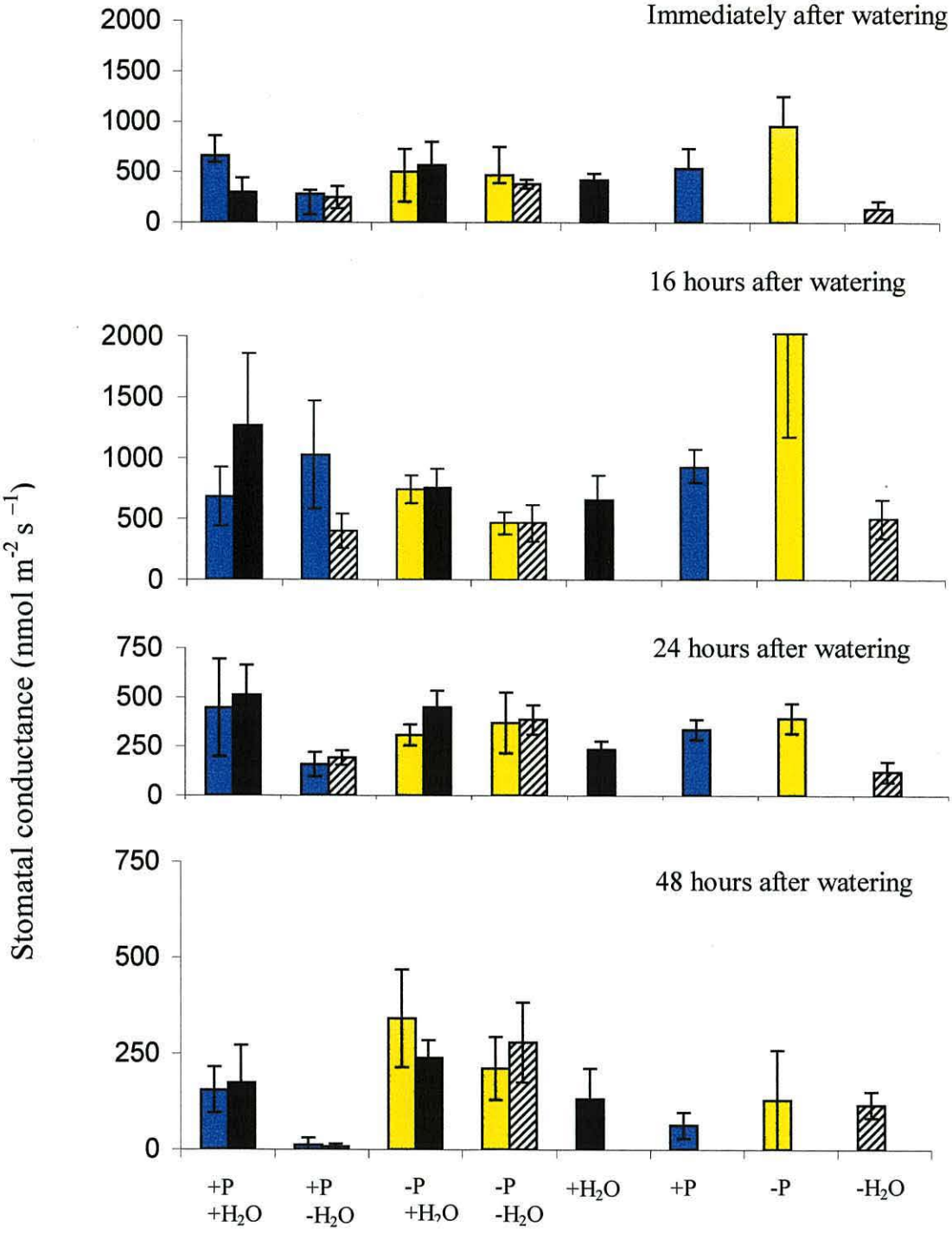


Fig. 4.12 *Comparison of mean stomatal conductance of paired ramets of A. stolonifera in which one of the pair was given either 1.33 mmol dm^{-3} phosphate or 0.133 mmol dm^{-3} phosphate and the partner was given either field capacity water or 25% of that volume. Stomatal conductance was measured at the times indicated after watering. +P (Blue bars) = high phosphate (1.33 mmol dm^{-3}); -P (yellow bars) = low phosphate treatment (0.133 mmol dm^{-3}); $+H_2O$ (black bars) = field capacity water; $-H_2O$ (cross-hatched bars) = 25% field capacity water. Error bars are ± one standard error of the mean. N = 4.*

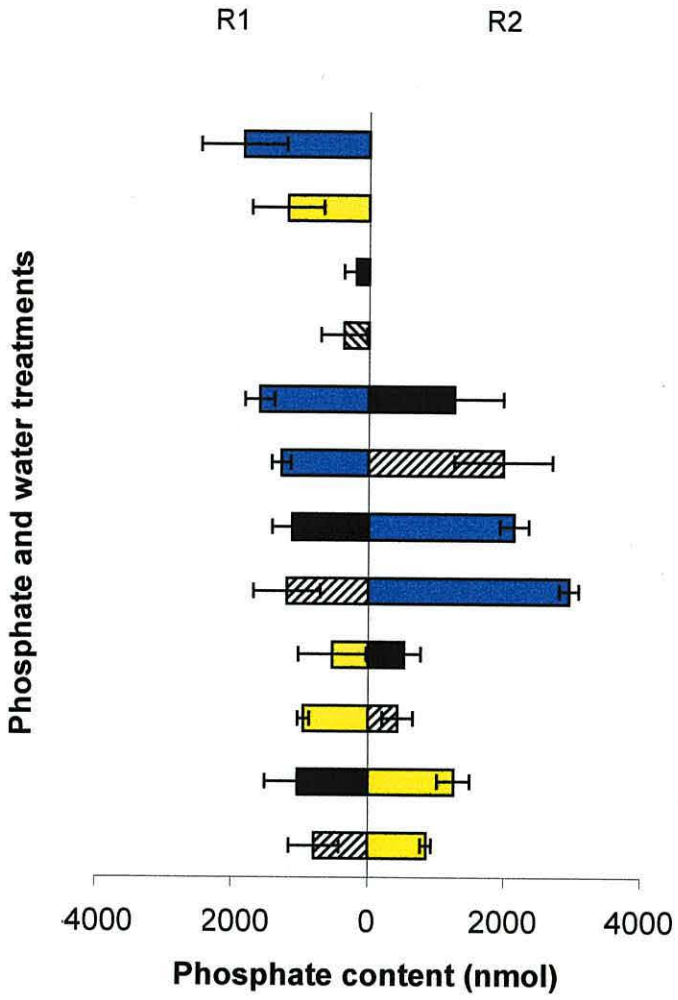


Fig 4.13 *Mean phosphorus content (nmol) of terminal 3 internodes, nodes and leaves of the shoots of single control ramets and of each of a pair of ramets of A. stolonifera after 30 days in which one partner was given a phosphate treatment and the other was given a water treatment. The bars on the left hand side show the mean phosphorus content of shoots of the older ramet R1 and the bars on the right hand side show the mean phosphorus content of the shoots of the younger ramets R2. +P (Blue bars) = high phosphate (1.33 mdm^{-3}); -P (yellow bars) = low phosphate treatment (0.133 mmol dm^{-3}); +H_2O (black bars) = field capacity water; -H_2O (cross-hatched bars) = 25% field capacity water. Error bars are ± one standard error of the mean. N = 4.*

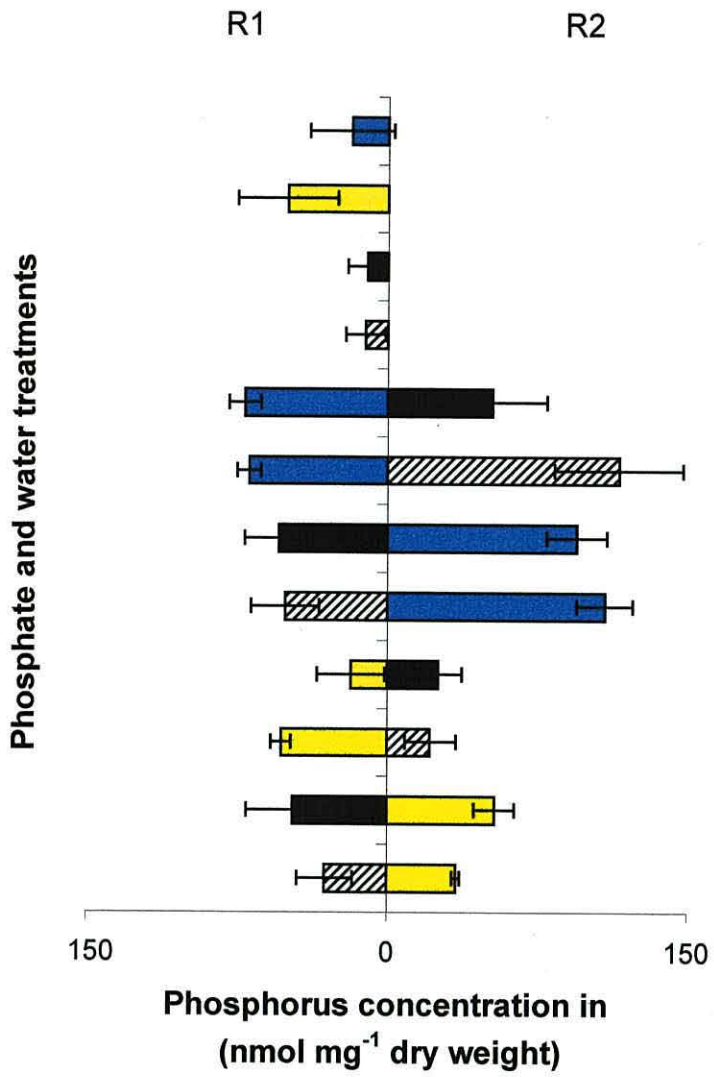


Fig 4.14 *Mean phosphorus concentration (nmol mg^{-1}) of terminal 3 internodes, nodes and leaves of the shoots of single control ramets and of each of a pair of ramets of A. stolonifera after 30 days in which one partner was given a phosphate treatment and the other was given a water treatment. The bars on the left hand side show the mean phosphorus concentration in shoots of the older ramet R1 and the bars on the right hand side show the mean phosphorus concentration in the shoots of the younger ramets R2.* +P *(Blue bars) = high phosphate (1.33 mdm^{-3});* -P *(yellow bars) = low phosphate treatment (0.133 mmol dm^{-3});* $+H_2O$ *(black bars) = field capacity water;* $-H_2O$ *(cross-hatched bars) = 25% field capacity water. Error bars are ± one standard error of the mean. N = 4.*

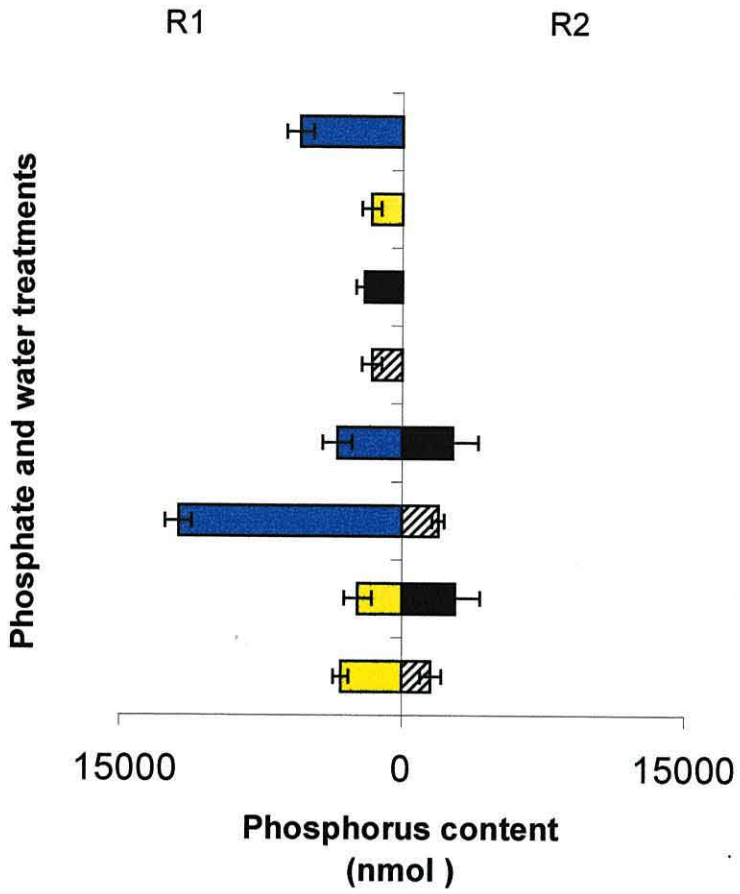


Fig. 4.15 *Mean phosphorus content (nmol) in roots of four single and four paired ramets of A. stolonifera after 30 days in which the R1 partner was given a phosphate treatment and the R2 ramet a water treatment. The bars on the left hand side show the mean phosphorus content in the roots of the older ramet R1 and the bars on the right hand side show the phosphorus content in the younger R2 ramets. +P (Blue bars) = high phosphate (1.33 mmol dm^{-3}); -P (yellow bars) = low phosphate treatment (0.133 mmol dm^{-3}); $+H_2O$ (black bars) = field capacity water; $-H_2O$ (cross-hatched bars) = 25% field capacity water. Error bars are ± one standard error of the mean. N = 4.*

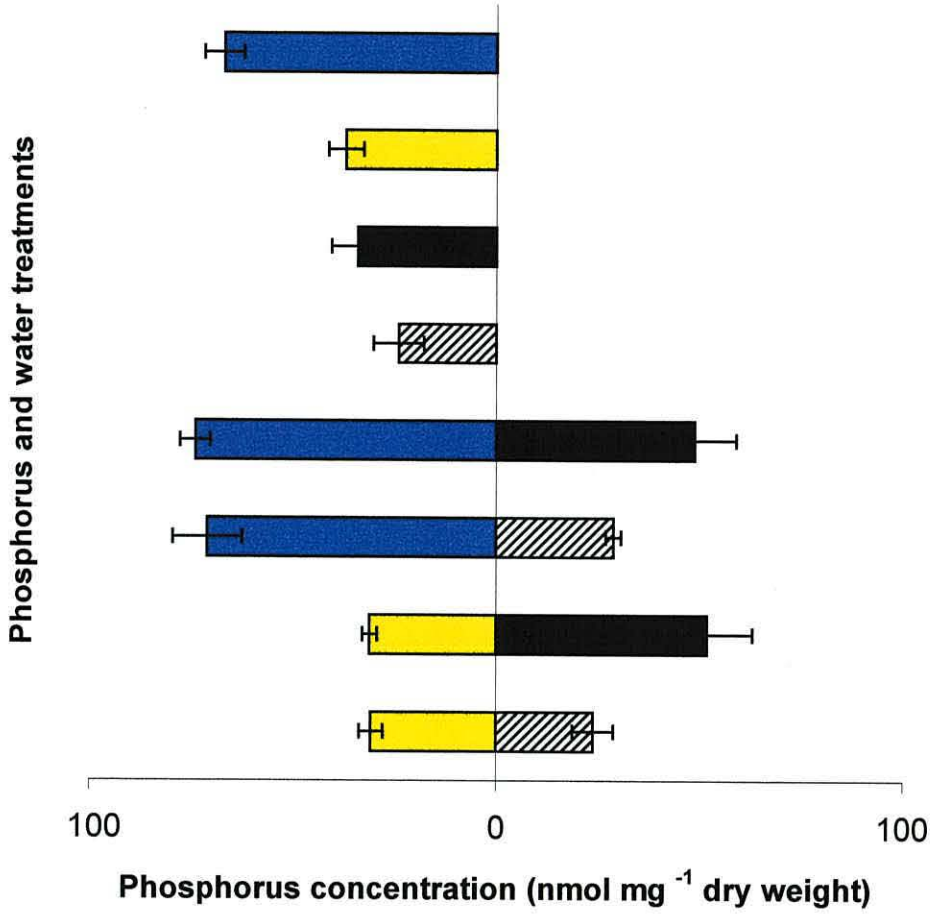


Fig. 4.16 *Mean phosphorus concentration (nmol mg^{-1} dry weight) in roots of four single and four paired ramets of A. stolonifera after 30 days in which the R1 partner was given a phosphate treatment and the R2 ramet a water treatment. The bars on the left hand side show the mean phosphorus concentration in the roots of the older ramet R1 and the bars on the right hand side show the phosphorus concentration in the younger R2 ramets. +P (Blue bars) = high phosphate (1.33 mmol dm^{-3}); -P (yellow bars) = low phosphate treatment (0.133 mmol dm^{-3}); +H_2O (black bars) = field capacity water; -H_2O (cross-hatched bars) = 25% field capacity water. Error bars are one standard error of the mean. N = 4.*

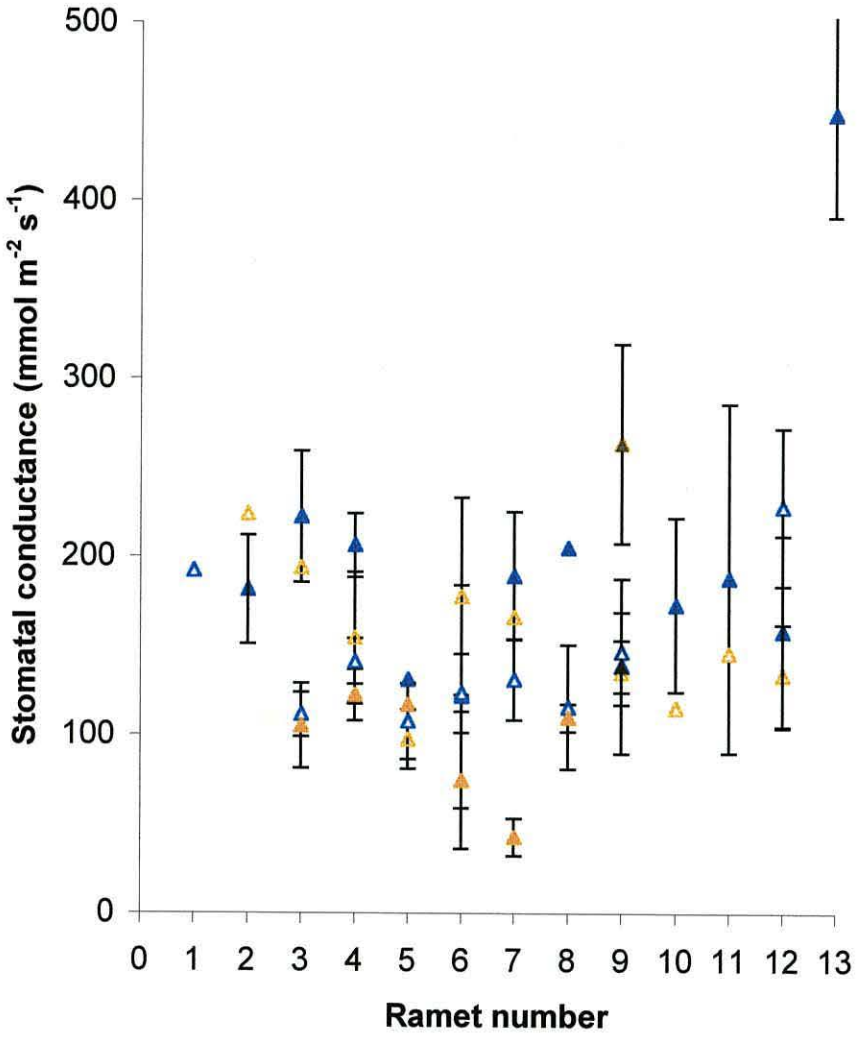


Fig. 4.17 *Mean stomatal conductance of the upper epidermis of the youngest fully-expanded leaf of successively younger rooted daughter ramets along stolons of A. stolonifera given two phosphate and two water treatments. Closed blue triangles = +P +H_2O; open blue triangles = +P -H_2O; closed yellow triangles = -P +H_2O; open yellow triangles = -P -H_2O. Error bars are ± one standard error of the mean. N = 4.*

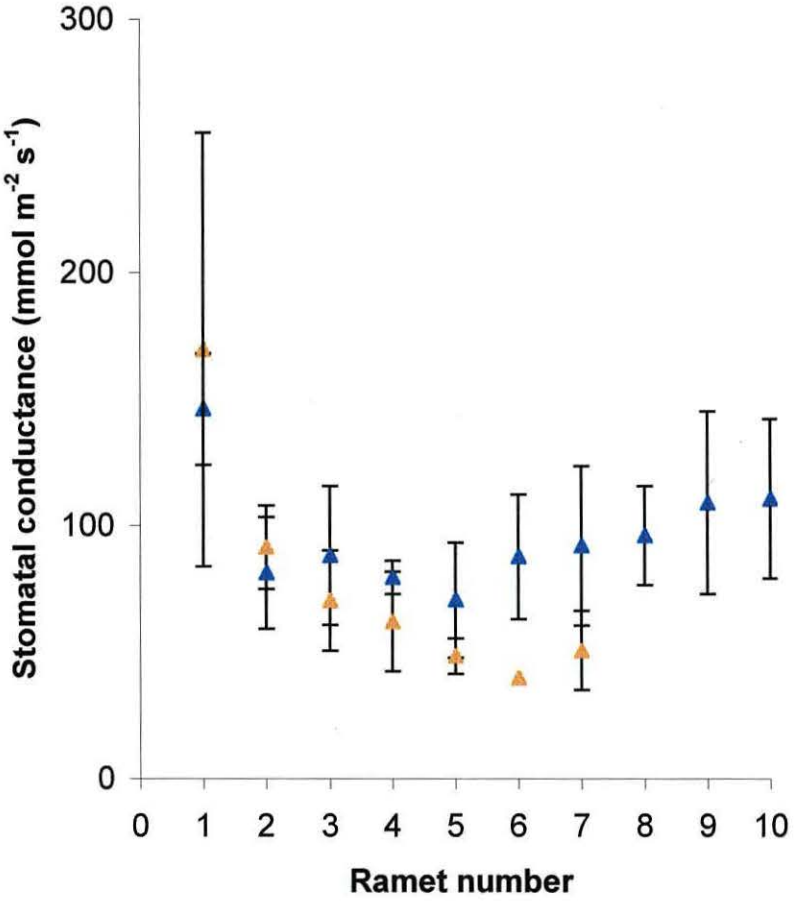


Fig 4.18 *Mean stomatal conductance of the upper epidermis of the youngest fully expanded leaf of successively younger unrooted daughter ramets along stolons of A. stolonifera in which the rooted ramet R1 was given one of two phosphate treatments* +P *(1.33 mmol* dm^{-3}*) phosphate (blue triangles) or* -P *(0.133 mmol* dm^{-3}*) phosphate (yellow triangles). Error bars are ± one standard error of the mean. N = 4.*

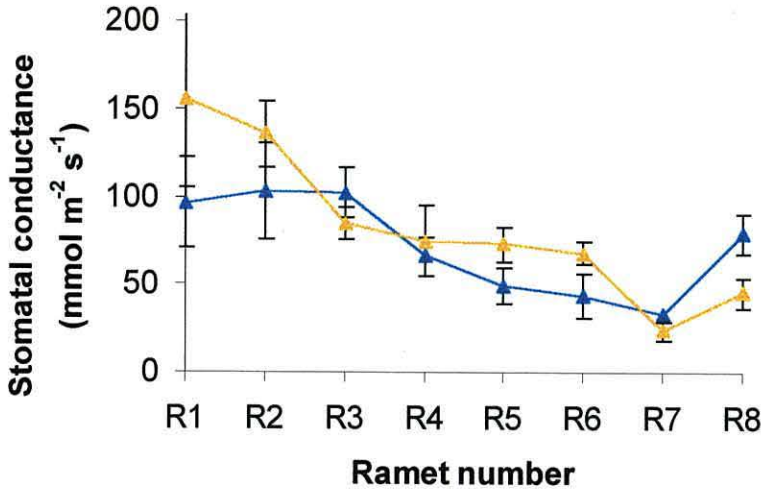


Fig. 4.19 *Mean stomatal conductance (mmol $m^{-2}s^{-1}$) of the upper epidermis of the youngest fully-expanded leaf of rooted ramets along stolons of A. stolonifera. The R1 ramet was given one of two phosphate treatments. +P = 1.33 mmol dm^{-3} phosphate(blue triangles); -P = 0.133 mmol dm^{-3} (yellow triangles). Each ramet along the stolon was given successively less water. Error bars are ± one standard error of the mean. N = 4.*

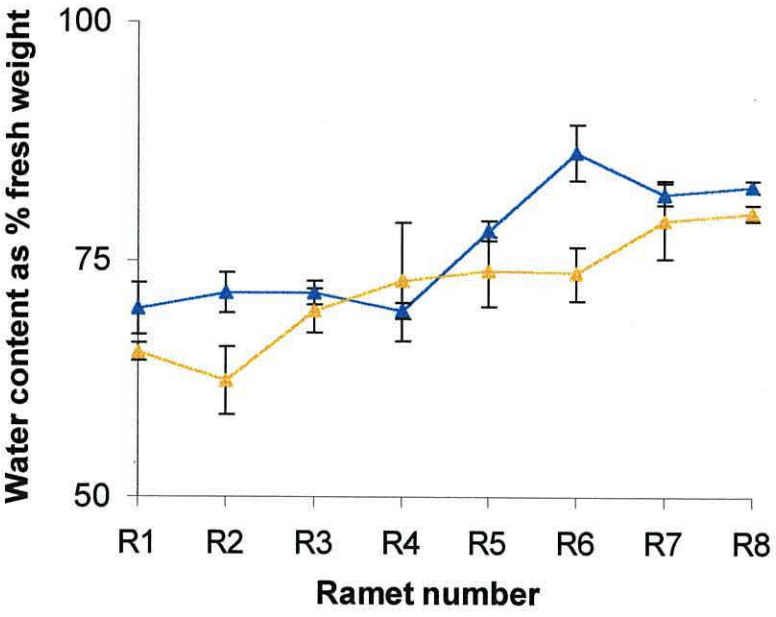


Fig. 4.20 *Mean water content as % fresh weight of ramets along stolons of A. stolonifera. The R1 ramet was given one of two phosphate treatments. +P (blue triangles) = 1.33 mmol dm^{-3} phosphate; -P (yellow triangles) = 0.133 mmol dm^{-3}. Each ramet along the stolon was given successively less water. Unrooted ramets were supplied with water from R1. Error bars are ± one standard error of the mean. N = 4.*

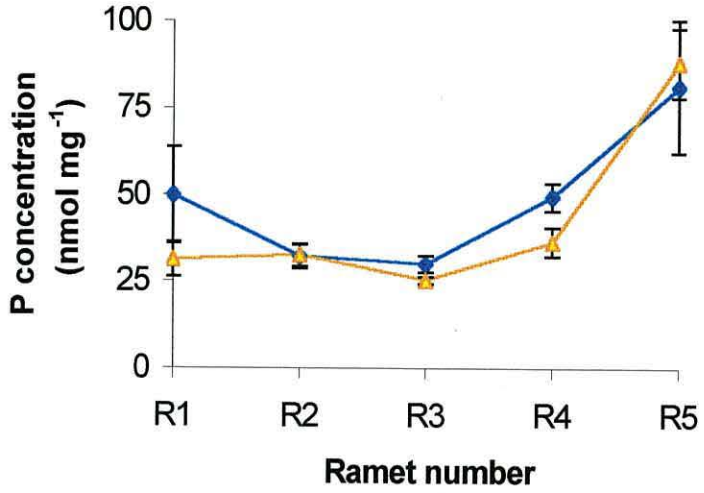


Fig. 4.21 *Mean phosphorus concentration (nmol mg^{-1}) in the shoots of rooted ramets along stolons of A. stolonifera. The R1 ramet was given one of two phosphate treatments. +P = 1.33 mmol dm^{-3} phosphate (blue triangles); -P = 0.133 mmol dm^{-3} (yellow triangles). Each ramet along the stolon was given successively less water. Error bars are ± one standard error of the mean. N = 4.*

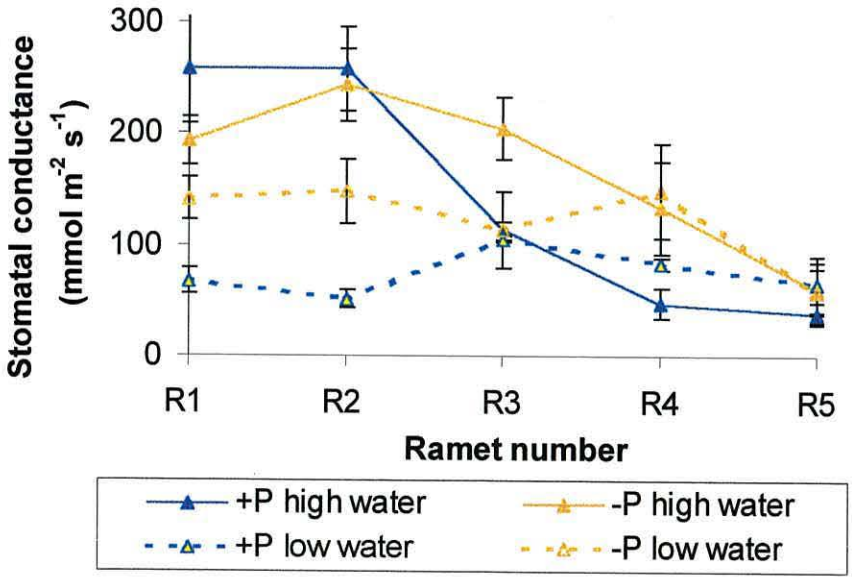


Fig. 4.22 *Mean stomatal conductance (mmol m^{-2} s^{-1}) of the upper epidermis of the youngest fully-expanded leaf of rooted ramets along stolons of A. stolonifera. Ramet R1 was given one of two phosphate treatments. +P = 1.33 mmol dm^{-3} phosphate; -P = 0.133 mmol dm^{-3}. Each ramet along the stolon was given successively less phosphate.* high water *= 50 cm^3 water;* low water *= 15 cm^3 water. Error bars are ± one standard error of the mean. N = 4.*

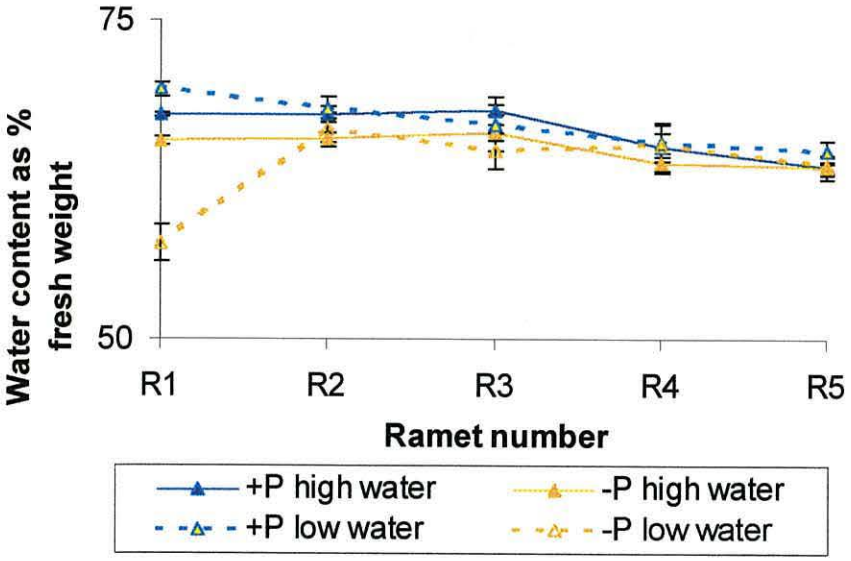


Fig. 4.23 *Mean water content expressed as % fresh weight of shoots of rooted ramets along stolons of A. stolonifera. Ramet R1 was given one of two phosphate treatments.* +P = *1.33 mmol* dm^{-3} *phosphate;* -P = *0.133 mmol* dm^{-3}. high water = *50* cm^3 *water;* low water = *15* cm^3 *water. Each ramet along the stolon was given successively less phosphate. Error bars are ± one standard error of the mean. N = 4.*

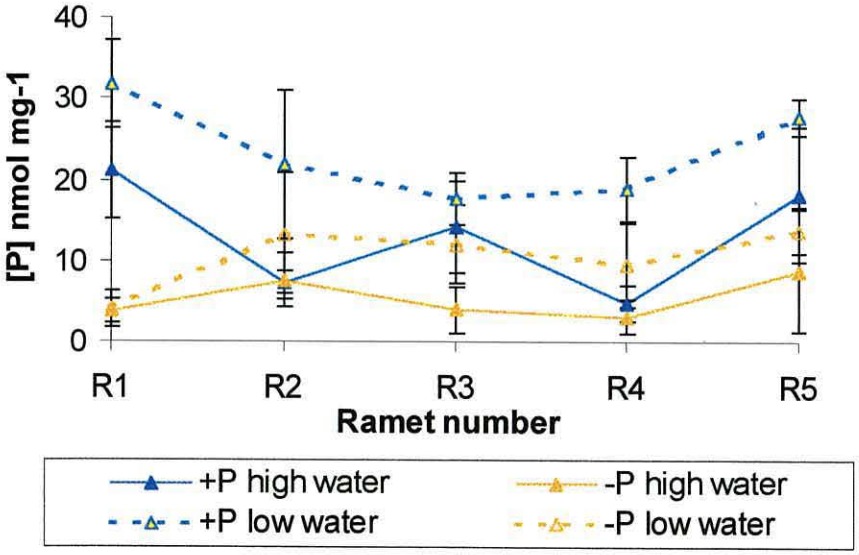


Fig. 4.24 *Mean phosphorus content expressed as nmol* mg^{-1} *dry weight of shoots of rooted ramets along stolons of A. stolonifera. Ramet R1 was given one of two phosphate treatments.* +P = *1.33 mmol* dm^{-3} *phosphate;* -P = *0.133 mmol* dm^{-3}. High water = *50* cm^3 *water;* low water = *15* cm^3 *water. Each ramet along the stolon was given successively less phosphate. Error bars are ± one standard error of the mean. N = 4*

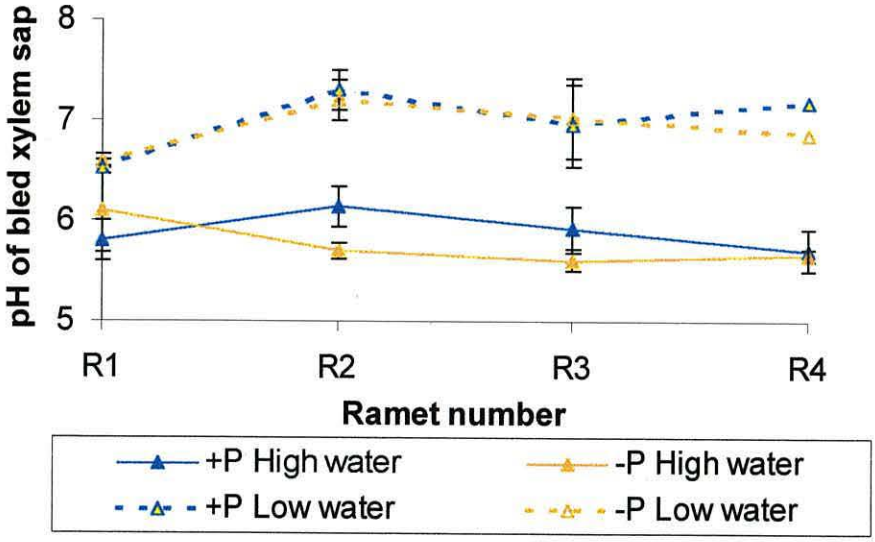


Fig. 4.25 *Mean pH of xylem sap bled from cut shoots of rooted ramets along stolons of A. stolonifera. Ramet R1 was given one of two phosphate treatments.* +P = *1.33 mmol dm^{-3} phosphate;* -P = *0.133 mmol dm^{-3}.* High water = *50 cm^3 water;* low water = *15 cm^3 water. Each ramet along the stolon was given successively less phosphate. Error bars are ± one standard error of the mean. N = 4*

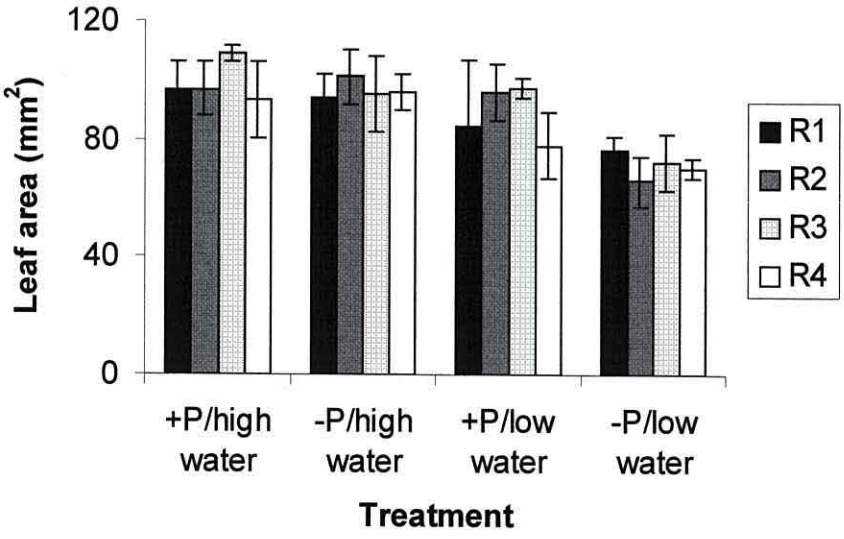


Fig. 4.26 *Area (mm^2) of youngest fully expanded leaf of each ramet on a stolon of A. stolonifera given the treatments shown.* +P = *1.33 mmol dm^{-3} phosphate;* -P = *0.133 mmol dm^{-3}.* High water = *50 cm^3 water;* low water = *15 cm^3 water. Each ramet along the stolon was given successively less phosphate. Error bars are ± one standard error of the mean. N = 4*

DISCUSSION

Is the movement of phosphate through A. stolonifera, rooted in sand, in the direction of the transpiration stream?

Pairs of ramets were grown in sand with one of the pair given either 1.33 mmol dm^{-3} phosphate or 0.133 mmol dm^{-3} phosphate and the sibling ramet given either high water, low water or allowed to dry out.

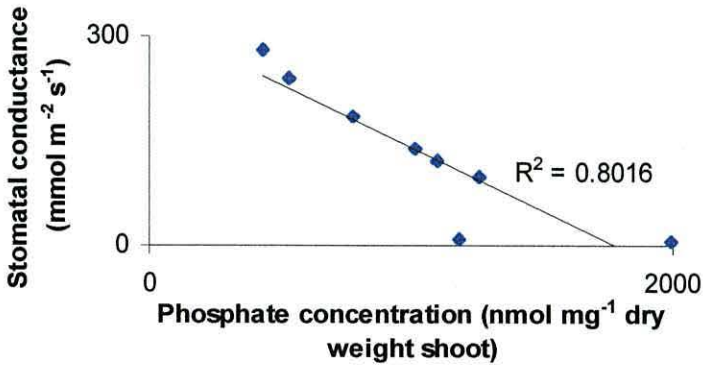


Fig. 4. 28 *The correlation between the phosphate concentration (nmol P mg^{-1} dry weight) of ramets with access to phosphate from a partner, and the stomatal conductance from the upper epidermis of the youngest fully expanded leaf on the ramet main stolon. N = 8; p =<0.05*

It is assumed that the dry weight of a ramet varies with the area of the leaves and hence the rate at which water is lost through the stomata. Since stomatal conductance correlates closely with the phosphate concentration in the leaves of ramets which had no access to phosphate except from an attached partner ramet (Fig. 4.28), it can be concluded that phosphate and water are moving together and thus the hypothesis that the movement of phosphate through *A. stolonifera*, rooted in sand, is in the direction of the transpiration stream is accepted.

Does a water-stressed ramet acquire more water and more phosphorus from an attached partner than would a well-watered ramet?

It was expected that if phosphate and water are moving together this would result in greater growth of water-stressed ramets than well-watered ramets if both relied on a partner to provide phosphate. This hypothesis is not supported by the data. The dry weight, number of main stolon leaves, number of branches and of basal

tillers was the same for ramets given high, low or no water when attached to partners given the same phosphate treatment. Shortage of water reduced the length of main stolons but low phosphate had no effect on the length of the main stolons, probably because the low P (0.133 mmol dm^{-3}) was still adequate for normal growth.

A comparison of the number of main stolon branches produced by R1 ramets again showed there to be the same number of branches for all the treatments provided. Amongst the R2 ramets there were significantly more branches on the main stolons of ramets given high phosphate than on those given low phosphate. Halsted and Lynch (1996) also found that a greater supply of phosphate resulted in more branches and basal tillers in a range of C_3 and C_4 plants and found an increase in the dry weight in plants supplied with phosphate as compared with those given reduced quantities of phosphate. The production of more branches would be an advantage to a clonal plant with a guerrilla habit (Clegg, 1975) allowing the clone to exploit resources by producing more rooted ramets in a patchy soil environment. Overall a close correlation was found between the P concentration in the shoot and the number of main stolon branches for the ramets given the three water and two phosphate treatments.

The production of more basal tillers would also allow foraging growth (Callaghan *et al* 1986; Slade & Hutchings, 1987; Jonsdottir & Callaghan, 1988). Although for all five treatments taken together there was little correlation between the P concentration in the shoot and the number of basal tillers, amongst the R1 ramets, those given high phosphate had a significantly ($p=0.003$) greater number of basal tillers than ramets with other treatments. Amongst the R2 ramets those given high phosphate had very significantly ($p<0.001$) more basal tillers than the ramets given low water or allowed to dry out.

No significant difference was found in the areas of the youngest fully expanded leaves of each ramet. Thus sharing of resources allows ramets given no

phosphorus to grow as well as ramets supplied with phosphorus as found for nitrogen in *Fragaria chiloensis* (Alpert & Mooney, 1986).

The shoots of ramets grown singly and given no water died, whereas their roots remained white, fleshy and apparently alive, presumably because without a shoot through which to lose water by transpiration they did not dry out during the course of the experiment. Single ramets grown with low water were slightly larger than those given high water. The former had longer main stolons, but the same number of main stolon leaves and hence slightly longer internodes. They also had a slightly greater dry weight. The explanation could be that before they were used to make single cuttings, the ramets had been supplied with nutrients by the parent ramets and that the residual nutrients were more diluted in the high water cuttings than in the low water.

Where ramets were deprived of water but attached to a partner their shoots survived. For ramets grown in pairs the whole ramets and their shoots given high, low or no water had the same dry weights but the dry weight of roots of ramets given high water was greater than that of those given low water and both had heavier roots than those given no water. Marshall & Anderson-Taylor (1992) found that an unrooted ramet on a complete stolon had a greater dry weight than a ramet rooted in sand presumably because a water potential gradient provided it with water and mineral nutrients. The water potential gradients set up in the pairs of ramets described in experiment 4.1 were presumably insufficient to transport phosphorus in such quantity. The dry weight of ramets given only water was greater if they were attached to a partner given phosphate than when they were grown singly with water. Hence the transfer of phosphorus into them must rely on mass flow of phosphate in a circulation within the vascular system which would be maintained as the ramet grew. Tanner & Beevers (1990) found that in maize (*Zea mays)*, movement of water through the xylem carried minerals and the rate of transport was accelerated by a high rate of transpiration. However they also found that the plants had greater fresh and dry weights when grown in low vapour pressure deficit (VPD) and concluded that transpiration is not

essential for transport of minerals. This observation was challenged by Smith (1991) because, even at high VPD, plants continue to transpire. However, Pederson & Sand-Jensen (1997) concluded that absence of transpiration in submerged *Mentha aquatica* did not restrict the transfer of nutrients from root to shoot. Fick's Law shows that the rate of diffusion is directly proportional to the concentration gradient but that although the rate of diffusion is rapid over short distances it is far too slow to account for the transfer of nutrients such as phosphate through a plant (Taiz & Zeiger, 1991). Mass flow driven by the negative hydrostatic pressure created by transpiration is the predominant mechanism by which water and dissolved nutrients are transferred throughout a plant through the xylem (Taiz & Zeiger, 1991).

Do ramets given high phosphate have a higher rate of stomatal conductance?

The stomatal conductance of the youngest fully expanded leaves was about four times greater on the adaxial epidermis than on the abaxial surface showing that most stomata lay in the upper epidermis. A number of authors have found that stomatal conductance is influenced by the nutrient status of plants. Radin & Eidenbock (1984) found that in cotton seedlings the rate of transpiration was decreased by 26% in plants grown in nutrient solution with low phosphate. The concentration of abscisic acid (ABA) increases in the leaves of plants subjected to drought conditions as a result of drying of the soil. This increase in ABA has been correlated with reduced stomatal conductance which would conserve water (Bates & Hall, 1981; Kramer, 1988; Davies & Zhang, 1991; McDonald & Davies, 1996). Radin (1990) showed that in cotton seedlings both N- and P-stressed plants had stomatal conductances similar to those of fully-nourished seedlings but a reduction in temperature reduced stomatal conductance in nutrient-stressed plants. Radin concluded that if ABA is a messenger from the roots then it is effective only in nutrient-stressed plants. A reduction in stomatal conductance in P-deficient mulberry (*Morus alba)* was reported by Sharma (1995) and in P-deficient wheat (*Triticum aestivum)* by Carvajal *et al.* (1996). Chapin (1990) considered that nutrient deficiency might bring about synthesis of

ABA. Tomos (1990) pointed out the effect of ABA of reducing the turgor pressure of stomatal guard cells. However, Munns & King (1988) found that stomata closed in the absence of ABA and that ABA, applied to unstressed wheat plants in the concentration in which it occurs in stressed plants, did not cause stomata to close.

In the *A. stolonifera* ramets studied, the stomatal conductance was slightly (but statistically non-significantly) greater in the single ramets given low phosphate. There was a significant reduction in the stomatal conductance of both partners in pairs of ramets where one partner was water-stressed and the other was given high phosphate. The effect was greater where the older ramet, R1, was water-stressed and the younger ramet, R2 had high phosphate. Reduction in stomatal conductance did not occur where there was water-stress and low phosphate, or in high phosphate ramets with no water-stress. This effect has been recorded in barley under drought conditions (Hak & Natr, 1984) in Douglas fir seedlings (Dosskey *et al.,* 1993) and in *Trifolium repens* (Dhananjay *et al.,* 2000). Reduced stomatal conductance could be attributed to the production, by the water-stressed ramet of ABA, and its transport to the partner. If this was the case it would appear that more ABA was transported acropetally than basipetally. The closure of stomata in *A. stolonifera* may be controlled by ABA and high phosphate together. This does not agree with the report by Radin (1990) that if ABA is a messenger from the roots then it is effective only in nutrient-stressed plants but Radin was working with cotton and there may be differences in the response in different species.

ABA is said to be synthesised if plants are wounded (Pena-Cortes *et al.,* 1990: Sanchez-Seranno *et al.,* 1991). If small amounts were made in response to cutting the main stolon to produce individual ramets, it could account for the observation that high phosphate ramets did not have the expected higher stomatal conductance than the low-P individuals although the available phosphate was distributed between the two ramets so neither had significantly more than the other. The same would be true for clonal fragments consisting of pairs of

ramets. If ABA were synthesised where the main stolons were cut and if it interacts with high phosphate then these pairs would have a lower stomatal conductance than low phosphate pairs. Such a difference can be seen in Fig. 4.10. Fig. 4.18 shows that where the stolon was not cut, the high phosphate unrooted ramets had a higher stomatal conductance than the low phosphate unrooted ramets although this difference was not evident in connected rooted ramets (Fig. 4.17). Where ramets were watered at intervals of less than 48 hours it was seen that stomatal conductance increased to a maximum several hours after watering, then decreased, possibly as all the plants suffered water-stress. By 48 h after watering the reduction in stomatal conductance was seen to occur in pairs of ramets given high phosphate and attached to a water-stressed ramet. Stomatal conductance was also reduced in the single ramet given high phosphate and may indicate that water-stress had occurred during this interval between watering and measurement of stomatal conductance. However Davies & Zhang (1991) stated that stomatal closure occurs before ABA production commences and stomatal closure is brought about by redistribution of ABA. The same authors considered the lag phase for the production of ABA to be 30–60 minutes. If it is conjectured that in *A. stolonifera,* to bring about the closure of stomata seen in high phosphate/low water ramets, an interaction between high phosphate and ABA must occur, it would be expected that the R1 ramets given high phosphate would have a greater concentration of phosphorus in the apices of the main stolon than occurred in the tips of low-phosphate ramets as illustrated by Fig. 4.14. This was not the case – the apices of the R1 ramets, both those given high phosphate and those given low phosphate, had the same concentration of phosphorus. The inference is, therefore, that high phosphate in the roots might promote production of a messenger substance in the roots, perhaps ABA, or a messenger substance which interacts with ABA in droughted plants. Alternatively, phosphate, circulating in the xylem and phloem, is itself the messenger substance. Fig 4.15 shows the greater amount of phosphorus in the roots of the high phosphate ramet attached to the water-stressed partner than in the high phosphate ramet attached to the well-watered R2 ramet. This difference is significant ($p=0.002$). The main stolon apex of the water-stressed R2 ramet, on

which stomatal conductance was measured, had no more phosphorus than any other of the R2 ramets, so where the interaction between phosphorus and water stress occurs to bring about stomatal closure, is unclear.

Do ramets given high P and low water reduce their rate of stomatal conductance?

In experiment 4.2 three methods were used to attempt to establish a gradient of water and phosphorus along a stolon. For Method 1 provision of water and phosphorus to the first ramet, R1 on a stolon did not result in a gradient of stomatal conductance in successively younger ramets towards the apex of the stolon so no analyses were made of water-content or phosphorus concentration. These resources were probably distributed along the length of the stolon as found in *A. stolonifera* by Marshall & Anderson-Taylor (1992).

The second method, in which daughter ramets were given successively less water, resulted in a reduction in stomatal conductance along the stolon of unrooted ramets but not in the stolon of rooted ramets. There was no difference in the water content, expressed as a percentage of the dry weight, between ramets given high or low phosphate or between rooted or unrooted ramets. Alpert & Mooney (1986) found that flow of water from a parent ramet supplied unrooted daughter ramets along long stolons in *Fragaria chiloensis*. In rooted ramets of *A. stolonifera* the water content and phosphorus concentration increased slightly towards the apices of the stolons. Stomatal conductance varied neither with water content nor with phosphorus concentration.

The third method, in which daughter ramets were given successively less phosphate, produced a decrease in the stomatal conductances of ramets along well-watered stolons, with decrease occurring more rapidly in high phosphate ramets than in low phosphate. As observed in pairs of ramets, the stolon given high phosphate and low water had the lowest stomatal conductance in ramets R1 and R2. Stomatal conductances remained the same for the ramets along the water-stressed stolons. This decrease in stomatal conductance was not related to

the water content of the stolons where, apart from the first ramet, R1, of the low-phosphate, low-water stolon there was no difference in the water content as shown in Fig. 4.23. There is a body of evidence to suggest that signals from roots in dry soil can reduce stomatal conductance without a change in the water potential of leaf cells (Bates & Hall, 1981; Davies & Zhang, 1991; Bunce, 1999).

The pH of xylem sap collected by bleeding from the cut stumps of the main stolon and basal tillers showed that the water-stressed ramets had a higher pH than the well-watered ramets. The pH of xylem sap of ramets given high phosphate and high water remained close to 6, the pH of the high phosphate Long Ashton nutrient solution provided, whereas the pH of the high phosphate, low water ramets rose to approximately 7. Conversely, the pH of low phosphate, well-watered ramets fell from 7 to nearer 6 and the low phosphate, low water ramets remained at a pH of about 7. The pH was not related to the pH of the nutrient solution provided, nor to the phosphorus concentration, nor to the percentage water within the tissues but to the water treatment given to the roots. Such alkalinity can be induced by the drying of the soil (Schurr & Gollen, 1990). Hartung & Davies (1991) suggested that development of an alkaline pH in the apoplast of droughted plants would draw ABA from the cytosol (since cytoplasmic pH values remain unaffected by drought stress) and allow it to be transported into the guard cells where it would bring about stomatal closure. Thus the first signal for stomatal closure would be a rise in pH. ABA would be redistributed and only if drought stress were prolonged would biosynthesis of ABA occur. Bacon *et al.* (1998) suggested that an increase in xylem sap pH in barley (*Hordeum vulgare*) acted as a drought signal reducing the rate of elongation of leaves, but there was no such reduction in the rate of elongation of the leaves of *A. stolonifera.* It has been suggested that high pH might be buffered by phosphorus in the tissues and prevent the action of ABA (Davies, pers. com.), the antithesis of what appears to be happening in *A. stolonifera.*

CONCLUSIONS

1. Phosphorus is carried in the xylem in the direction of the transpiration stream.
2. A reduction of phosphate from 1.33 mmol dm^{-3} to 0.133 mmol dm^{-3} in the supply to *A. stolonifera* rooted in sand does not affect the length of the main stolon, the number of main stolon leaves but does reduce the number of main stolon branches and the number of basal tillers produced.
3. The rate of transpiration was greater from the adaxial than from the abaxial surface of the leaves indicating that *A. stolonifera* bears more stomata on the adaxial than on the abaxial surface of its leaves.
4. If ramets are subjected to water stress those with access to high phosphate close their stomata; both partners are affected even if only one is water-stressed. This reduction in stomatal conductance is more closely related to the phosphorus concentrations in the tissues of the root than that in the apex of the shoot where stomatal conductance was measured.
5. Reduction in stomatal conductance in response to drought does not reduce the final dry weight in plants with access to high concentrations of phosphate.
6. If water and phosphate are supplied to the oldest ramet on a stolon these resources are distributed to younger ramets.
7. Even if water to the roots of ramets is restricted, the water content, expressed as percentage of the dry weight, is not significantly different from that of well-watered ramets.
8. pH values in the xylem sap rise in response to dry conditions experienced by the roots.

Chapter 5

How does internal phosphorus status alter the uptake and transport of ^{32}P through paired ramets and whole stolons of *Agrostis stolonifera*?

INTRODUCTION

The experiments described in Chapter 2 provide indirect evidence for the transfer of phosphorus from the roots of a ramet with adequate external supplies of phosphate to either a younger or to an older sibling ramet lacking adequate supplies of phosphate.

In non-clonal plants phosphorus is transported from the roots to the shoots through the xylem (Milthorpe & Moorby, 1969; Marschner, 1985; Jeschke *et al.,* 1997; Cui & Caldwell 1998; Schachtman *et al.,* 1998). This is the case also for clonal plants where the xylem sap is translocated towards the apex of the main stolon but is supplemented by water and inorganic nutrients taken up by the nodal roots of ramets along the main stolon between the roots of the parent and the tip of its main stolon. After arrival in the leaves some phosphorus is recirculated in the phloem – a conclusion first drawn by Hartig (1861). That a circulation of inorganic ions, including phosphate, occurs in non-clonal plants has been reported by later authors (Biddulph *et al.*, 1958; Bieleski & Ferguson, 1983; Ahmad *et al.,* 1984; Mimura, 1995) and the movement of phosphate through the phloem confirmed by Mimura *et al.* (1996), Jeschke *et al.* (1997) and Schachtman *et al.* (1998).

Milthorpe & Moorby (1969), reviewing the transport of Pi through plants, stated that entry into the vacuoles of root cells of tomato appears to be a rapid process and subsequent loss from the vacuoles is slow. Of the phosphorus within the root approximately 90-95% is in the vacuoles and 5-10% is in the cytoplasm. The "metabolic" cytoplasmic fraction consists of phosphorus in the cytosol and in the cytoplasmic organelles - in the case of mesophyll cells of leaves, particularly in the chloroplasts. The vacuolar pool acts as a storage pool

maintaining the concentration in the cytoplasm. Confirmation of this distribution of phosphorus is reported by Lauer *et al.* (1989), Lee & Ratcliffe (1993), Theodorou & Plaxton (1993) and Leigh (1997). Ahmad *et al.* (1984) showed that in barley leaves 86% of the phosphorus is in the vacuoles and of the 14% in the cytoplasm, 6% is in the chloroplasts, the P-translocator in the chloroplast envelope allowing rapid exchange of phosphorus between the cytosol and chloroplast. Foyer & Spencer (1986) used ^{31}P-Nuclear Magnetic Resonance (NMR) to study the partitioning of Pi in barley leaves and found that the vacuolar concentration changes greatly in response to reduction in the concentration of phosphate in the external medium. In contrast the concentration of phosphorus in the cytoplasm is little affected, ensuring a constant supply of phosphorus to the chloroplasts and permitting rapid photosynthesis to continue. Only when the plants are grown for extended periods in a growth medium devoid of phosphate are reductions observed in the concentration of phosphorus in the cytoplasm, a conclusion confirmed by Lee & Ratcliffe (1993). Lauer *et al.* (1989), also using ^{31}P-NMR to investigate partitioning of phosphorus between the cytoplasm and vacuoles of living cells of soybean, came to the same conclusions as Foyer & Spencer (1986) and suggested that the vacuolar pool of phosphorus is only filled after the demands for metabolic and cytoplasmic phosphorus have been met, but that the vacuolar pool would be mobilised before the phosphorus in the cytoplasm. Mimura (1995) showed that the concentration of phosphorus in the cytoplasm remains constant only while external conditions remained more-or-less unchanged; hence cells of *Acer,* grown in suspension, accumulate Pi in the vacuole and increase the cytoplasmic concentration of Pi when starved of sucrose, as organic phosphate molecules are broken down to release inorganic phosphate. In *Chara* 50% of the inorganic phosphate taken up in darkness is esterified to organic phosphate in the light (Mimura, 1995). However these changes are small when compared with the changes in the concentration of phosphorus in the vacuoles. Ahmad *et al.* (1984) traced the flux of ^{32}P in both rust-infected and uninfected leaves of barley in order to determine the rate of uptake of phosphorus into cell compartments, as well as the sizes of the cytoplasmic and vacuolar pools of phosphorus. They concluded that the P

concentrations in the cytoplasm were more easily regulated by exchange across the plasmalemma with the apoplast than by exchange with the vacuole.

Plants grown in low phosphate continue to produce new leaves as phosphorus from older leaves is remobilised and translocated to the developing leaves (Bieleski & Ferguson, 1983; Mimura *et al.*,1996). Mimura (1995) used "imaging plates" (Fuji Film, Japan) which are more sensitive than X-ray film, to follow the redistribution of phosphorus from older to younger leaves and found that ^{32}P moves from the first (oldest) leaf to the second and to the third, redistribution which he believed to occur through the phloem. The same author also found that the concentration of phosphorus in the apoplast remains constant, suggesting that a homeostatic mechanism must be at work, and postulated that a phosphate regulon, analogous to that known to exist in bacteria and yeasts, might exist in higher plants. Mimura (1995) concluded that the tonoplast normally has a low permeability to phosphate but becomes more permeable in conditions of phosphate-deprivation and suggested that cytoplasmic Pi may be over-estimated as a result of leakage and contamination from organelles such as the vacuole.

A faster rate of uptake of ^{32}P, per g dry weight of root per unit time, was observed in P-stressed tomato and barley than in P-sufficient plants by Clarkson & Scattergood (1982) and in potato by Cogliatti & Clarkson (1983). Drew *et al.* (1984) showed more rapid depletion of phosphate from nutrient solution by P-stressed barley, and Anghioni & Barber (1980) found an increased rate of depletion of nutrient solution by *Zea mays* (per cm length of root) given an initial five days in high phosphate followed by up to six days without phosphate. A number of authors have reported a higher rate of transport of phosphorus through P-deprived compared with P-supplied plants (Raghotama 1999) e.g. in tomato and barley (Clarkson & Scattergood, 1982) whereas Jeschke *et al.* (1997) found that in P-deficient castor bean there is "drastic curtailment" of both uptake and translocation of phosphorus through the xylem, probably because of reduction in the rate of uptake and transport of water through the xylem.

Several studies of movement of phosphorus through clonal plants have been made using radioisotopes of phosphorus (Hoshino, 1974; Anderson-Taylor, 1982; Noble & Marshall, 1983; Chapman & Hay, 1993; Kemball & Marshall, 1994). Unidirectional movement of ^{32}P towards the apex in *Trifolium repens* was reported by Hoshino (1974) and of ^{33}P in *Carex arenaria* by Noble & Marshall (1983). For clonal plants growing in a patchy environment, especially those with a "guerrilla" growth form (Lovett Doust, 1981), transfer of resources may be acropetal or basipetal towards a sibling ramet under stress (Pitelka & Ashmun, 1985). However, Noble & Marshall (1983) found only limited basipetal transport of ^{33}P in *C. arenaria,* which has such a growth form, and nutrient deficiency was not relieved by basipetal transport of nutrients. Anderson-Taylor (1982) showed that phosphorus taken up by the roots of a ramet of *A. stolonifera* may pass acropetally to the next younger ramet but that most is retained in the shoot of the fed ramet. Younger ramets along a stolon tend to retain the ^{32}P in their leaves but where it is exported most passes to the apex of the stolon and only a trace moves basipetally. Young, unbranched roots of *T. repens* export phosphorus to the main stolon whereas older nodal roots direct more to the branch arising from the same node (Chapman & Hay, 1993). The same authors found that the pattern of distribution differs between two genotypes within the same species. Lötscher and Hay (1996) also investigated the translocation of ^{32}P in two genotypes of *Trifolium* and found that where ramets are widely spaced on a stolon, with unrooted nodes between them, there is a difference in the ability of the two genotypes to transport ^{32}P from the side of the fed root to branches on the opposite side. Kemball & Marshall (1995) supplied ^{32}P to the roots of ramets at various points along the main stolon of *T. repens.* They found only traces of ^{32}P proximal to the fed root whereas ^{32}P applied to the roots of a ramet halfway along a secondary branch stolon appears in the primary branch both proximal and distal to the secondary branch. Labelled phosphorus from the same fed root also appears in the main stolon from which the branch arose, but within the main stolon most movement is acropetal to the branch and only a trace basipetal.

Pi is transported from the roots in the xylem (Marschner, 1995; Schachtman *et al.*, 1998) hence it is assumed that acropetal transport of Pi in *A. stolonifera* is in the xylem. Recirculation of P from leaves is in the phloem (Ahmad *et al.*, 1984; Hay & Sackville Hamilton, 1996; Jeschke *et al.*, 1997; Kockenberger *et al.*, 1997; Schachtman *et al.*, 1998) hence basipetal transport in *A. stolonifera* is assumed to be in the phloem. Phosphate transfers from xylem to phloem (Headley *et al.*, 1988, Marschner, 1995; Hay & Sackville Hamilton, 1996). Milthorpe and Moorby (1969), reviewing experiments with barley, castor bean and sugar cane, suggested that phloem carries as much P as xylem whereas Jeschke *et al.* (1997) believed that in castor bean, translocation of phosphate in the phloem is 50% of the flow found in the xylem and Marschner (1995) reported six times more P (as Pi) in the xylem as Po in the phloem. Thus bidirectional movement of phosphate is possible in the same plant organ (Quereshi and Spanner, 1971; Marshall, 1990).

This chapter tests the hypotheses that

- phosphate supplied to the roots of one of a pair of connected ramets of *A. stolonifera,* grown in heterogeneous or homogeneous phosphate environments is transferred from ramets given high to those given low phosphate
- phosphorus is transported from the roots to the shoot in the xylem
- phosphorus transported from the roots to the youngest fully expanded leaf on the main stolon of the unfed ramet, circulates out of the leaf
- phosphorus recirculated from a leaf is transported in the phloem
- the phosphorus status of a ramet alters the allocation between the cell vacuole and the cytoplasm, of phosphorus arriving in a leaf
- pre-treatment with high or low phosphate affects the speed at which ^{32}P is transported through the main stolon of a plant
- the internal P status alters the rate of uptake of phosphate into the roots and the flux of P into connected, paired ramets of *A. stolonifera*

MATERIALS AND METHODS

Agrostis stolonifera plants, propagated from the clone maintained at Pen y Ffridd field station, University of Wales, Bangor, were grown in John Innes No 1 potting compost in a glasshouse until they had produced a main stolon which was cut off to encourage the production of tillers. The third and fourth nodes of the basal tillers were gently scarified to encourage rooting, the tillers laid on wet paper and the nodes covered with strips of paper which were kept damp until roots and shoots about 5 cm long had developed at both nodes. The paired ramets, still attached by the original internode, were then cut from the basal tiller, and grown hydroponically as described in chapter 2 and summarised in Table 5.1.

Table 5.1 *Phosphate treatments given to paired ramets of A. stolonifera grown hydroponically.* +P = *Long Ashton nutrient solution with 1.33 mmol* dm^{-3} *phosphate;* -P = *Long Ashton nutrient solution with 0.007 mmol* dm^{-3} *phosphate.*

Treatment of pair	Phosphate treatment given to R1 the older ramet	Phosphate treatment given to R2 the younger ramet
1. (+P -P)	+P	-P
2. (-P +P)	-P	+P
3. (+P +P)	+P	+P
4. (-P -P)	-P	-P

Four replicates of each of the treatments shown in Table 5.1. were used and the paired ramets were grown for 10 days in a Conviron E15 controlled environment cabinet with a photosynthetic photon flux density (PPFD) (measured using a Skye Quantum sensor, Model SKP 200) of 500 μmol m^{-2} s^{-1} at plant height, supplied by three 250 watt HQI/NDL lamps. A photoperiod of 16 h light/8 h dark was given with a temperature of 20 °C in the light period and 16 °C in the dark and a vapour pressure deficit (VPD) of 0.83 kPa at 20 °C and 0.64 kPa at 16 °C. After 10 days growth each shoot consisted of a main stolon approximately 30 cm long with up to five basal tillers.

The pairs of ramets were transferred to a light bank providing a PPFD of 120 μmol m^{-2} s^{-1}. The roots of each of the two ramets were inserted into test tubes containing 15 cm^3 of the same Long Ashton nutrient solution as that in which they had been grown. Aluminium shielding 5 mm thick was placed between the test tubes containing the roots of the R1 and R2 ramets.

In Experiments 5.1 to 5.4 the movement of ^{32}P was monitored by means of a Geiger-Müller tube (GM Centronic ZP 1430). In each experiment 370 kBq ^{32}P as carrier-free orthophosphate in dilute hydrochloric acid was fed to the roots of one of the pair. The dilute hydrochloric acid ensured that the orthophosphate remained in solution. The amount of phosphate added was in the order of picogrammes so there was no significant effect on the concentration of phosphate supplied to the fed root. In each of Experiments 5.5 to 5.7, after feeding with ^{32}P, the plants were dried and weighed. The radioactivity of weighed subsamples was then counted by liquid scintillation in water using Cerenkov radiation, with correction for half-life and counting efficiency in a LKB-Wallac 1215 Rackbeta II liquid scintillation counter.

Experiment 5.1 To test the hypothesis that phosphate supplied to the roots of one of a pair of connected ramets of *A. stolonifera*, grown in heterogeneous or homogeneous phosphate environments is transferred from ramets given high to those given low phosphate

Paired ramets were grown as described above. The roots of the ramet to be fed radiophosphate were immersed in 15 cm^3 Long Ashton nutrient solution to which was added 370 kBq ^{32}P. A GM tube was attached to the youngest fully expanded leaf of the shoot to be monitored and connected to a rate meter (Scaler Ratemeter 7R7 Type 5251, Nuclear Enterprises, Ltd.) from which a trace was produced on a chart recorder during three to four hours. The measurements summarised in Table 5.2 were made on different pairs of ramets. Three or more replicates of each test were carried out. A representative set of results is shown for each pair of ramets tested.

Table 5.2 *Table of treatments of paired ramets connected by the parent internode, with the roots of one ramet fed ^{32}P. In each case the fed root was given 15 cm^3 of the Long Ashton nutrient solution in which it had been grown containing 370 kBq ^{32}P. The youngest fully expanded leaf on the main stolon was monitored by means of a Geiger-Müller tube.* R1 = *older ramet;* R2 = *younger ramet.* +P = *1.33 mmol dm^{-3} phosphate;* -P = *0.007 mmol dm^{-3} phosphate.*

Ramet fed ^{32}P		Ramet monitored	
R1	+P	R2	-P
R1	-P	R2	+P
R1	+P	R2	+P
R1	-P	R2	-P
R2	-P	R1	+P
R2	+P	R1	-P
R2	+P	R1	+P
R2	-P	R1	-P

Experiment 5.2 To test the hypothesis that phosphorus is transported from the roots to the shoot in the xylem

If a shoot is placed in darkness the stomata close and transpiration is reduced. The reduction in transpiration reduces the rate at which materials are transported through the xylem. If radiophosphorus is transported through the xylem the reduction in transpiration will cause a trace on a chart-recorder, of the amount of radioactivity in a leaf, to level off but to continue to rise again once light is restored to the leaf. Paired ramets were arranged as described for Experiment 5.1. The roots of the fed ramet were provided with 370 kBq ^{32}P and a Geiger-Müller tube attached to the youngest fully expanded leaf of the main stolon of the monitored ramet. When a trace was produced showing that radiophosphorus had arrived in the leaf the whole of the shoot bearing the monitored leaf was covered in aluminium foil so light was excluded. The foil cover was removed and replaced at intervals and monitoring continued for up to four hours.

Experiment 5.3 To test the hypothesis that phosphorus transported from the roots to the youngest fully expanded leaf on the main stolon of the unfed ramet, circulates out of the leaf

Paired ramets were arranged as described for Experiment 5.1. Approximately 20% of the fibrous root system was separated from the rest of that of an R1 ramet but remained attached to the plant. These separated roots were provided with 370 kBq ^{32}P. The remaining roots of the R1 ramet and the roots of the R2 ramet were inserted in test tubes of 15 cm^3 of the Long Ashton nutrient solution in which they had been grown. A Geiger-Müller tube was attached to the youngest fully expanded leaf of the R2 ramet. After one hour the fed roots of the R1 ramet were removed from the plant and the chart recorder traced the movement of the phosphorus in the youngest fully expanded leaf of the R2 ramet for the next 24 hours.

Experiment 5.4 To test the hypothesis that phosphorus recirculated from a leaf is transported in the phloem

If a shoot is cooled, the transport through the phloem slows down or ceases because the viscosity of the phloem contents increases as the temperature drops. This will cause a trace on a chart-recorder, of the radioactivity in the part of a plant being monitored, to level off but to start to rise again as the build-up of material above the sieve plates forces material through into the next sieve tube element (Farrar, 1988). Paired ramets were arranged as described for Experiment 5.1. An area, approximately 1 cm long, across the width of the adaxial surface of the youngest fully expanded leaf on the main stolon of the younger ramet (R2), which had been grown in low phosphate, was abraded using Carborundum© paste. The abraded leaf was supported on a microscope cavity-slide and a 0.144 cm^3 droplet of orthophosphate containing 370 kBq ^{32}P placed on the abraded surface. A GM tube was attached to the base of the same main stolon. The fed and monitored ramet was attached to an older ramet, R1, which had been grown with high phosphate. When a trace was produced, showing that radiophosphorus had been transferred from the abraded leaf to the base of the main stolon, the stolon was cooled rapidly between the fed leaf and the monitor.

Cooling was achieved by attaching paired brass plates cooled by a flow of water at 5 °C through 0.5 mm diameter copper tubing welded to them. Each plate measured 8 x 1.5 cm and they were placed across the main stolon. This arrangement cooled a section of stolon 1.5 cm long for 90 minutes.

Experiment 5.5 To test the hypothesis that the phosphorus status of a ramet alters the allocation between the cell vacuole and the cytoplasm, of phosphorus arriving in a leaf

Paired ramets were grown for four weeks with homogeneous or heterogeneous phosphate environments to the roots as summarised in Table 5.1. Sixteen pairs of connected ramets with similar size and development were set up with their roots in test tubes of the Long Ashton nutrient solution in which they had been grown to give four replicates of each of treatments numbered 1-4 in Table 5.1. The roots of each of the R1 ramets were fed 370 kBq ^{32}P as carrier-free orthophosphate in dilute hydrochloric acid and held in the Long Ashton nutrient solution containing ^{32}P for six days during which time the test tubes were topped up with nutrient solution. The youngest and the oldest fully expanded leaves on the main stolon of the fed ramet and of the attached sibling ramet were removed. These leaves were cut into 5 mm lengths giving approximately square leaf-segments which were skewered on fine needles. The segments were immersed in 20 cm^3 deionised water in a scintillation vial and left for 1 minute at 20 °C to allow efflux of ^{32}P from the leaf segments into the water. The leaf segments were then transferred to a second vial and left in water for 2 minutes. The process was repeated so the leaf segments were submerged in water for 1, 2, 4, 10, 20, 40, 60, 120, 240 and 480 minutes. The leaves were finally transferred to 20 cm^3 water at 80 °C for 5 minutes. The eluted leaf segments were added to 20 cm^3 deionised water in scintillation vials. In order to measure the efflux of ^{32}P from the roots into the surrounding water 20 cm^3 of the water surrounding the roots of R2, the unfed ramets, was also removed. The ^{32}P in the water in each vial was counted by Cerenkov radiation.

Experiment 5.6 To test the hypothesis that pre-treatment with high or low phosphate affects the speed at which ^{32}P is transported through the main stolon of a plant

Eight individual ramets of *A. stolonifera* were grown in a controlled environment cabinet for 14 days until each had grown a main stolon with 8 – 10 nodes. The roots of four were given full Long Ashton nutrient solution with 1.33 mmol dm^{-3} phosphate, the remaining four had Long Ashton nutrient solution with 0.007 mmol dm^{-3} phosphate. The plants were transferred to fresh nutrient solution under a light bank providing a PPFD of 120 μmol m^{-2} s^{-1} and the roots of each given 370 kBq ^{32}P as carrier-free orthophosphate in dilute hydrochloric acid. The roots were fed for 120 minutes then removed from the nutrient solutions containing ^{32}P. The main stolons of two of the plants given high and two of the plants given low phosphate were cut at each node and the internodes placed in 20 cm^3 deionised water for counting by Cerenkov radiation. The second two in each set had their roots placed in fresh, Long Ashton nutrient solution with the same concentration of phosphate as that in which they had been grown. The ^{32}P was "chased" for 30 minutes after which the stolons of these plants were also cut at the nodes. The activity in the internodes was counted by Cerenkov radiation.

Experiment 5.7 To test the hypothesis that the internal P status alters the rate of uptake of phosphate into the roots and the flux of P into connected, paired ramets of *A. stolonifera*

Paired ramets were grown for one week with homogeneous or heterogeneous phosphate environments to the roots as summarised in Table 5.1. Sixteen pairs of connected ramets with similar size and development were set up with their roots in test tubes of the Long Ashton nutrient solution in which they had been grown to give four replicates of each of treatments numbered 1-4 in Table 5.1. The roots of each of the older (R1) ramets were fed 370 kBq ^{32}P for 5 hours and 30 minutes. The pairs were cut into individual ramets and the root and shoot of each separated. The plant organs were dried and weighed, and weighed subsamples placed in vials containing 20 cm^3 deionised water for counting by Cerenkov radiation. The experiment was repeated with the roots of the younger ramet (R2) fed ^{32}P. Weighed subsamples were analysed for their phosphorus

RESULTS

Experiment 5.1 tested the hypothesis that phosphate, supplied to the roots of one of a pair of connected ramets of *A. stolonifera,* grown in heterogeneous or homogeneous phosphate environments to the roots, is transferred from ramets given high to those given low phosphate. Radioactivity appeared in the youngest fully expanded leaf on the main stolon of a ramet attached to a partner whose roots were fed ^{32}P, within 1 – 4 hours from feeding. Fig. 5.1.1 indicates that phosphate was transferred from a ramet grown in low phosphate (0.007 mmol dm^{-3}) to one grown in high phosphate (1.33 mmol dm^{-3}) (Fig. 5.1.1d), but not *vice versa* (Fig. 5.1.1c). Fig. 5.1.1c shows the chart recorder trace for a period of 192 minutes during which time the roots of an older R1 ramet were fed ^{32}P and the younger R2 ramet monitored; the trace was allowed to run for 24 hours and no radiation was detected in the monitored leaf in that time. This is probably due to the insensitivity of the GM tubes as when the experiment was repeated, the youngest fully expanded leaves, both those monitored and those on the main stolon of the fed ramet, were counted by Cerenkov radiation and there was found to be a small quantity of ^{32}P present in the monitored leaves of the R2 low phosphate ramets attached to a R1 high phosphate partner (Table 5.3). There was rapid transfer of ^{32}P from a low phosphate ramet to a high phosphate sibling (Fig. 5.1.1d) and also between ramets given homogeneous high or low phosphate (Fig 5.1.1a and b).

Table 5.3 *^{32}P expressed as mean counts $minute^{-1}$ mg^{-1} dry weight (± 1 standard error of the mean) in the youngest fully expanded leaf on the main stolon of each of a pair of connected ramets of A. stolonifera. The roots of the older ramet were fed 370 kBq ^{32}P for 330 minutes before the youngest fully expanded leaves were removed for counting by Cerenkov radiation. n=3.*

	R1	R2	R1	R2	R1	R2	R1	R2
	+P	-P	-P	+P	+P	+P	-P	-P
cpm mg^{-1}	791 ± 175	142 ± 84	11299 ± 3849	4688 ± 4212	464 ± 56	494 ± 248	7761 ± 3923	2417 ± 2088

A similar pattern of basipetal distribution of ^{32}P occurred when the roots of the younger, R2 ramet were fed and the youngest fully expanded leaf on the main

stolon of the R1 ramet monitored. Again no transfer of ^{32}P was recorded into a low phosphate ramet from a high phosphate partner as shown in Fig 5.1.2c but Cerenkov radiation showed the presence of a small amount of ^{32}P (65 counts minute^{-1}). The partners of all other R2 ramets showed the transport of ^{32}P in their youngest fully expanded leaf as shown in Fig 5.1.2a, b and d.

Experiment 5.2 tested the hypothesis that P is carried from the roots to the shoot in the xylem. The transport of ^{32}P from the roots of an older ramet (R1) into the youngest fully expanded leaf of an attached younger ramet (R2) was shown to take place in the xylem, in pairs in which the R2 ramet had previously been supplied with Long Ashton nutrient solution containing1.33 mmol dm^{-3} phosphate (Fig 5.2a and b). Transport in the xylem also occurred from a younger (R2) ramet into an older ramet (R1) grown in high phosphate (Fig. 5.2c). In each of these pairs, when the shoot was darkened and the rate of transpiration slowed, this was accompanied by a flattening of the trace as transport of ^{32}P into the monitored leaf slowed down or stopped. Again the rate of transfer of ^{32}P was greatest from a low-phosphate, R1 ramet into a high phosphate, R2 ramet (Fig 5.2a).

Experiment 5.3 tested the hypothesis that P transferred from the roots to the youngest fully expanded leaf on the main stolon of the unfed ramet circulates out of the leaf. When ^{32}P was fed to part of the roots system of an R1 ramet, the trace produced by monitoring the youngest fully-expanded leaf on the main stolon of the R2 ramet showed that ^{32}P was entering the monitored leaf and it continued to do so for 4 hours after the fed roots were removed (Fig 5.3). After that time the trace dropped indicating that ^{32}P was being recirculated from the monitored leaf.

Experiment 5.4 tested the hypothesis that phosphorus recirculated out of a leaf is transported in the phloem. Fig. 5.4 shows the trace produced of the radioactivity at the base of the main stolon of a low-phosphate R2 ramet when its youngest fully expanded leaf was abraded and fed 370 kBq ^{32}P. A cold block at 5 °C was

applied between the fed leaf and the monitor the trace showed a levelling in the amount of activity, followed by an increase, showing that the ^{32}P was transported from the fed leaf in the phloem.

Experiment 5.5 tested the hypothesis that the phosphorus status of a ramet alters the allocation of phosphorus arriving in a leaf between the cell vacuole and the cytoplasm. Table 5.4 shows the calculation of $\log_{10}$ cumulative counts minute^{-1} remaining in the leaf segments after each time interval in water into which the ^{32}P was eluted. The $\log_{10}$ values were plotted as shown in Fig. 5.5.1, the values for the R1, fed ramet in the pair given **+P** -P treatment. These ramets had previously been grown with the older, R1, ramet given 1.33 mmol dm^{-3} phosphate and the younger, R2, ramet given 0.007 mmol dm^{-3} phosphate. The trendline was fitted to last three points of Fig 5.5.1. The intercept of the trendline with the y axis gives the amount of ^{32}P washed from the cytoplasm – that above the intercept, whilst that below the intercept gives the ^{32}P eluted from the vacuole (Farrar & Farrar, 1985). Table 5.4 shows the ^{32}P diffusing from the cytoplasm and vacuoles of the youngest fully expanded leaf on the R1 partner (the fed ramet) in the **+P** -P pair shown in Fig. 5.5.1. Table 5.4 also shows the ^{32}P diffusing from the youngest fully expanded leaves on the R1 ramets of pairs given -P +P, +P +P and -P -P treatments. The same calculation was carried out for the youngest fully expanded leaves on the R2 partner ramets to the fed R1 ramets and also for the oldest leaves on both partners.

The percentage phosphorus in the cytoplasm and vacuoles for the youngest and oldest leaves on both partners is summarised in Table 5.5

There was no significant difference in the percentage phosphate distributed between the vacuoles and the cytoplasm of the youngest leaves for any of the treatments given to the R1 and R2 ramets. The oldest leaves of the R1 ramets had a greater allocation to the cytoplasm than in youngest leaves and the oldest leaf of the R2 ramet in the -P **+P** pair also had a greater allocation to the cytoplasm than the youngest leaf given the same treatment.

Table 5.4 *Calculation of log_{10} counts $minute^{-1}$ (cpm) remaining in leaf segments soaked in water for exponentially increasing time intervals. The log_{10} values were plotted as in Fig. 5.5.1 and from the last three plots the intercept with the y axis was derived. Radioactivity above the intercept shows the ^{32}P in the cytoplasm whilst that below the intercept gives the ^{32}P in the vacuole.*

Time (min)	R1 +P of +P-P cpm	cumulat -ive total	log 10	R1 -P of -P+P cpm	cumulat -ive total	log 10	R1 +P of +P+P cpm	cumulat -ive total	log 10	R1 -P of -P-P cpm	cumulat -ive total	log 10
1	176	8106	3.909	387	15786	4.198	32	1577	3.198	414	19033	4.280
2	48	7930	3.899	133	15399	4.188	16	1545	3.189	125	18619	4.270
4	60	7882	3.897	133	15266	4.184	6	1529	3.185	175	18494	4.267
8	46	7822	3.893	109	15133	4.180	3	1523	3.183	122	18319	4.263
15	37	7776	3.891	78	15024	4.177	9	1520	3.182	107	18197	4.260
30	34	7739	3.889	74	14946	4.175	7	1511	3.179	137	18090	4.257
60	23	7705	3.887	73	14872	4.172	0	1504	3.177	135	17953	4.254
120	19	7682	3.885	50	14799	4.170	0	1504	3.177	88	17818	4.251
240	14	7663	3.884	27	14749	4.169	0	1504	3.177	61	17730	4.249
480	8	7649	3.884	19	14722	4.168	3	1504	3.177	28	17669	4.247
Hot water	7641	7641	3.883	14703	14703	4.167	1501	1501	3.176	17641	17641	4.247
Total cumulative cpm	8105			15786			1578			19033		

The percentage phosphorus in the cytoplasm and vacuoles of the youngest and oldest leaves on both partners is summarised in Table 5.5.

Table 5.5 *Calculation of percentage ^{32}P partitioned to vacuoles and cytoplasm of youngest and oldest leaves on the main stolon of A. stolonifera. The roots of the older ramet R1 were fed 370 kBq ^{32}P for 6 days. The youngest fully expanded leaf and the oldest leaf of the main stolon of the R1 and R2 ramets were removed. ^{32}P was removed by efflux from leaf segments into water, at exponential time intervals from 1 to 480 minutes. The radioactivity, expressed as counts minute^{-1}, was plotted and the intercept derived as shown in Fig. 5.5.1. Total counts above the intercept represent the ^{32}P in the cytoplasm whilst counts below the intercept represent ^{32}P in the vacuole. n = 4.*

R1	R2	Log_{10} Intercept	cpm at intercept	Total cpm	cpm in cytoplasm	cpm in vacuole	%P in cytoplasm	%P in vacuole
Youngest fully expanded leaf of R1								
+P	-P	3.8859	7679	8105	426	7679	5.26	94.74
-P	+P	4.1699	14788	15786	998	14788	6.32	93.68
+P	+P	3.1775	1505	1578	73	1505	4.63	95.37
-P	-P	4.2526	17890	19033	1143	17890	6.01	93.99
Youngest fully expanded leaf of R2								
+P	-P	3.2813	1911	2030	119	1911	5.86	94.14
-P	+P	3.2710	1866	1937	71	1866	3.66	96.34
+P	+P	2.1891	155	168	13	155	7.74	92.26
-P	-P	3.5822	3821	4004	183	3821	4.57	95.43
Oldest leaf of R1								
+P	-P	3.6348	4313	5154	841	4313	16.32	83.68
-P	+P	3.5374	3447	4148	701	3447	16.90	83.10
+P	+P	2.9622	917	1046	129	917	12.33	87.67
-P	-P	3.7401	5497	5941	444	5497	7.47	92.53
Oldest leaf of R2								
+P	-P	2.7815	605	664	59	605	8.89	91.11
-P	+P	2.9521	896	1094	198	896	18.10	81.90
+P	+P	1.6629	46	48	2	46	4.17	95.83
-P	-P	3.0698	1174	1211	37	1174	3.06	96.94

The amount of phosphorus *per* mg dwt shoot was determined by chemical analysis and the allocation to cytoplasm and vacuoles calculated and the data are shown in Table 5.6.

Table 5.6 *Calculation of the amount of phosphorus partitioned to vacuoles and to cytoplasm of youngest and oldest leaves on the main stolon of A. stolonifera. Plants were grown in high phosphate (1.33 mmol dm^{-3}) or low phosphate (0.007 mmol dm^{-3}) for 28 days . The roots of the older ramet R1 were fed 370 kBq ^{32}P for 6 days. The % P in the vacuole and cytoplasm were calculated as shown in Table 5.5. The amount of phosphorus (nmol mg^{-1}) in the shoots was established by analysis using the phospho-molybdenum blue method following acid digestion. The last column shows the ratio of P in the cytoplasm and vacuole.*

	R1	R2	%P in cytoplasm	%P in vacuole	nmol P mg dry weight shoot $^{-1}$ in cytoplasm	nmol P mg dry weight shoot $^{-1}$ in vacuole	Ratio of [P] in cytoplasm: vacuole C:V
Treatment			Youngest fully expanded leaf of R1				
1	+P	-P	5.26	94.74	9 ± 1a	162 ± 17a	0.056
2	-P	+P	6.32	93.68	4 ± 0.3b	56 ± 5b	0.071
3	+P	+P	4.63	95.37	7 ± 0.5a	144 ± 9a	0.049
4	-P	-P	6.01	93.99	2 ± 0.4c	24 ± 6c	0.083
Treatment			Youngest fully expanded leaf of R2				
5	+P	-P	5.86	94.14	5 ± 1a	76 ± 13a	0.066
6	-P	+P	3.66	96.34	7 ± 1a	183 ±15b	0.038
7	+P	+P	7.74	92.26	11 ± 1b	134 ± 7c	0.082
8	-P	-P	4.57	95.43	2 ± 0.3c	37 ± 7d	0.054
Treatment			Oldest leaf of R1				
9	+P	-P	16.32	83.68	28 ± 3a	142 ± 15a	0.197
10	-P	+P	16.90	83.10	10 ± 1b	50 ± 4b	0.200
11	+P	+P	12.33	87.67	19 ± 1c	132 ± 9a	0.144
12	-P	-P	7.47	92.53	2 ± 0.5d	24 ± 6b	0.083
Treatment			Oldest leaf of R2				
13	+P	-P	8.89	91.11	7 ± 1a	74 ± 13a	0.095
14	-P	+P	18.10	81.90	34 ± 3b	155 ± 13b	0.219
15	+P	+P	4.17	95.83	6 ± 0.3a	139 ± 8b	0.043
16	-P	-P	3.06	96.94	1 ± 0.2a	38 ± 7c	0.026

These data were analysed by ANOVA followed by Tukey's Honestly Significant *post hoc* test. Ramets given high phosphate to their roots had significantly more phosphorus in their cells than those given low phosphate. There were significant differences in the amounts of phosphorus *per* mg dwt shoot in the cytoplasm and vacuoles for the treatments given. The letters after each value show differences between the amount of phosphorus in the cytoplasm and vacuoles for each treatment within the group of four treatments in each column. For the youngest

leaves on the R1 the allocation of P to the cytoplasm and vacuoles was the same for the two R1 ramets given +P ('a' in Table 5.6).

The ratio of the P *per* mg dwt shoot in the cytoplasm and vacuole (C:V) are shown in Table 5.6. In the youngest leaves of the R1 ramet the highest C:V ratio of phosphorus was in the two sets of -P ramets. This indicates that the vacuoles were releasing P into the cytoplasm to maintain the concentration there. For the youngest leaves on the R2 ramets the highest C:V ratio in the heterogeneous pairs was in the low P ramets in the +P **-P** pair but was also high in the +P **+P** pair. It is possible that these had so much phosphorus that it could not easily be taken into the replete vacuole. The low ratio in the -P **+P** pair might be because these +P ramets do not support the R1 ramet but grow rapidly and thus may utilise their cytoplasmic phosphate supplies more quickly than the vacuole can supply it. The cytoplasm to vacuole ratio in the oldest leaves was much higher than in the youngest leaves, suggesting that as these leaves senesced their Pi was transported to the youngest developing leaves and the vacuoles were re-supplying the cytoplasm. In the R2 -P oldest leaves of the -P -P pair the ratio could be low because there was insufficient P present for the vacuoles to send P into the cytoplasm whilst the R2 -P oldest leaves in the +P -P pair had P supplied from the partner. Thus the hypothesis that the concentration of P in the cytoplasm is maintained at the expense of that in the vacuoles as the leaf ages is supported by the data.

The amount of P *per* mg dwt shoot in the cytoplasm and in the vacuoles of the youngest and oldest leaves were compared separately by means of student's t-test (SPSS for Windows 1998). In the older, R1, ramets the phosphorus content in the cytoplasm of the youngest leaves was highly significantly different from that in the cytoplasm of the oldest leaves ($p<0.001$) for pairs given **+P** -P, **-P** +P and **+P** +P treatments but not for those given **-P** -P. There was no significant difference in the amount of P *per* mg dwt shoot in the vacuoles in either R1 or R2 ramets for any of the four treatments. However between the cytoplasm of the oldest and youngest leaves of the R2 ramets there were significant differences in

the amounts of P *per* mg dwt shoot in the pairs given -P **+P** and +P **+P** treatments as summarised in Table 5.7

The amounts of P *per* mg dwt shoot in the vacuole and the cytoplasm (calculated from Table 5.6) was plotted against that in the pair of ramets (total P mg^{-1} in root and shoot of both ramets) (Fig 5.5.2). This shows the different loading of phosphorus into the cytoplasm and vacuole. It also indicates that above approximately 25 nmol mg^{-1}, P accumulates in the vacuole and that the larger percentage of phosphorus in the vacuole is not simply due to the greater volume occupied in the cell by the vacuole.

Table 5.7 *Significance of the difference between the amount of P per mg dwt shoot in the cytoplasm and vacuoles of youngest and oldest leaves on the main stolon of paired connected ramets of A. stolonifera given the phosphate treatments shown.*

Treatment	R1	R1	R2	R2
	Cytoplasm	Vacuole	Cytoplasm	Vacuole
+P -P	**	nsd	nsd	nsd
-P +P	**	nsd	**	nsd
+P +P	**	nsd	**	nsd
-P -P	nsd	nsd	nsd	nsd

** $p \leqslant 0.001$

Experiment 5.6 tested the hypothesis that pre-treatment with high or low phosphate affects the speed at which ^{32}P is transported through the main stolon of a plant. This experiment investigated the speed at which ^{32}P is transported through stolons previously given high phosphate compared with those given low phosphate. The results of counting the activity in successive internodes of fed and "chased" stolons of *A. stolonifera* by Cerenkov radiation are shown in Fig. 5.6. This figure shows that ^{32}P moved only two internodes in 30 minutes (0.4 ± 0.04 cm min^{-1}) in the ramets given high (1.33 mmol dm^{-3}) phosphate, whereas it travelled four internodes in the same time (0.7 ± 0.08 cm min^{-1}) in the stolons given low (0.007 mmol dm^{-3})

given low (0.007 mmol dm^{-3}) phosphate. The figure also shows that the stolons of plants grown for 14 days in 1.33 mmol dm^{-3} phosphate transported more ^{32}P than those grown in 0.007 mmol dm^{-3} phosphate.

Experiment 5.7 tested the hypothesis that the internal P status alters the rate of uptake of phosphate into the roots and the flux of P into connected, paired ramets of *A. stolonifera.* The efficiency of counting ^{32}P by Cerenkov radiation in water in glass scintillation vials is 42.7% (Kolb, 1979). The rate of decay of 1 Bq of radioactive substance is 1 decay s^{-1} (1 dps). 1 kBq thus gives 25620 counts minute $^{-1}$ (cpm) corrected for efficiency. The kBq ^{32}P present in each root and shoot of the pair of joined ramets can be found from the cpm of Cerenkov radiation.

Table 5.8 *Total nmol Pi entering roots and shoots of paired ramets of A. stolonifera during feeding ^{32}P to the roots of the partner marked*

Treatment (R1 root fed)	Total nmol Pi entering during 5 h 30 min. R1 root	R1 shoot	R2 shoot	R2 root	Total for pair of ramets
+P -P	247.28	52.58	12.05	14.36	326.27
-P +P	11.52	5.80	1.03	0.10	18.45
+P +P	321.41	54.78	14.11	18.53	408.83
-P -P	13.96	4.80	0.67	0.09	19.52
Treatment (R2 root fed)	Total nmol Pi entering during 5 hours R1 root	R1 shoot	R2 shoot	R2 root	Total for pair of ramets
+P -P	0.13	9.94	11.11	7.68	28.86
-P +P	0.52	23.33	529.27	358.35	911.47
+P +P	0.34	8.34	95.59	680.76	785.03
-P -P	0.04	0.61	18.47	11.16	30.28

From the concentration of inorganic phosphate (Pi) in the Long Ashton nutrient solution supplied and the volume of nutrient solution, the specific activity of the 370 kBq ^{32}P nmol $^{-1}$ Pi in the high and low phosphate Long Ashton solutions is derived and from that the nmol Pi entering each root and shoot of the paired ramets. Table 5.8 summarises the nmol Pi entering each root and shoot.
Table 5.9 summarises the average rate of uptake of Pi during each hour of feeding, making the assumption that the rate of uptake is constant.

Table 5.9 *Mean rate hour^{-1} of uptake of Pi by roots and shoots of paired ramets of A. stolonifera during feeding ^{32}P to the roots of one partner marked*

Treatment (R1 root fed)	Total nmol Pi entering during 5 h 30 min. R1 root	R1 shoot	R2 shoot	R2 root	Total for pair of ramets
+P -P	44.96	9.56	2.19	2.61	59.32
-P +P	2.09	1.05	0.19	0.02	3.35
+P +P	58.44	9.96	2.56	3.37	74.33
-P -P	2.54	0.87	0.12	0.02	3.55
Treatment (R2 root fed)	Total nmol Pi entering during 5 hours R1 root	R1 shoot	R2 shoot	R2 root	Total for pair of ramets
+P -P	0.03	1.99	2.22	1.54	5.78
-P +P	0.10	4.67	105.85	71.67	182.29
+P +P	0.07	1.67	19.12	136.15	157.01
-P -P	0.01	0.12	3.69	2.23	6.05

The values shown in Tables 5.8 and 5.9 are for entire roots and shoots.

Table 5.10 shows the mean rate of uptake of Pi hour^{-1} mg^{-1} dry weight

Table 5.10 *Mean rate (hour^{-1} mg dry weight plant organ^{-1}) of uptake of Pi into roots and shoots of paired ramets of A. stolonifera during feeding ^{32}P to the roots of one partner marked*

Treatment (R1 root fed)	Total nmol Pi entering h^{-1}mg dry weight^{-1} (during 5 h 30 min). R1 root	R1 shoot	R2 shoot	R2 root	Total for pair
+P -P	8.65	0.22	0.08	0.47	9.42
-P +P	0.23	0.02	0.005	0.002	0.257
+P +P	3.56	0.29	0.11	0.38	4.34
-P -P	0.26	0.02	0.003	0.002	0.285
Treatment (R2 root fed)	Total nmol Pi entering h^{-1}mg dry weight^{-1} (during 5 h). R1 root	R1 shoot	R2 shoot	R2 root	Total for pair
+P -P	0.004	0.04	0.06	0.31	0.414
-P +P	0.009	0.06	1.23	4.78	6.079
+P +P	0.008	0.03	0.37	10.47	10.878
-P -P	0.001	0.003	0.07	0.20	0.274

Subsamples of the dried root and shoot material were analysed for their phosphorus content. The phosphorus content, dry weight and the flux of Pi into each compartment are summarised in Table 5.11 for pairs of ramets in which the older ramet, R1 was fed ^{32}P and in Table 5.12 for the reciprocal arrangement in which the roots of the younger ramet, R2, were fed ^{32}P.

Table 5.11 *The amount of Pi organ $^{-1}$, dry weight, phosphorus pool as nmol mg^{-1} and flux of Pi for each compartment of paired, ramets of A. stolonifera joined by the parental internode. P pool found by chemical analysis. The roots of the older ramet, R1marked ✪, were fed 370 kBq ^{32}P for 5 hours 30 minutes*

	R1		R2	
P pool (nmol)	5881	+P Shoot	1468	-P Shoot
Dry weight (mg)	42.5±6.4		27.6±11.4	
[P] (nmol mg^{-1})	138		53	
P flux (nmol h^{-1})		*9.35* →	*2.21* ↓	
P flux (nmol h^{-1})		↑ *44.98*	*2.59*	
[P] (nmol mg^{-1})	178		50	
Dry weight (mg)	5.2±0.5		5.5±2.3	
P pool (nmol)	928	+P Root✪	276	-P Root

P pool (nmol)	2798	-P Shoot	6008	+P Shoot
Dry weight (mg)	50.4±18.5		39.0±10.8	
[P] (nmol mg^{-1})	56		154	
P flux (nmol h^{-1})		*1.01* →	↓*0.20* ↓	
P flux (nmol h^{-1})		↑ *2.14*	*0.02*	
[P] (nmol mg^{-1})	62		292	
Dry weight (mg)	9.3±3.3		7.7±1.8	
P pool (nmol)	579	-P Root✪	2246	+P Root

P pool (nmol)	5816	+P Shoot	4318	+P Shoot
Dry weight (mg)	34.5±10.6		23.1±9.0	
[P] (nmol mg^{-1})	169		187	
P flux (nmol h^{-1})		*10.01* →	↓ *2.54* ↓	
P flux (nmol h^{-1})		↑ *58.38*	*3.34*	
[P] (nmol mg^{-1})	270		345	
Dry weight (mg)	16.4±5.7		8.8±4.2	
P pool (nmol)	4421	+P Root✪	3036	+P Root

P pool (nmol)	1595	-P Shoot	2419	-P Shoot
Dry weight (mg)	43.0±1.9		42.7±16.5	
[P] (nmol mg^{-1})	37		57	
P flux (nmol h^{-1})		*0.86* →	↓*0.13* ↓	
P flux (nmol h^{-1})		↑*2.50*	*0.02*	
[P] (nmol mg^{-1})	62		49	
Dry weight (mg)	9.6±1.9		9.4±5.0	
P pool (nmol)	598	-P Root✪	465	-P Root

Table 5.12 *The amount of Pi organ $^{-1}$, dry weight, phosphorus pool as nmol mg^{-1} and flux of Pi for each compartment of paired, ramets of A. stolonifera joined by the parental internode. P pool found by chemical analysis. The roots of the younger ramet, R2 marked ✪, were fed 370 kBq ^{32}P for 5 hours*

	R1		R2	
P pool (nmol)	7262	+P Shoot	2383	-P Shoot
Dry weight (mg)	51.4±3.7		35.4±3.5	
[P] (nmol mg^{-1})	141		67	
P flux (nmol h^{-1})		↓*2.06* ←	*2.12*	
P flux (nmol h^{-1})		*0.03*	↑*1.52*	
[P] (nmol mg^{-1})	378		80	
Dry weight (mg)	6.8±3.3		4.9±2.7	
P pool (nmol)	2571	+P Root	392	-P Root✪

P pool (nmol)	3997	-P Shoot	13642	+P Shoot
Dry weight (mg)	76.0±5.8		86.1±13.6	
[P] (nmol mg^{-1})	53		158	
P flux (nmol h^{-1})		↓*4.56* ←	*105.90*	
P flux (nmol h^{-1})		*0.10*	↑*73.13*	
[P] (nmol mg^{-1})	63		303	
Dry weight (mg)	11.6±3.4		15.3±1.8	
P pool (nmol)	728	-P Root	4638	+P Root✪

P pool (nmol)	9627	+P Shoot	9781	+P Shoot
Dry weight (mg)	59.0±2.9		51.6±9.5	
[P] (nmol mg^{-1})	163		190	
P flux (nmol h^{-1})		↓*1.77* ←	*19.09*	
P flux (nmol h^{-1})		*0.07*	↑*134.02*	
[P] (nmol mg^{-1})	265		350	
Dry weight (mg)	8.5±2.0		12.8±3.2	
P pool (nmol)	2250	+P Root	4474	+P Root✪

P pool (nmol)	1872	-P Shoot	2817	-P Shoot
Dry weight (mg)	47.8±7.7		52.5±4.9	
[P] (nmol mg^{-1})	39		54	
P flux (nmol h^{-1})		↓*0.14* ←	*3.68*	
P flux (nmol h^{-1})		*0.01*	↑*2.26*	
[P] (nmol mg^{-1})	58		43	
Dry weight (mg)	12.9±2.7		11.3±1.7	
P pool (nmol)	742	-P Root	484	-P Root✪

The pairs of ramets used to determine the flux into each compartment from R1 to R2 were given the treatments shown in Table 5.1 for 168 hours before feeding with Long Ashton nutrient solution containing ^{32}P. Those used to determine the flux of phosphate from R2 to R1 were given the same treatments for 240 hours. Phosphate is taken up by the roots of both ramets. Although as the roots increased in size their rate of uptake of phosphate would increase, by using the calculated rates of uptake a maximum rate of influx from both roots for a feeding period of 173 hours can be derived as shown in Table 5.13.

Table 5.13 *Total flux of Pi (nmol mg dry weight of plant organ^{-1}) into each compartment of paired ramets of A. stolonifera during pre-treatment and during feeding. Values derived from the sum of the rates of flux nmol mg^{-1} h^{-1} in Figs 5.10 and 5.11 for the roots and shoots of both ramets calculated for 178 h.*

	R1 root	R1 shoot	R2 shoot	R2 root
+P -P	1540	46	25	139
-P +P	43	14	220	851
+P +P	635	57	85	1931
-P -P	46	4	13	36

Some radioactivity remained in the leaf segments and in the Long Ashton nutrient solution to which ^{32}P fed to the roots of R1 ramets. Radioactivity was also found in the Long Ashton nutrient solution surrounding the roots of the R2, unfed, ramets. The residual activity is summarised in Table 5.14

Table 5.14 *Table of residual phosphate which was not eluted from leaf segments when ^{32}P was fed to the roots of R1.*
yfel = youngest fully expanded leaf.

kBq ^{32}P remaining in leaf segments				
Treatment	+P -P	-P +P	+P +P	-P -P
R1 yfel	0.06	0.17	0.01	0.26
R2 yfel	0.02	0.02	0.001	0.05
R1 oldest leaf	0.13	0.08	0.03	0.13
R2 oldest leaf	0.02	0.03	0.002	0.03
kBq ^{32}P still remaining in spiked Long Ashton of 370 kBq originally supplied to roots of R1				
	18	2.4	17	1.3
kBq ^{32}P at end of experiment in Long Ashton supplied to unfed roots of R2, present due to efflux from roots of R2				
	0.02	0.11	0.02	0.05

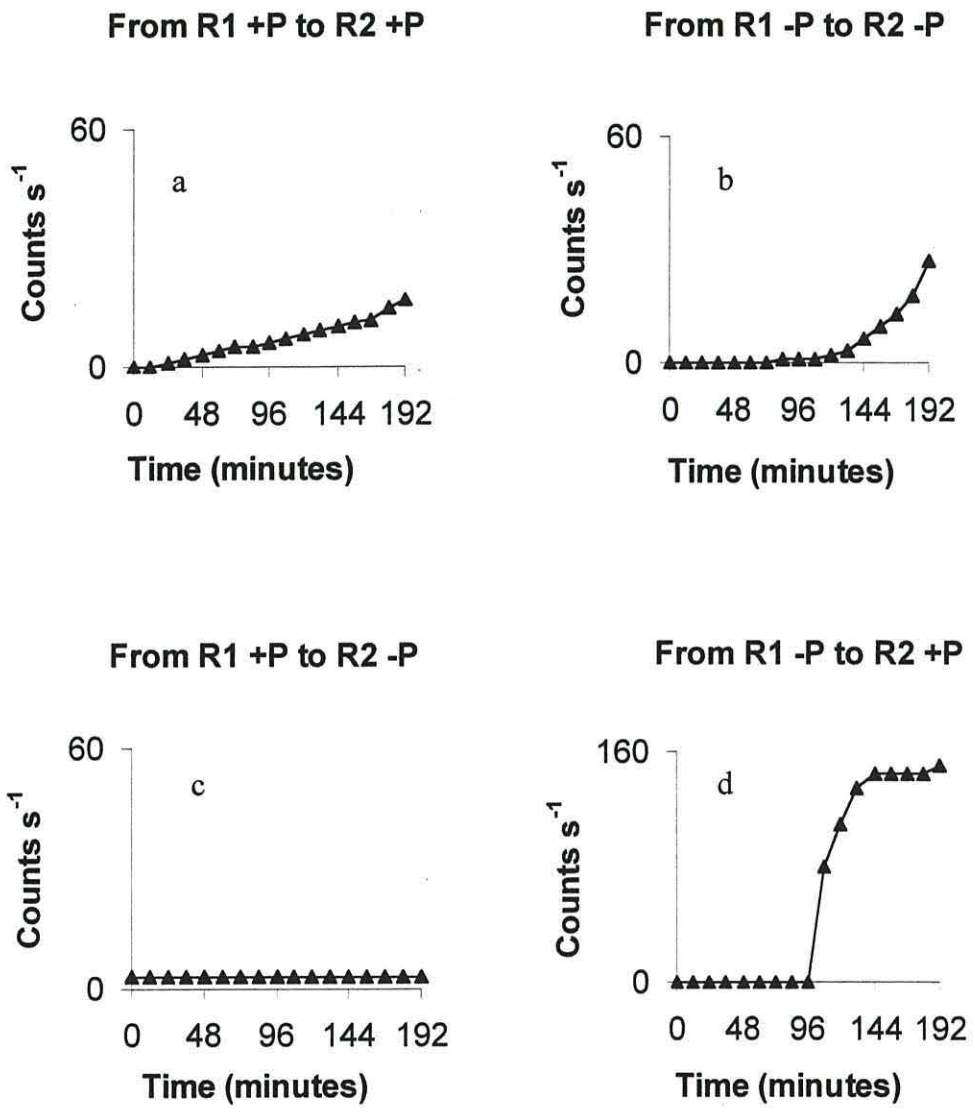
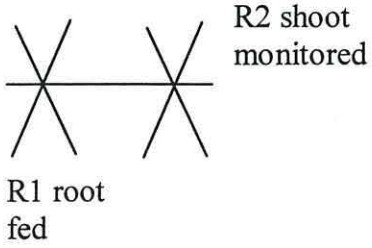


Fig. 5.1.1 *Counts s^{-1} above background radiation, monitored by a GM tube attached to the youngest fully expanded leaf on the main stolon of the younger ramet (R2) of ^{32}P transported from the fed roots of the older ramet (R1) for four different phosphate treatments.* +P = *1.33 mmol dm^{-3} phosphate;* -P = *0.007 mmol dm^{-3} phosphate. 370 kBq ^{32}P fed to roots of R1 ramet in each case.*



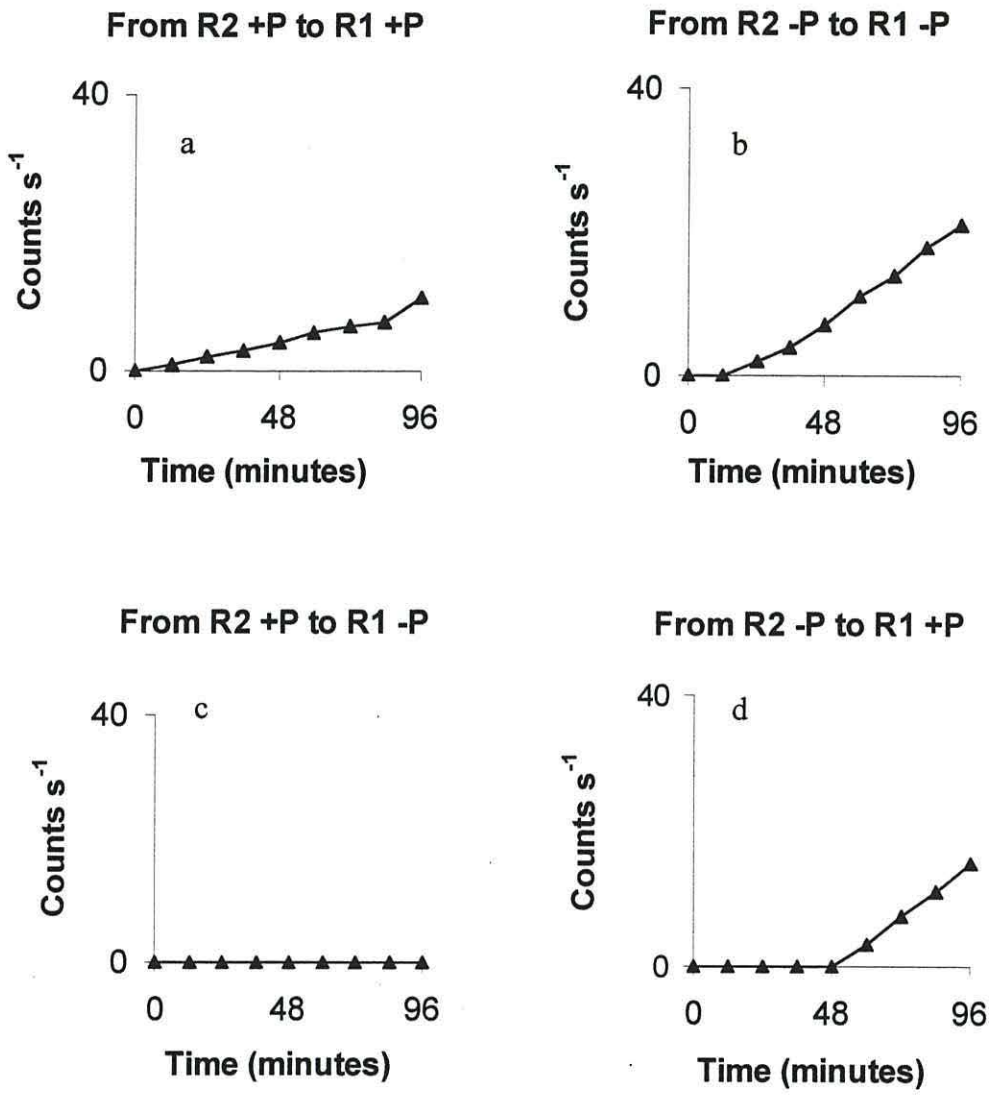
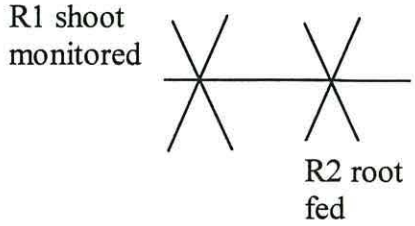


Fig 5.1.2 *Counts s^{-1} above background radiation, monitored by a GM tube attached to the youngest fully expanded leaf on the main stolon of the older ramet (R1), of ^{32}P transported from the fed roots of the younger ramet (R2) for four different phosphate treatments. +P = 1.33 mmol dm^{-3} phosphate; -P = 0.007 mmol dm^{-3} phosphate. 370 kBq ^{32}P fed to roots of R1 ramet in each case.*



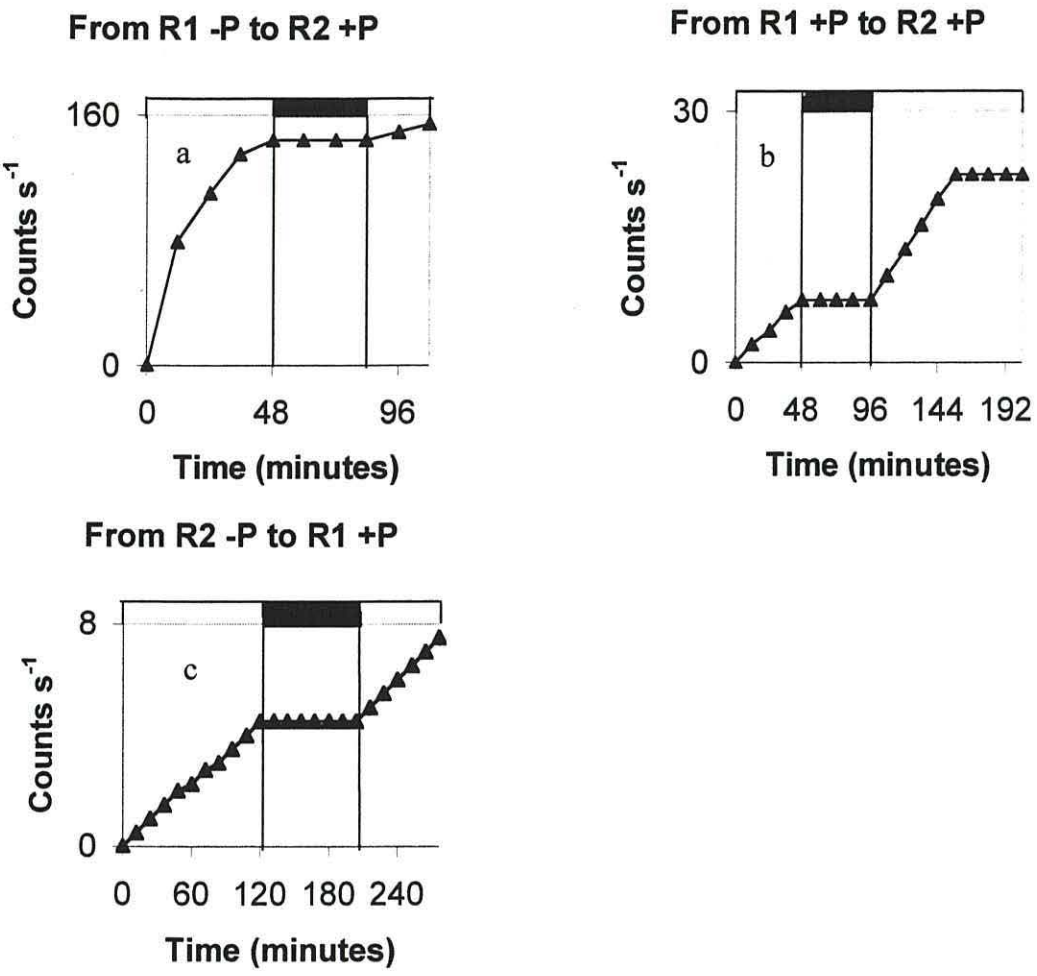


Fig. 5.2 *Counts s^{-1} above background radiation, monitored by a GM tube attached to the youngest fully expanded leaf of one ramet, of ^{32}P transported from the fed roots of the sibling ramet, for three different phosphate treatments. The monitored ramet was darkened with aluminium foil between the times indicated by the black bar. +P = 1.33 mmol dm^{-3} phosphate; -P = 0.007 mmol dm^{-3} phosphate. 370 kBq ^{32}P fed to roots of R1 ramet in each case.*

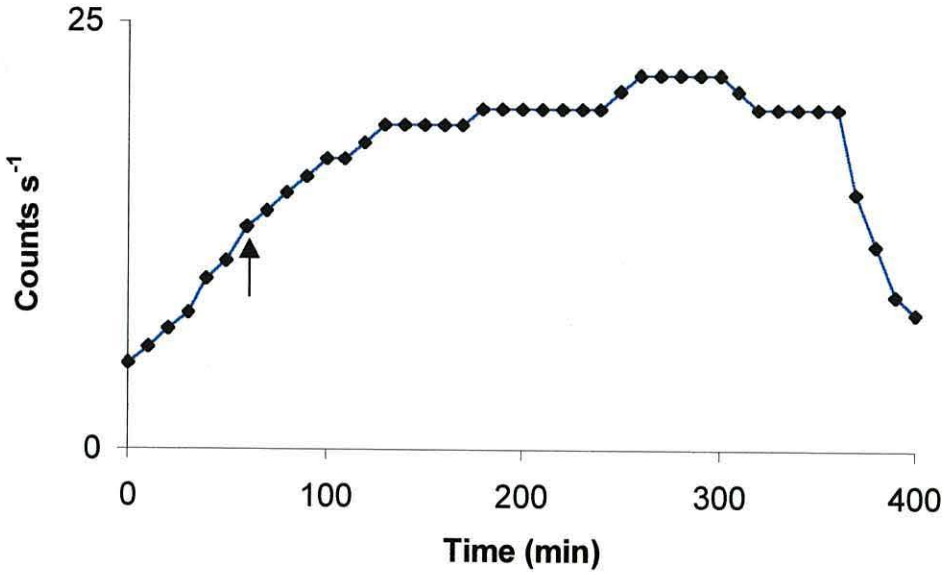


Fig 5.3 *Transport of ^{32}P from the roots of an R1 ramet fed 370 kBq radiophosphate to the youngest fully expanded leaf on the R2 ramet. The fed root was removed after 1 hour at the point marked ↑ and the ^{32}P continued to enter the leaf. After 300 minutes the trace showed a reduction in the radioactivity in the leaf monitored with a GM tube.*

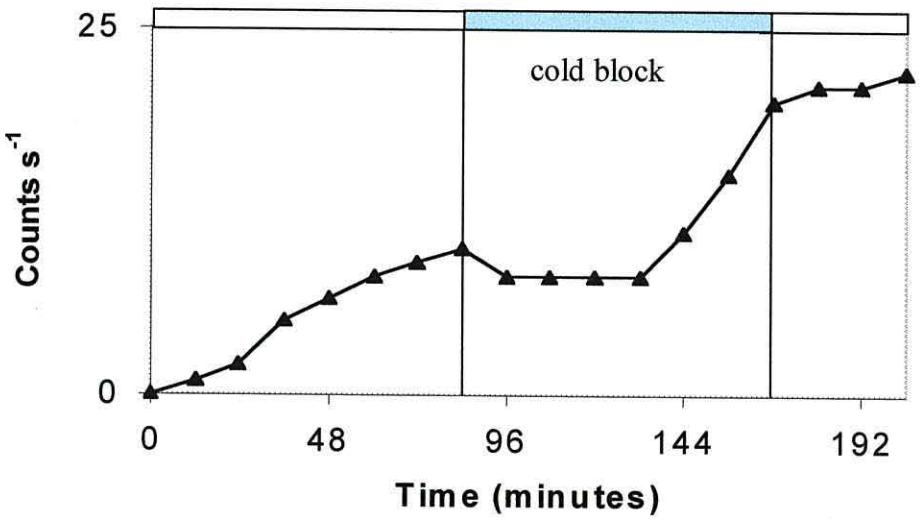


Fig. 5.4 *Transport of ^{32}P from the abraded youngest fully expanded leaf on the main stolon of R2 -P ramet into its main stolon. The base of the main stolon was monitored with a GM tube. A cold block was applied between the monitor and the fed leaf 90 minutes after monitoring began and removed after a further 90 minutes.*

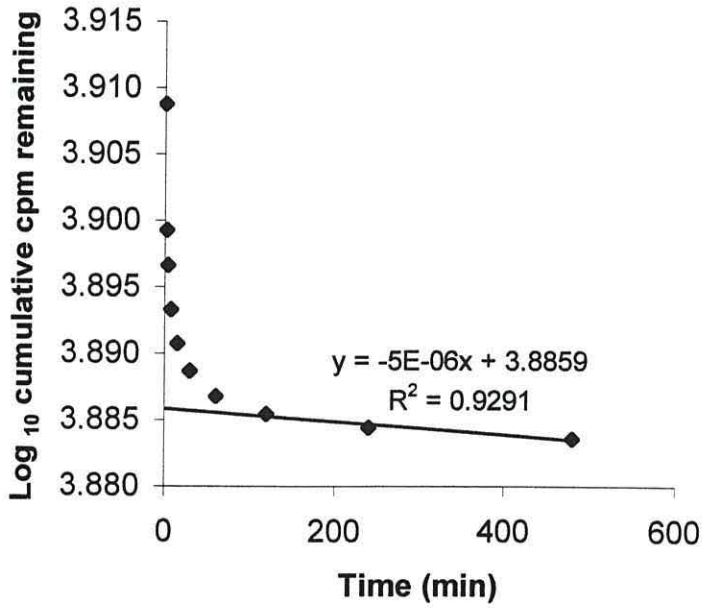


Fig. 5.5.1 *Log_{10} cumulative counts minute^{-1} of ^{32}P remaining in leaf segments over time intervals from 1 to 480 minutes. The equation for the trend line of the last three points gives the intercept with the y axis. Radioactivity above the intercept indicates the amount of ^{32}P in the cytoplasm whilst that below the intercept indicates the ^{32}P in the vacuole.*

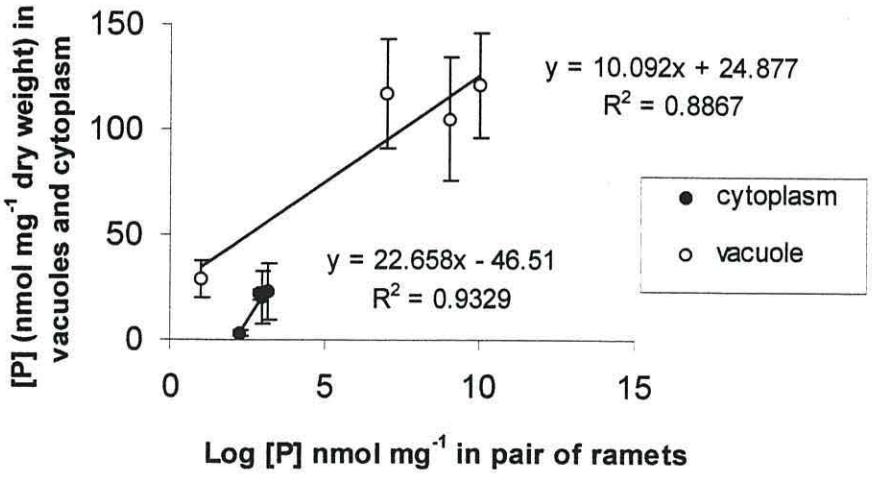


Fig. 5.5.2 *Mean total phosphorus concentration (nmol mg^{-1} dry weight) in each pair of ramets for each of the treatments* +P -P; -P +P; +P +P; -P -P *compared with the mean phosphorus concentration in the vacuoles and cytoplasm calculated as the mean of the youngest and oldest leaves on the R1 and R2 ramets for each treatment shown in Table 5.6.*

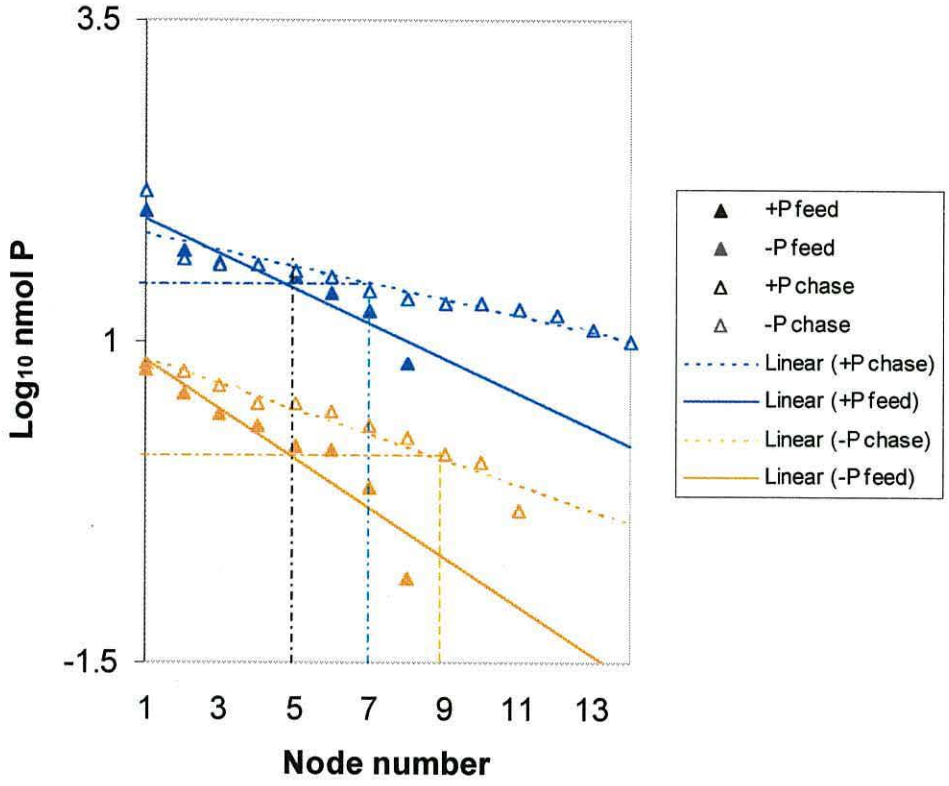


Fig. 5.6 *Log_{10} nmol P calculated from the counts minute $^{-1}$ ^{32}P in each internode along the length of stolons of A. stolonifera. The rooted ramet was fed 370 kBq ^{32}P for 120 minutes. Half the plants were then removed and their main stolon cut into separate internodes for counting by Cerenkov radiation. The second half were "chased" with fresh Long Ashton nutrient solution for 30 minutes before their main stolons too were cut into internodes for counting by Cerenkov radiation. The broken lines show the number of internodes over which a flux of phosphate travelled from node number 5 in 30 minutes.*

DISCUSSION

Is phosphate transported from one ramet of a connected pair to the other?

When ^{32}P was fed to the roots of an older ramet, radioactivity was detected in the youngest fully expanded leaf on the main stolon of an attached younger ramet but only if the roots of the younger ramet were given the same amount, or less, phosphate in the growth medium. There was insufficient transfer, during 24 hours, of ^{32}P from the roots of a ramet given high phosphate to the shoot of a sibling given low phosphate, to be detectable by a GM tube, although Cerenkov scintillation showed that a small amount of ^{32}P was in fact transferred. This was also the case if the roots of a high phosphate younger ramet were fed ^{32}P and the youngest fully expanded leaf on the main stolon of an attached older low phosphate ramet was monitored.

Shading the shoot of a ramet reduced the rate of transpiration until the shade was removed, showing that P was carried in the xylem, that its rate of movement depended on the rate of flow of water in the transpiration stream and that phosphate was transferred both acropetally and basipetally in paired ramets of *A. stolonifera.* Acropetal transfer of ^{32}P was observed in *Trifolium repens* by Hoshino (1974) and in *Carex arenaria* by Noble & Marshall (1983), but in both these cases transport was studied in whole stolons made up of a number of ramets and with the stolon apex in place. Production of new leaves at the main stolon apex requires supplies of phosphorus i.e the apex is a strong sink for phosphorus and can take P from the roots of a ramet at a distance along the main stolon, and in the absence of the parent main stolon tip the apices of the new stolons of the two sibling ramets would compete for phosphorus. That basipetal movement of ^{32}P is possible in whole stolons was demonstrated by Anderson-Taylor (1982) but basipetal movement was limited in the presence of the main stolon apex.

Does P status regulate the movement of water and P through the xylem?

Paired ramets given homogeneous phosphate treatments showed transfer of ^{32}P from the fed ramet to the partner suggesting an exchange of phosphate between the ramets which were of similar sizes. Where paired ramets were given heterogeneous phosphate treatments there was rapid net transfer of P towards the high phosphate partner acropetally (from R1 to R2). In the heterogeneous pairs the R2 ramet grown in high phosphate and having a larger shoot would be transpiring more rapidly and thus water and P would be conducted through the xylem towards the larger ramet. The high P shoots were also more branched than the low P and the extra apices would also cause the shoots to have a higher demand for phosphate than the relatively unbranched low P shoots. There was basipetal transfer of ^{32}P from low P younger (R2) ramets towards a high P R1 ramet but basipetal transfer was not as rapid as acropetal. Where the ramets were given homogeneous treatments there was reciprocal transfer of P but the shoots were more evenly matched so the net exchange was less than for heterogeneous treatments.

Fig. 5.6 shows that although a small amount of P was transported more rapidly through low P ramets, larger amounts were transported more slowly in +P ramets. Drought experiments reported in Chapter 4 showed that stomatal conductance was only altered where one ramet in a pair was given high phosphate and its partner was water-stressed, in which case stomatal conductance was markedly reduced in both ramets. For ramets given adequate water there was no difference in stomatal conductance of water vapour between ramets given high or low phosphate. The rate of movement of P through the transpiration stream is not regulated by control of stomatal conductance in *A. stolonifera* fragments. Jeschke *et al.* (1996) showed that water is taken up more rapidly by the roots of *Ricinus* plants given high phosphate than those given low phosphate. The rate of uptake of water is highest at the apices of roots of barley and decreases with distance from the tip (Sanderson, 1983). Ramets of *A. stolonifera*

given low phosphate have long, comparatively unbranched roots with few apices. P status could therefore be responsible, by altering root morphology, for differences in the rate of uptake and movement of P through the xylem.

Does P circulate within the xylem and phloem of ramets?

The transport of ^{32}P through the xylem, into the youngest fully expanded leaf, recirculation from the leaf and transport out of the leaf through the phloem is demonstrated in Figs. 5.2 to 5.4. The length of stolon which was cooled in Experiment 5.4 measured only 1.5 cm and this is shorter than reported in similar experiments (Farrar, 1988). The main stem of *A. stolonifera* is slender and this may be the reason why phloem transport was inhibited when only a short length was cooled. In clonal fragments consisting of pairs of ramets, recirculation of phosphorus occurs, as has been reported for non-clonal plants by Pate *et al.* (1985), Mimura *et al.* (1996), Jeschke *et al.* (1997) and Schachtman (1998). Milthorpe & Moorby (1969) reported that the concentration of phosphate ions was comparable in both xylem and phloem and that the presence of phosphate ions in the phloem was a result of transfer to the phloem for retranslocation from the leaf. There are many reports of the transfer of P and other materials between xylem and phloem including those of Headley *et al.* (1988); Marschner (1995) and Hay & Sackville Hamilton (1996). Bieleski (1973), reviewing the transport of P stated that Pi is taken into the phloem from the xylem along the length of the stem of deciduous trees. Carbon and nitrogen are exchanged between xylem and phloem of *Lupinus albus* (Pate & Atkins, 1983). Pate & Jeschke (1995) report the exchange of materials through specialised transfer cells between xylem and phloem of *Senecio vulgaris*, and amino acids have been observed to be exchanged between xylem and phloem (Van Bel, 1984). Köckenberger *et al.* (1997) used NMR to establish the exchange of water between xylem and phloem along the length of vascular bundles.

Does the vacuole acts as a reservoir of P, supplying the cytoplasm and maintaining a constant concentration there at the expense of the concentration of P in the vacuole?

Experiment 5.5, in which ^{32}P was eluted from leaf segments, gave results for the allocation of P between vacuole and cytoplasm in agreement with results reported in the literature (e.g. Milthorpe & Moorby, 1969; Lauer *et al.,* 1989; Mimura *et al.,* 1990). However, not all the phosphorus shown by chemical analysis to be present would be in the symplasm, some would be in the apoplast and some in the vascular system so the values for phosphorus content of the cytoplasm and vacuoles shown in Table 5.6 are an over-estimate and show only the likely ratios of phosphorus in these compartments. The amount of phosphorus (nmol mg dry weight of the shoot^{-1}) in the vacuoles differed depending on the external supply of phosphate to the roots, in agreement with the findings for barley leaves of Foyer & Spencer (1986) but the amount of P *per* mg dwt shoot in the cytoplasm was not held at a constant value. This was generally slightly higher in ramets given high phosphate than in those given low phosphate except for the oldest leaves of the younger (R2) ramets. Experiment 5.5 tested the hypothesis that the phosphorus concentration in the cytoplasm would remain constant whatever the age of the leaf but that the concentration in the vacuoles would fall in older leaves as the vacuole acted as a reservoir of P, keeping the concentration in the cytoplasm constant as P recirculated from the older, senescing leaves. In fact, for R1 ramets, the amount of P *per* mg dwt shoot in the cytoplasm of older leaves was almost three times that in young leaves for ramets with access to high phosphate. Since the allocation of available phosphate was unchanged, with approximately 95% allocated to the vacuole and 5% allocated to the cytoplasm, the plants were probably not short of phosphate, and changes in allocation might be seen after more than seven days of growth since observable differences between high and low phosphate ramets were not apparent until about twelve days of treatment with nutrient solution containing high or low phosphate. Foyer & Spencer (1986) and Lee & Ratcliffe (1993) also found little change in the concentration of P in the cytoplasm until plants had been deprived of phosphate for an extended period. However, the ratio of P in

the cytoplasm and vacuoles changed. Older leaves had a higher ratio as the vacuoles lost phosphate and the cytoplasm gained it as senescing leaves mobilised their P reserves to supply developing leaves. Thus the vacuole acts as a reserve of P supplying the cytoplasm. The mean phosphorus in the cytoplasm and in the vacuoles of both oldest and youngest leaves of the R1 and the R2 ramets was plotted against the total phosphorus in the roots and shoots of the pair of ramets – the internal phosphorus environment of the plant (Fig 5.5.2). In the shoot there was always more P in the vacuoles than in the cytoplasm but never more than 25 nmol mg^{-1} dry weight in the cytoplasm whereas the amount of P in the vacuoles increased as the total P in the shoot increased. Thus 25 nmol mg^{-1} dry weight shoot appears to be the threshold for loading the vacuoles. The lowest value for the vacuoles is that of the pair given -P -P. The mean total P per unit dry weight of the shoot for these ramets was 29 nmol mg^{-1} dry weight, just above that for loading the cytoplasm. Where high P was available, either through a ramet's own roots as in the +P +P pairs or through the roots of a partner as in the +P -P and -P +P pairs, there was more P in the vacuoles. This suggests that the allocation of P between the cytoplasm and the vacuoles is controlled, that the vacuole is probably replenished only when the concentration in the cytoplasm is satisfied, as suggested for soybean by Lauer *et al.* (1989) and that the larger percentage of phosphorus in the vacuole was not due simply to the greater volume in the cell occupied by the vacuole.

Does the internal P status alter the rate of uptake of phosphate by the roots?

Comparison of the rates of uptake (nmol h^{-1} mg dry weight root $^{-1}$) by the roots of fed ramets (Table 5.10) shows that the amount of phosphate taken up by the roots is greater in P-sufficient ramets from Long Ashton nutrient solution containing 1.33 mmol dm^{-3} phosphate than in low-P ramets in 0.007 mmol dm^{-3} phosphate. Anghioni & Barber (1980) found that when maize plants were transferred from high phosphate to low phosphate their rate of uptake of phosphate was increased by 55%. Other authors who have reported increased

rate of uptake of phosphorus by P-deprived plants include Ullrich-Eberius *et al.* (1981 and 1984) working with *Lemna gibba*. Clarkson & Scattergood (1982) found an increase in the rate of uptake of ^{32}P by both tomato and barley plants, followed by a decline in the rate of uptake as symptoms of deficiency appeared. However, when phosphate supplies were restored the authors noted such a rapid rise in the rate of uptake that the same plants accumulated toxic concentrations of P. Similar enhanced rates of uptake by roots on restoration of the P-supply are reported in barley by Drew *et al.* (1984), and Drew & Saker (1984) and in potato plants by Cogliatti & Clarkson (1983). All the above experiments involved a switch at some stage from high phosphate to low and sometimes back again to high, whereas *A.stolonifera* ramets were grown in the experiments described in this chapter in either high or low phosphate throughout. Raghothama (1999), reviewing the effects of low phosphate, concluded that in general a low phosphate supply to the roots results in enhanced uptake but that this is due to the significant increase in the number of root hairs in plants grown in soil. *A. stolonifera* ramets grown hydroponically had no root hairs. For plants in a heterogeneous environment those roots entering a rich source of phosphate would increase their rate of uptake as the roots proliferated (Raghothama, 1999). In *A. stolonifera* a result of high external phosphate is an increase in root branching. Where the developing roots leave the stele the endodermis is perforated and this may allow faster uptake of minerals into the xylem (Bieleski, 1973).

Is P transported more rapidly through stolons of ramets supplied with high phosphate than with low phosphate?

Whether phosphorus is transported more rapidly through P-sufficient or P-deficient plants appears to vary depending on the plant and hence probably on the vascular architecture. Figure 5.6 shows that the rate of transport of ^{32}P through whole stolons of *A. stolonifera* appeared higher for low phosphate than for high phosphate plants. This is in agreement with the findings of Clarkson & Scattergood (1982) in tomato and barley, and Raghothama (1999), whereas Cogliatti & Clarkson (1983) found a reduction in the rate of transport in potato

and Jeschke *et al.* (1997) found that in P-deficient castor bean there was "drastic curtailment" of both uptake and translocation of phosphorus through the xylem. Table 5.10 shows that although some ^{32}P may have moved more rapidly through P-deprived *A. stolonifera*, the total amount of phosphorus moved (nmol), and also the amount of phosphorus expressed as a percentage of the flux of phosphorus into the fed root, was greater in P-sufficient than in P-deficient ramets as shown in Table 5.16. However if the amount of phosphorus translocated from the fed root to the shoot of each ramet is expressed as a percentage of the phosphorus pool in the root the results are clear and here the lowest percentage of phosphorus transferred to the shoot was from the R2 ramet in the pair given homogenous high phosphate treatment (+P +P). Thus discrepancies in the reported rates of transport in high and low P plants may be the result of comparing different parameters.

Table 5.16 *Flux of phosphorus from the fed root into its shoot as a percentage of the phosphorus (a) entering root (b) present in the root*

	P flux into shoot (nmol mg^{-1} h^{-1})	(a) P flux into root (nmol mg^{-1} h^{-1})	(a) % flux phosphorus translocated to shoot	(b) P pool in fed root (nmol)	(b) % pool phosphorus translocated to shoot
Treatment	From R1 fed root				
+P -P	0.22	8.65	2.5	928	0.024
-P +P	0.02	0.23	8.7	579	0.003
+P +P	0.29	3.56	8.1	4421	0.007
-P -P	0.02	0.26	7.7	598	0.003
	From R2 fed root				
-P **+P**	0.06	0.31	19.4	392	0.15
+P **-P**	1.23	4.78	25.7	4638	0.27
+P **+P**	0.37	10.47	3.5	4474	0.008
-P **-P**	0.07	0.20	35.0	484	0.14

Table 5.16 shows that R1 ramets given high phosphate had a larger pool of phosphate in their roots than ramets given low phosphate and that a higher proportion of the pool of phosphate was transferred to the shoots by high phosphate ramets. Amongst R2 ramets, again those grown in high phosphate

had a larger pool of phosphate but only the R2 ramet in the -P+**P** pair transported a larger proportion of the phosphate pool to the shoot than the low phosphate ramets although the roots of the R2 in the homogeneous high phosphate pair (+P +**P**) transported a greater quantity of P to the shoot than did the low-P ramets. The observation made by Clarkson *et al.* (1978) and Raghothama (1999), that low phosphate ramets tend to keep more phosphate in their roots than do high phosphate ramets was not seen in *A. stolonifera.*

How does internal P status alter the rate of efflux of P from the roots?

From Table 5.14 it is apparent that not all the ^{32}P taken into the leaves was eluted from them either during 8 hours in cold water or 5 minutes in hot water. The residual phosphate will have been incorporated into the phospholipid of membranes, phosphorylated enzymes and nucleic acids (Ahmad *et al.*, 1984). The same table also shows that not all the radiophosphate had been taken up from the nutrient solution in which it was fed to the roots and that there was less remaining in the low phosphate Long Ashton solutions where it was in less competition for uptake with the non-radioactive isotope. Efflux of ^{32}P occurred from the unfed roots of all the pairs into the nutrient solution provided to their roots; the greatest efflux was from the low-phosphate R2 ramets attached to a +P partner fed ^{32}P where the low P status allowed increased efflux and the high P partner provided phosphate which was subsequently lost.

CONCLUSIONS

1. Phosphorus was transferred from one of a pair of joined ramets to a sibling either proximal or distal to it but only towards a stronger or equally strong sink
2. Phosphorus was carried from the roots of a ramet through the xylem to the leaves and circulated out of the leaves in the phloem.
3. The ratio of phosphorus in the cytoplasm to that in the vacuoles increased as the vacuolar P was mobilised and transferred to the cytoplasm. The leaves of older ramets had significantly more phosphorus in the

cytoplasm than the leaves of younger ramets (except for those given homogeneous low phosphate). Ramets given high phosphate to their roots had more P in their leaves. Where a pair of ramets was given heterogeneous P, treatments the -P ramet contained more P than a ramet which was one of a pair given homogeneous low P.

4. High phosphate ramets transferred more phosphorus to the shoot in unit time than did low phosphate ramets. These latter transferred small quantities of phosphorus more rapidly than did high phosphate ramets.
5. Efflux of phosphate from the roots was greater from low-P ramets than from high P ramets.

Chapter 6

The distribution of photoassimilates in clonal fragments of *Agrostis stolonifera* L. given contrasting phosphate treatments

INTRODUCTION

An effect of phosphorus deficiency is that the dry weight of roots is relatively unaffected or even enhanced, despite a lower dry weight of shoots, consequently there is an increase in root:shoot dry weight ratio. This is due to increased partitioning of photosynthates to the roots (Fredeen *et al.*, 1989, 1990; Cakmak *et al.*, 1994; Ciereszko *et al.*, 1996; Jeschke *et al.*, 1996). Qiu & Israel (1992), however, suggest that this increase in root:shoot dry weight ratio is a result of increased efficiency in the use of carbohydrates in the roots of low-phosphate plants rather than greater export to their roots. Fredeen *et al.* (1989) showed that although growth rate is reduced in soybean plants in low phosphate, the concentration of carbohydrates is greater in the roots of phosphate-deficient than of phosphate-sufficient plants.

Ciereszko *et al.* (1996) noted that more carbohydrate is transported from the leaves to the roots of bean plants (*Phaseolus vulgaris)* grown in low phosphate than in high phosphate and suggested that such an increase might be an early indication of phosphate-stress. The observation (Chapter 2) that the phosphorus concentration in shoots is most closely correlated with the R:S ratio of dry weights suggests that the increase in R:S ratio as plants are deprived of phosphate is due to the phosphate status of the shoot and to changes induced by low phosphate on the partitioning of photosynthates to the roots. Shading the shoot of barley plants reduces the rate of photosynthesis and brings about a consequent reduction in the amount and the proportion of carbohydrate allocated to the roots, which results in greater growth of the shoot than the root (Minchin *et al.* 1994). Partitioning to roots and shoots depends not only on the ability of the roots and shoots to use incoming assimilate but also on the supply of photosynthate available (Minchin *et al.* 1994). In fact it is argued that the term

'sink strength' has little meaning and that growth, maintenance and storage of carbohydrate in the roots depends on the flux of carbon from the leaves (Farrar, 1996a).

Clonal plants with intact connections may transfer materials from ramet to ramet and hence share resources, including photoassimilates, so that ramets growing in resource-poor or patchy environments and lacking a resource are supported by ramets growing in an abundant supply of that resource (Pitelka & Ashmun, 1985; Marshall, 1990; Stuefer *et al.*, 1996). The vascular connections between leaves, stems and roots determine whether or not it is possible for carbohydrates, minerals and water to be transported between plant organs (Price *et al.*, 1992). Young ramets begin as net importers of resources. Import ceases as they develop their own leaves, stems and roots but may be re-established if the ramet is damaged or stressed by lack of a resource. Even when connections persist between ramets in a clone, resources may not be shared if the plant is made up of 'integrated physiological units' (IPUs) defined by Watson & Casper (1984) as 'that level of morphological organisation within which the assimilation, distribution and utilisation of carbon is regulated'.

Sharing of photosynthates has been studied in whole stolons of *A. stolonifera*. (Anderson-Taylor, 1982; Jinks, 1986). Most assimilate labelled with ^{14}C remained in the fed shoot, with a small amount translocated to the roots of the fed shoot but only traces to other parts of the stolon. This distribution was little altered by either defoliating or shading other parts of the stolon in the short term (3 days) but over 14 days more ^{14}C-assimilate appeared in the roots of adjacent ramets if the shoots of these were shaded or defoliated. Thus sharing of photosynthate in *A. stolonifera* was limited, at least in the short term. The pattern of distribution of ^{14}C in clones of *Trifolium repens*, as developing leaves became sources rather than sinks, differed from that seen in clones of *A. stolonifera*. In *Trifolium* ^{14}C moved to all parts of the shoot and to the roots, although less assimilate was exported to the main stolon and more to the roots as

the plant grew (Chapman & Hay, 1993; Chapman *et al.*, 1992; Kemball & Marshall, 1995).

In clonal plants manipulation of the source leaves might be expected to have less obvious effects on sinks than are seen in non-clonal plants, as stressed ramets may be supported by unstressed siblings (Pitelka & Ashmun, 1985; Marshall, 1990). Kemball *et al.* (1992) compared the distribution of ^{14}C in *Galium aparine* (a non-clonal plant) with that in *Trifolium repens* (a clonal plant). They found that shading reduced the rate of growth in both species but that, whereas if a branch of *Galium* is darkened it soon dies, a branch of *Trifolium* is supported by photoassimilates from undarkened branches. The same authors reported that only a trace of the ^{14}C supplied to the main axis of the non-clonal plant is transferred to a basal branch, whether or not it was shaded, whereas in the clonal plant more than 30% of labelled assimilate is exported to such a branch.

The distribution of ^{14}C was studied in *Carex arenaria* with defoliated ramets (Noble & Marshall, 1983) and in intact clonal systems (D'Hertefeldt & Jonsdottir, 1999). Noble & Marshall (1983) found, in autoradiographic studies, that labelled assimilate is translocated from a fed shoot to several roots and to a small shoot along the rhizome as well as to the rhizome apex. In glasshouse studies the isotope appears in all defoliated shoots and in the entire root system but in the field, with ^{14}C fed to a flowering shoot, there is no movement of assimilate into defoliated shoots. D'Hertefeldt & Jonsdottir (1999) analysed the translocated ^{14}C assimilate quantitatively and found significant transfer of ^{14}C into roots proximal to the fed shoot as ^{14}C is translocated both basipetally and acropetally, the latter especially for older rhizomes.

In non-clonal plants shortage of a resource may result in partitioning to the organ responsible for gathering that resource i.e. specialisation for shortage, (Thornley & Johnson, 1990; Minchin *et al.*, 1994) whereas clonal plants may show division of labour such that if two resources are in short supply in different parts of the clone, enhanced growth occurs in those organs able to exploit a rich

resource i.e. specialisation for abundance in a heterogeneous environment. A heterogeneous environment can bring about morphological changes (Alpert & Mooney, 1986; Friedman & Alpert, 1991; Stuefer, *et al.*, 1994 and 1996; Stuefer, 1998). Clonal plants may alter the allocation of resources to roots and shoots in order to maximise the uptake of a resource in a rich patch (Stuefer *et al.*, 1996). Paired ramets of *Fragaria chiloensis*, in which one ramet was given high nitrogen and low light and the partner the reciprocal treatment, supported each other so the final dry weight of the pair was more than 50% greater if the connection was left intact than if they were separated and unable to exchange resources (Friedman & Alpert, 1991; Alpert & Stuefer, 1997). Division of labour also occurred in *Trifolium repens.* Where one ramet was provided with high light and low water and the partner ramet had the reciprocal treatment, the ramet in high light and low water increased allocation of carbohydrate to the shoot whereas the ramet in low light but high water had a significantly greater allocation to the roots i.e. specialisation for abundance (Stuefer *et al.*, 1996).

Plants lacking phosphorus may increase the export of carbohydrates to their roots to increase their rate of growth and hence their ability to take up phosphate from a poor source, but their ability to do so varies from species to species. Manipulation of the source leaves results in changes to the distribution of carbohydrate, but in intact clonal plants there is often support for ramets short of carbohydrates either because of defoliation or shading.

This chapter tests the hypotheses that

- ^{14}C will be transported from a fed shoot to all parts of a clonal fragment consisting of a pair of connected ramets
- the export of ^{14}C from shoots in clonal fragments of *A. stolonifera* consisting of connected pairs of ramets will be greater from low P than from high P shoots
- more ^{14}C will be allocated to the roots of low P than of high P ramets.

MATERIALS AND METHODS

To test the hypotheses that the export of ^{14}C from shoots in clonal fragments of *A. stolonifera* consisting of connected pairs of ramets will be greater from low P than from high P shoots and that more ^{14}C will be allocated to the roots of low P than of high P ramets

Agrostis stolonifera plants, from the clone maintained at Pen y Ffridd field station, University of Wales, Bangor, were grown in John Innes No 1 potting compost in a glasshouse until they had produced a main stolon. This was cut off to encourage the production of tillers. The third and fourth nodes of the basal tillers were gently scarified to encourage rooting, the tillers laid on wet paper and the nodes covered with strips of paper. The paper was kept damp until roots and shoots about 5 cm long had developed at both nodes. The paired ramets, still attached by the original tiller internode, were then cut from the rest of the tiller, and grown hydroponically as described in Chapter 2 and summarised in Table 6.1.

Table 6.1 *Phosphate treatments given to paired ramets of A. stolonifera grown hydroponically.* +P = *Long Ashton nutrient solution with 1.33 mmol dm^{-3} phosphate;* -P = *Long Ashton nutrient solution with 0.007 mmol dm^{-3} phosphate.*

Treatment of pair	Phosphate treatment given to R1, the older ramet	Phosphate treatment given to R2, the younger ramet
1. (+P -P)	+P	-P
2. (-P +P)	-P	+P
3. (+P +P)	+P	+P
4. (-P -P)	-P	-P

The paired ramets were grown for 10 days in a Conviron E15 constant environment cabinet as described in Chapter 2, Experiment 2.1. After 10 days each shoot consisted of a main stolon approximately 30 cm long with up to five basal tillers.

Pairs of ramets were transferred to a light bank providing a PPFD of 120 μmol m^{-2} s^{-1} at plant height. A Melinex feeding chamber was placed round the whole shoot of the older ramet, R1, and a 0.01 cm^3 droplet containing 185 kBq ^{14}C sodium hydrogen carbonate ($NaH^{14}CO_3$) was injected into an open-ended tube containing excess hydrochloric acid, within the feeding chamber. Labelled carbon dioxide ($^{14}CO_2$) was released inside the feeding chamber (Colvill & Marshall, 1981). The injection hole was sealed with adhesive tape and a carbon dioxide-fixing period of 45 min allowed. The Melinex feeding chambers were then removed in a fume hood and the plants returned to the Conviron growth cabinet for 24 h. The plants were harvested, the paired ramets separated and the root and shoot of each ramet dried and weighed separately. Weighed subsamples were then combusted in a Harvey biological sample-oxidiser OX400 for 4 min at 3000 °C. The $^{14}CO_2$ liberated was absorbed in Oxysol, and activity counted as disintegrations per minute (dpm) in a LKB-Wallac 1215 Rackbeta II liquid scintillation counter. The experiment was repeated with the shoot of the younger ramet, R2, fed $^{14}CO_2$. From the Long Ashton nutrient solution surrounding the roots of each ramet one cm^3 was removed and added to 25 cm^3 deionised water. The activity in the solutions was counted to determine the efflux of ^{14}C from the roots of ramets.

In both repeats of the experiment four replicates of each treatment were used. The ramets in the heterogeneous pairs were compared with their control counterparts in the homogeneous pairs and the results analysed statistically by ANOVA followed by the *post hoc* test for Least Significant Difference.

RESULTS

The mean dry weights of the four replicates of the ramets in the pairs given each of the four phosphate treatments are shown in Fig. 6.1 for the pairs in which the shoot of the R1 ramet was fed $^{14}CO_2$ and in Fig. 6.2 where the R2 shoot was fed.

The allocation of ^{14}C to the root and shoot of each ramet in the pair is expressed as a percentage of the total activity (Bq) in the pair and this is shown in Table 6.2 (a) for the pairs in which the older ramet, R1, was fed ^{14}C and (b) for the reciprocal treatment in which the younger, R2, ramet was fed.

Table 6.2 *Allocation of ^{14}C to the root and shoot of both ramets expressed as a percentage of the total activity in the pair when the shoot of one ramet was fed 185Bq ^{14}C for 45 minutes and the ramets were harvested after 24 hours. The fed shoot is marked ✪. The final column shows the ratio of ^{14}C allocated to the root* (R) *and shoot* (S) *of the unfed ramet.*

Treatment	(a) Shoot of older ramet, R1, fed ^{14}C				
(fed ramet in bold)	Shoot R1✪	Root R1	Shoot R2	Root R2	R2:S2
+P -P	97.6 ±0.3	1.1 ±0.4*	0.4 ±0.1	0.9 ±0.4	2.3 ±0.4
-P +P	94.0 ±2.1	5.2 ±2.1	0.6 ±0.1	0.1 ±0.1	0.2 ±0.07
+P +P	95.4 ±1.2	2.9 ±0.3	1.6 ±1.3	0.1 ±0.02	0.1 ±0.05
-P -P	94.4 ±1.5	3.9 ±1.0	0.5 ±0.1	1.3 ±1.2	2.6 ±1.4
	(b) Shoot of younger ramet, R2, fed ^{14}C				
Treatment	Shoot R1	Root R1	Shoot R2✪	Root R2	R1:S1
+P **-P**	0.6 ±0.2	1.1 ±0.5	96.4 ±0.9	1.9 ±0.5	1.8 ±0.9
-P **+P**	0.6 ±0.2	0.3 ±0.3	91.9 ±1.2 *	7.2 ±0.9**	0.5 ±0.3
+P **+P**	0.3 ±0.2	0.4 ±0.3	95.3 ±1.8	4.0 ±1.4*	1.3 ±0.7
-P **-P**	0.4 ±0.1	0.4 ±0.3	95.3 ±0.5	4.0 ±0.8*	1.0 ±0.6

* $p \leqslant 0.05$; ** $p \leqslant 0.005$

The percentage of the ^{14}C translocated from the fed shoot of the R1 ramet, passing to its own roots and to the shoot and root of the attached ramet are shown in Table 6.2. Most of the small amount (6% or less) exported from the fed shoot

of the R1 ramets was supplied to its own root and less than 2% was allocated to the partner R2 ramet. Where a R1 +P shoot was fed $^{14}CO_2$ most of the exported ^{14}C went to its own root but of the small amount exported from the fed shoot, significantly more went to the roots of the -P partner R2 ramets ($p<0.05$). When a R1-P shoot was fed, least went to the roots of the R2+P partner, in fact more labelled assimilate went to the roots of R2 -P ramets irrespective of whether the 'donor' was a +P or -P ramet. There was no significant difference between the amount of ^{14}C exported by +P or -P shoots.

Where the R2 shoot was fed, again most of the exported ^{14}C went to the root of the fed shoot. R2 +P shoots sent more assimilate to the shoots than to the roots of a -P partner. Of the ^{14}C sent to the R1 ramets most went to the roots of the +P ramet in the **+P** -P pair (to the ramet shown in bold) and least went to the roots of the R1 -P ramet in the **-P** +P pair. Significantly more ^{14}C was exported to the R1 ramet in the **+P** -P pair than in any other pair and significantly less to the roots of the fed -P R2 ramet in the heterogeneous pair ($p<0.05$). Slightly more ^{14}C was exported by -P than by +P shoots but only in heterogeneous pairs.

Where the R1 shoot was fed, the R:S ratio of allocation was greater in each case for a -P R2 ramet. Where the R2 shoot was fed the greatest R:S ratio was in the +P R1 ramet of the **+P** -P pair and the least in the -P R1 ramet in the **-P** +P pair.

Table 6.3 shows the percentage distribution of the small amount of the labelled photoassimilate exported from the fed shoot and transported to the root of the fed ramet and to the shoot and root of the partner ramet. Of the labelled photoassimilate transported to the R2 ramet the highest proportion was exported to the partner by the R1 of the **+P** -P pair where of the 2.4% exported a little over half went to the R2 ramet. Where the R2 shoot was fed, a smaller proportion was exported to the R1 partner. Here between 10.1 and 41.7% of the exported ^{14}C passed out of the fed ramet into the older ramet.

Table 6.3 *Percentage distribution of total activity of ^{14}C exported to the root of the fed ramet and to the root and shoot of the unfed partner when the shoot of one ramet was fed 185Bq ^{14}C for 45 minutes and the ramets were harvested after 24 hours. The fed ramet is marked ✪.*

	(a) Shoot of older ramet, R1, fed ^{14}C ✪			
Treatment	Root R1	Shoot R2	Root R2	% of export going to R2
+P -P	48.2 ± 15.9	18.0 ± 3.4	33.8 ± 13.4	51.8 ± 15.9
-P +P	82.9 ± 8.0	15.8 ± 7.1	3.0* ± 1.7	18.8 ± 8.7
+P +P	74.0 ± 15.0	24.8 ± 15.4	1.2* ± 0.4	26.0 ± 15.4
-P -P	73.6 ± 12.7	11.8 ± 5.7	15.3 ± 12.5	27.1 ± 12.4
	(b) Shoot of younger ramet, R2, fed ^{14}C ✪			
Treatment	Shoot R1	Root R1	Root R2	% of export going to R2
+P -P	15.9* ± 1.7	25.8 ± 8.0	54.6* ± 7.7	41.7* ± 11.0
-P +P	6.7 ± 1.3	3.3 ** ± 2.5	90.1 ± 3.2	10.1 ± 3.1
+P +P	6.1 ± 1.6	5.5** ± 2.7	88.3 ± 3.4	11.6 ± 3.1
-P -P	8.4 ± 2.4	9.6* ± 8.5	81.5 ± 10.5	17.9 ± 10.7

* = $p < 0.05$; ** = $p < 0.005$

These results are summarised in Table 6.4

Table 6.4 *The fate of 185 Bq ^{14}C fed to the shoot of R1 and R2 ramets for 45 minutes and the ramets harvested after 24 h.*

R1 shoot fed ^{14}C	R2 shoot fed ^{14}C
94% to 98% ^{14}C remained in fed shoot	92% to 96% ^{14}C remained in fed shoot
48% - 83% of exported ^{14}C sent to own root	55% - 90% of exported ^{14}C sent to own root
19% - 50% of exported ^{14}C to R2	10% - 42% of exported ^{14}C to R1
More likely to donate carbohydrate	Less likely to donate carbohydrate

Table 6.4 continued

R1 shoot fed ^{14}C	R2 shoot fed ^{14}C
R1 +P shoot sent more to R2 -P roots than to R2 +P roots	R2 +P shoot did not send more to R1-P roots than to R1 +P roots
R1 -P shoot sent more to R2 -P roots than to R2 +P roots	R2 -P shoot sent more to R1 +P roots than to R1 -P roots
R1 +P & -P shoots sent more to R2 -P roots than shoots	R2 -P shoots sent more to R1 +P root than shoot only in the +P -P heterogeneous pair
R1 +P & -P shoots sent more to R2 +P shoots than roots	R2 shoots given homogeneous treatments sent same proportion to shoots and roots of R1
R1 -P shoot sent least to +P partner	R2 -P shoot sent most to +P partner

The activity expressed as Bq ^{14}C mg dry weight^{-1} of each plant organ where the R1 shoot was fed is shown in Fig. 6.3. There was no significant difference between the tissue specific activities of the shoot of the unfed R2 ramets for any of the treatments but the concentration of ^{14}C in the roots of the R2 ramet was greater in the roots given -P (Fig.6.3). The roots of the ramet given -P in the +P **-P** treatment had a higher specific activity than the R2 ramet in the -P+**P** pair ($p = 0.07$) and in the +P +**P** pair ($p = 0.057$). Although the R2 ramet in the -P -P pair had a higher specific activity than either of the R2 ramets given +P, the difference was not significant. The activity expressed as Bq ^{14}C mg dry weight^{-1} of each plant organ where the R2 shoot was fed is shown in Fig 6.4 When the R2 shoots were fed there was no difference between the specific activities of the shoots or roots for any of the treatments.

Efflux of ^{14}C from the roots

Although between 31 and 44 dpm were recorded for the Long Ashton solution surrounding the roots of ramets this was not significantly different from the mean background count (46.5 dpm) for both the fed or unfed ramets in each of the experiments so any efflux of ^{14}C from the roots of either the fed or unfed ramets was below detection level.

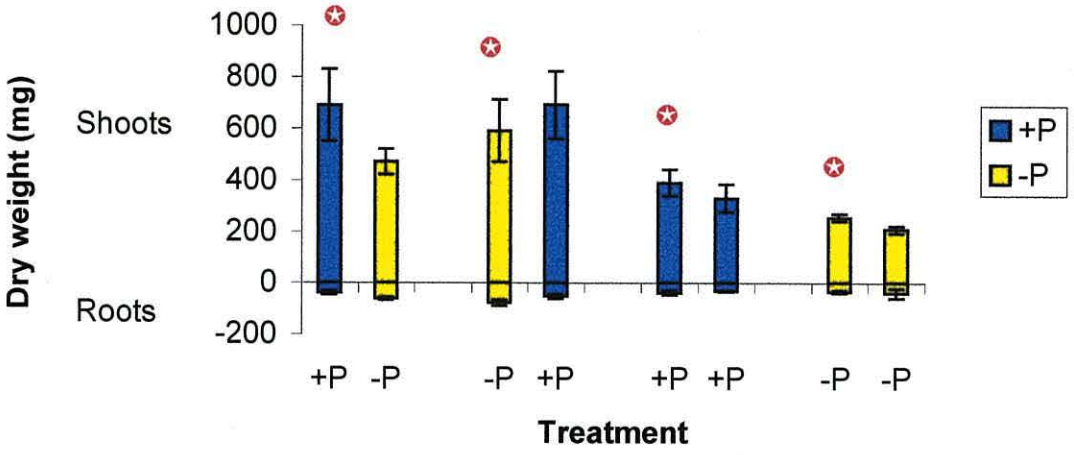


Fig 6.1. *Dry weights of roots and shoots of paired, joined ramets of A. stolonifera grown for 10 days in either 1.33 mmol dm^{-3} phosphate* (+P) *or 0.007 mmol dm^{-3} phos*phate (-P). *Shoots of the older ramet were fed with 185 kBq ^{14}C (marked ✪) for 45 minutes and the paired ramets were harvested 24 hours later. Error bars are 1 standard error of the mean. N = 4*

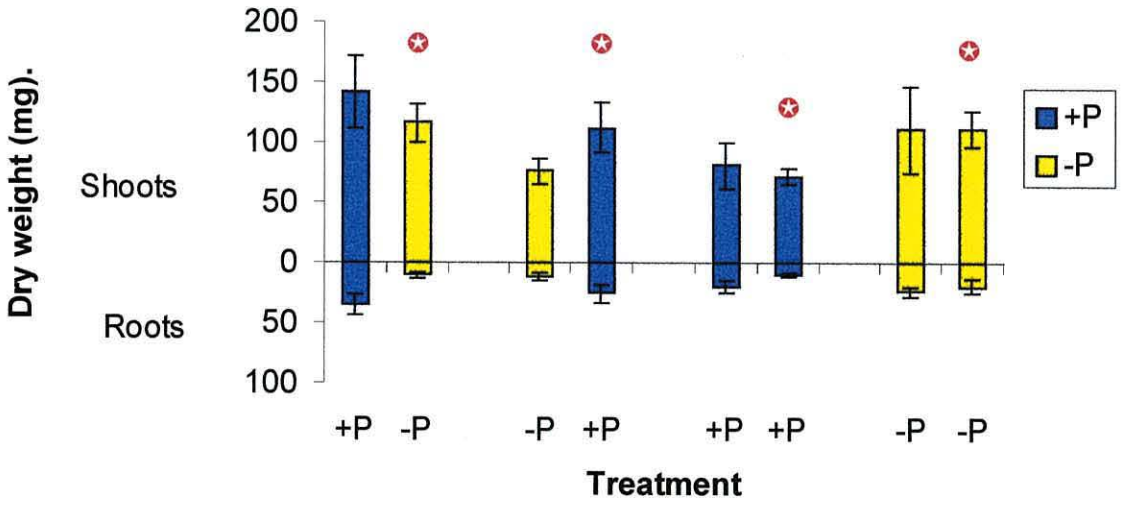


Fig 6.2. *Dry weights of roots and shoots of paired, joined ramets of A. stolonifera grown for 10 days in either 1.33 mmol dm^{-3} phosphate* (+P) *or 0.007 mmol dm^{-3} phos*phate (-P). *Shoots of the younger ramet were fed with 185 kBq ^{14}C (marked ✪) for 45 minutes and the paired ramets were harvested 24 hours later. Error bars are 1 standard error of the mean. N = 4*

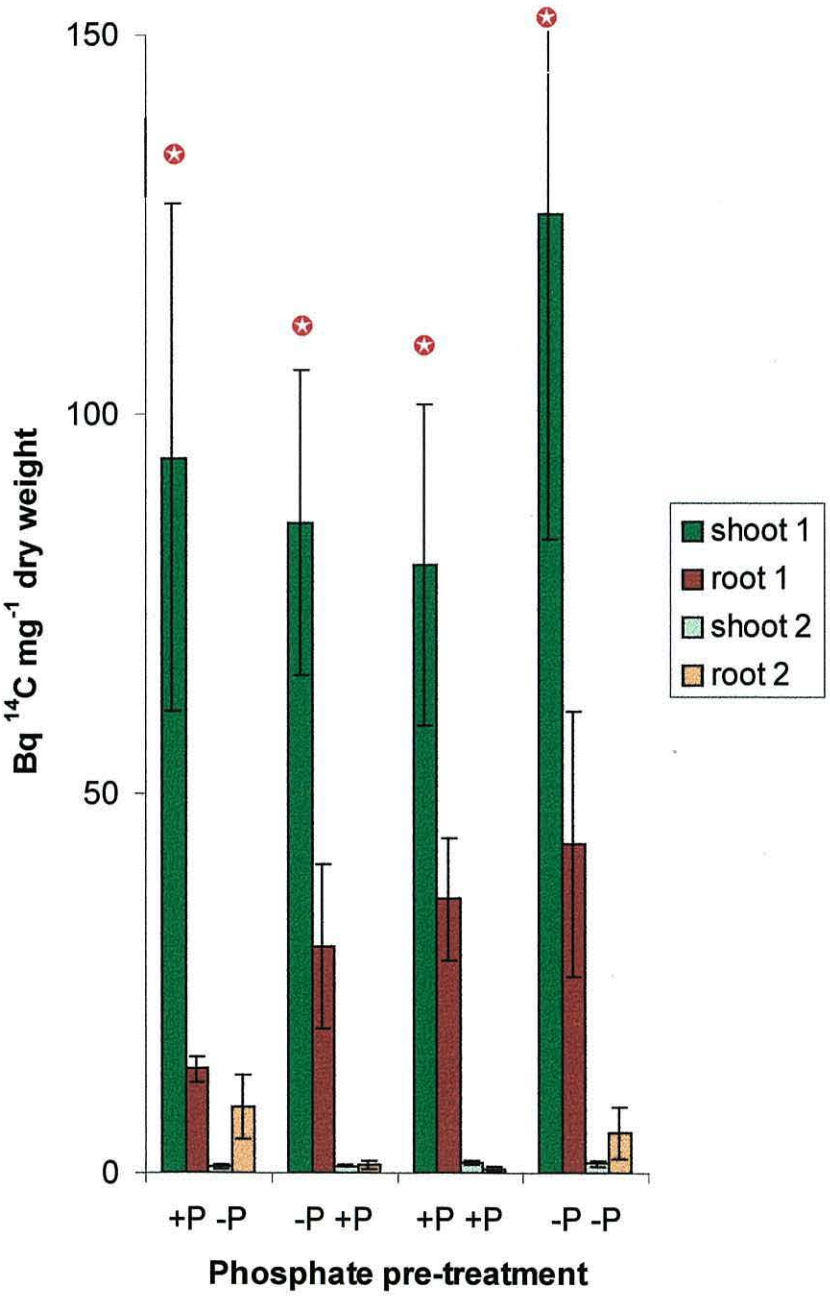


Fig 6.3 *Specific activity of ^{14}C expressed as Bq ^{14}C mg dry weight^{-1} in roots and shoots of paired, joined ramets of A. stolonifera when the shoot of the older ramet (marked ✪) was fed $^{14}CO_2$ for 45 minutes and the paired ramets were harvested 24 hours later. Error bars are 1 standard error of the mean. N = 4*

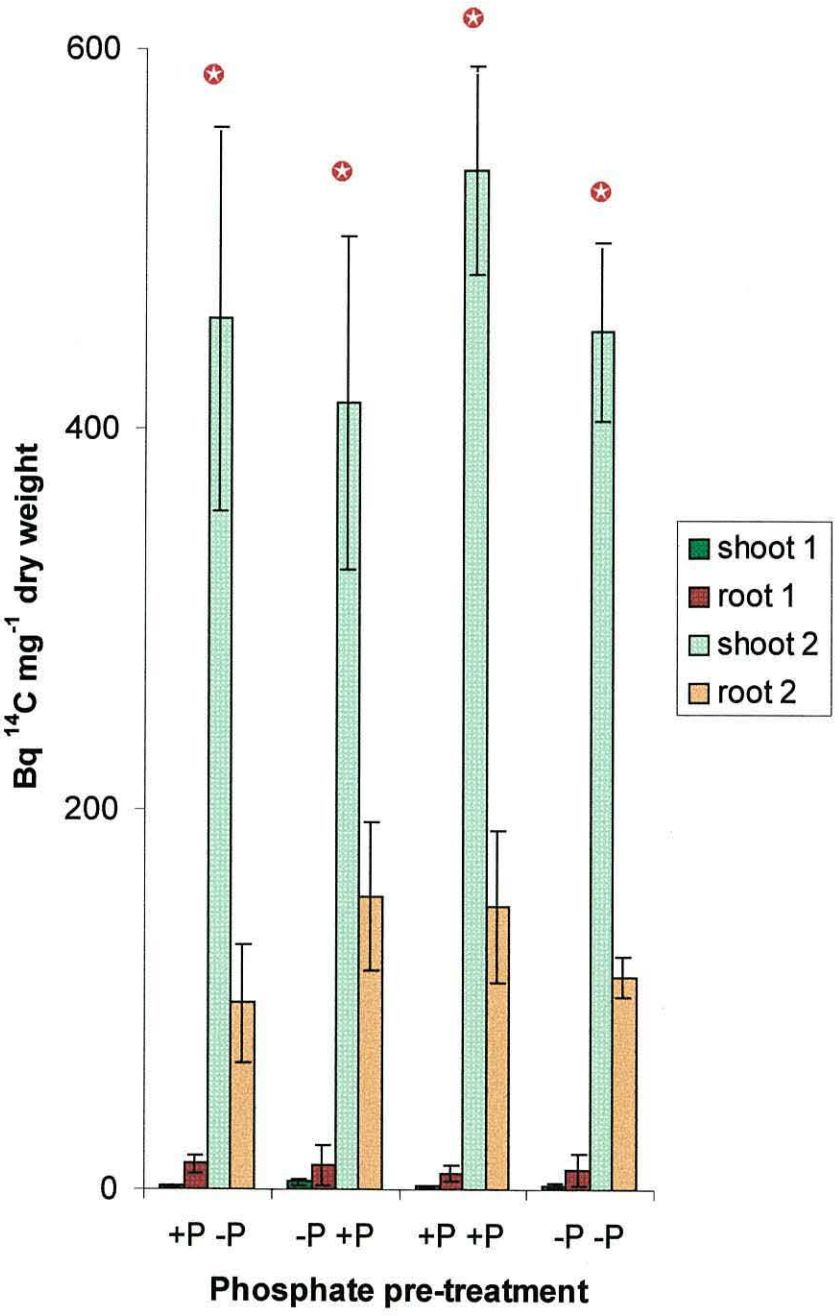


Fig 6.4 *Specific activity of ^{14}C expressed as Bq ^{14}C mg^{-} dry weight^{-1} in roots and shoots of paired, joined ramets of A. stolonifera when the shoot of the younger ramet (marked ✪) was fed $^{14}CO_2$ for 45 minutes and the paired ramets were harvested 24 hours later. Error bars are 1 standard error of the mean. N = 4*

DISCUSSION

A surprisingly small amount of the total assimilate labelled with ^{14}C was translocated from the fed shoot to the nodal roots of the fed shoot - between 1 and 5 % where the R1 ramet was fed and between 2 and 7 % where the R2 ramet was fed. Where the R1 was fed, slightly more went to its own roots if the ramet was P-deprived but this did not occur if a -P R2 ramet was fed. Only a trace - 1% to 2% - had been translocated into the attached ramet at the end of 24 h, either acropetally or basipetally. Since the vascular connections are known to exist between ramets it must be that each ramet is self-sufficient for carbohydrate. This is in agreement with Anderson-Taylor (1982), who reported that ^{14}C, fed to a shoot of *A. stolonifera,* appeared in only trace quantities in the short term, in other parts of a clone consisting of a number of ramets. Stressing other ramets by defoliating or shading did not alter this distribution. Such stress induced the translocation of ^{14}C-assimilate into the roots of stressed ramet after a longer period of 14 days. Thus the hypothesis that photoassimilates are transported to all parts of the clonal fragment is only partly supported for the 24 hours from feeding ^{14}C until harvest in clonal fragments of *A.stolonifera.*

Anderson-Taylor (1982) found that of the total ^{14}C fixed by a branch of an intact stolon of *A. stolonifera,* 88% remained in the fed shoot. When just two rooted ramets were examined in the present study, of the labelled carbon present in the pair of ramets over 90% was found to be in the fed shoot but these ramets were harvested after only 24 hours compared with the 72 hours before harvest in Anderson-Taylor's study. Jinks (1986) reported that the youngest leaves developing at the apex were supported with labelled carbohydrate from a nearby fed shoot. Such young leaves would be a sink for, rather than a source of, carbohydrate but the shoots of the two connected ramets in the present study were both sources of carbohydrate and approximately the same size and thus neither was in need of support from the other. Kemball and Marshall (1995) commented on the distribution of ^{14}C from a source leaf to all parts of a clone of white clover *(Trifolium repens)* with the roots of the parent a major sink for exported assimilate. The branch stolons of *Trifolium* are comparable with the

daughter ramets of *A. stolonifera* except that the branches described in their study were not rooted but supplied with water and minerals by the roots of the parent plant. This may explain their continued interdependence for photo-assimilates but *Trifolium* is said to show a high degree of clonal integration i.e. to share resources between ramets and to show support by resource-rich ramets for stressed siblings. Chapman *et al.* (1992) found that in differentially defoliated clones of *T. repens* there was strong reciprocal exchange of radiolabelled carbohydrate between parent and daughter ramets, with up to 40% of ^{14}C exported from the leaves of the parent to the branches and up to 50% translocated from branch stolons to the parent. Branch to branch translocation of assimilate was also recorded. Such integration in *Carex bigelowii* is developmentally programmed and non-plastic (Jonsdottir & Callaghan, 1988, 1989; Jonsdottir & Watson, 1997) so old, subterranean ramets take up water and minerals which are distributed to the rest of the clone but are supported with photoassimilates from the two or three youngest ramets, the only ones to bear photosynthetic shoots.

When the R1 shoot of *A. stolonifera* was fed, more ^{14}C was allocated to the roots than to the shoots of -P R2 ramets whereas more was allocated to the shoots than to the roots of attached +P ramets (Table 6.2 a). Only 0.1% of the total ^{14}C present in the pair appeared in the roots of the attached ramet if this sibling was P-sufficient whereas if the partner ramet had been phosphorus-deprived there was between 0.9% and 1.3%. When the shoot of the R2 ramet was fed $^{14}CO_2$ most of the labelled assimilated exported from the fed shoot appeared in the roots of the +P ramet in the pair given -P **+P** and least in the roots of the fed -P ramet in the +P **-P** pair. The subsamples of *A. stolonifera* used for scintillation counting were from the finely divided material taken from the entire root system, and since low-phosphate roots tend to have fewer branches than do high-phosphate roots, it is possible that there was more ^{14}C present in the root tips of the low-P ramets. Farrar *et al.* (1995) showed that most of the ^{14}C translocated into the roots of barley accumulated in the tips of the roots. Ciereszko *et al.* (1996) found 100% more ^{14}C labelled carbohydrate (mainly as sugar) in

phosphate-deficient bean plants and (1999) reported that low phosphate within bean plants enhanced the uptake of exogenous sugar into the apical meristem and zone of elongation of the roots. Growth experiments reported in Chapter 2 showed that low phosphate resulted in roots which were less branched but longer than +P roots. Farrar *et al.* (1995) suggested that increased turgor leading to increased elongation of the roots could be linked to increased import of carbohydrate into the roots.

Table 6.5 shows that where the shoots of the older, R1 ramets were fed ^{14}C, the total Bq appearing in the roots of -P partner ramets was very much greater than the amount appearing in the roots of +P ramets. However when the fed shoot was on the younger, R2 ramet the only R1 roots to acquire more of the total ^{14}C were those of the +P ramet in the **+P** -P pair. Similar amounts of ^{14}C were fixed by the ramets in (a) and (b)

Table 6.5 *Total Bq ^{14}C for roots, shoots, whole ramets and pairs of ramets given the phosphate treatments shown when the shoot of one ramet was given 185 Bq ^{14}C for 45 minutes and the ramets were harvested after 24 hours.*

(a) Shoot of R1 fed ^{14}C								
Bq	+P	-P	-P	+P	+P	+P	-P	-P
Shoot	52739	255	44624	298	33820	189	29668	117
Root	546	541	2401	60	1053	17	1448	400
Total	53285	796	47025	358	34873	206	31116	517
Pair	54081		47383		35079		31633	
(b) Shoot of R2 fed ^{14}C								
Bq	+P	-P	-P	+P	+P	+P	-P	-P
Shoot	266	58015	284	41935	132	37540	182	48621
Root	545	836	191	3370	166	1614	189	2050
Total	811	58851	475	45305	298	39154	371	50671
Pair	59662		45780		39452		51042	

Since there was no difference between the rate of photosynthesis in high and low P ramets (Chapter 2) this indicates that the greater allocation of carbon assimilate to the roots is more a function of their P-status than of the amount of carbohydrate available for partitioning. From Table 6.5 (b) it can be seen that where the R2 shoot was fed ^{14}C, the total for the heterogeneous pairs was not

significantly different but most ^{14}C was allocated to the roots of the +P ramet in the -P **+P** pair and there was no significant difference between the amounts allocated to the roots of the unfed R1 ramets either +P or -P. Hence whatever the signal from the -P roots, ^{14}C assimilate was allocated preferentially to the roots of a P-deprived distal ramet but not to a proximal older ramet lacking phosphorus. The specific activity (Bq mg^{-1}) of ^{14}C in the roots of the fed ramets and their partners (Figs. 6.3 and 6.4) shows that the larger amount of radioactivity was not a result of a larger root holding a greater amount of ^{14}C – the concentration of labelled assimilate was greater in the unfed roots of R2 ramets grown in low P.

Farrar & Minchin (1991) found that the partitioning of labelled photoassimilates between the two halves of split roots of barley correlated with the ratio of the dry weight of each half. A comparison of the partitioning of photoassimilates between the roots of R1 and R2 ramets in each pair showed a strong correlation between the ratios of the dry weight and total activity for the roots of R1 ramets given homogeneous phosphate treatment to their roots and whose shoots were fed $^{14}CO_2$ (Table 6.6). There was no correlation between the distribution of assimilate and root dry weight ratio for pairs of ramets given heterogeneous treatments or where the R2 ramet was given $^{14}CO_2$.

Table 6.6. *The correlation between the ratio of the dry weights of the roots of R1:R2 ramets in each pair and the ratio of total activity (dpm) in the roots for each treatment. n = 4*

	Correlation	
	R1 ramet fed	R2 ramet fed
+P -P	$r = 0.767, p > 0.1$	$r = 0.361, p > 0.1$
-P +P	$r = 0.214, p > 0.1$	$r = 0.683, p > 0.1$
+P +P	$r = 0.966, p < 0.05$	$r = 0.472, p > 0.1$
-P -P	$r = 0.999, p < 0.05$	$r = 0.256, p > 0.1$

Where paired ramets were given the same treatment, photoassimilate was distributed from the shoot of the older ramet *pro rata* to the roots of the pair. For ramets given contrasting phosphate treatments this was not the case. Neither was ^{14}C distributed according to the ratio of dry weights of the roots from the shoot of a younger ramet to an older. The roots of adjacent ramets can be

compared with split roots of a unitary plant but whereas in a unitary plant there is a single shoot, in a clonal fragment there were paired shoots differing in P-content because their roots have had different treatments. The P-status of the shoot alters the R:S ratio of dry weights but does not affect the rate of photosynthesis. However low P results in a smaller shoot so there are fewer photosynthetising leaves. The roots of pairs of ramets given homogeneous phosphate treatments (+P +P and -P -P) may be considered to be 'equivalent sinks' (Minchin *et al.,* 1994) having the same dry weight, the same P concentration, presumably the same growth rate, so the parameters determining the dry weight gain may be assumed to be the same for the roots of both partners. Thus ^{14}C will be unloaded at the sinks in equal proportions and because the dry weights of the sinks are the same this will be in proportion to their dry weight, at least in the short term of the experiment described and where delivery is from the shoot of an older ramet. Implicit in the correlation between ^{14}C and dry weight is that there are free vascular connections from the fed shoot of R1 to the roots of both R1 and R2 ramets. Allocation from the shoot of a younger ramet to both roots may not be so near equality if the basipetal vascular connections are more restricted. The dry weights and P concentration in the roots of pairs given heterogeneous treatments show that the roots in these pairs are not equivalent sinks and the supply of ^{14}C taken up is not distributed *pro rata.* More ^{14}C is allocated to the roots of low-P ramets than to high P. Thus partitioning of ^{14}C between the roots of +P and -P ramets is influenced at least in part by the P-status of the roots.

Farrar & Minchin (1991) found efflux of ^{11}C from the roots of barley after 40 minutes, mostly due to respiration. There was no measurable increase in activity above background in the bathing nutrient solution surrounding the roots of either high or low phosphate ramets, of either older or younger ramets in the 24 hours between feeding and harvesting *A. stolonifera* but this may be a reflection of the very low concentration of labelled carbohydrate in the *Agrostis* roots. This suggests that the small amount of labelled assimilate found in the roots of high P

ramets was not the result of rapid respiration of imported labelled assimilate and loss of labelled carbon dioxide to the bathing medium, but of lower import into these roots than into -P roots.

CONCLUSIONS

1. Very little ^{14}C is exported from the shoot in which it is fixed to other parts of a clone of *A. stolonifera.*
2. Photoassimilates are translocated to the roots of a ramet of *A. stolonifera* whose shoot is fed ^{14}C but less than 2% is translocated to adjacent ramets.
3. The phosphorus status of a ramet does not alter the distribution of photoassimilates significantly but there is a tendency for more assimilate to pass into low-phosphate roots of both the fed shoot and of a younger, low-phosphate, attached ramet.
4. The allocation of photoassimilate to roots is correlated with the ratio of the dry weight of the roots of R1 ramets given homogeneous phosphate treatment but not with those given heterogeneous treatments and not if the fed ramet is the younger.
5. The daughter ramets of *A. stolonifera* are more-or-less independent of each other with regard to carbohydrates. The small amounts of activity found in the unfed ramet shows that the phloem connections are intact but that ramets have a separate carbon economy.

Chapter 7

General Discussion

Previous studies have examined the allocation of resources in non-clonal plants and in whole stolons of clonal plants including *Agrostis stolonifera* (Burd, 1947; Asher & Loneragan, 1967; Milthorpe & Moorby, 1969; Clarkson *et al.,* 1978; Ericsson & Ingestad, 1988; Chapin *et al.,* 1989; Marshall & Anderson-Taylor, 1992 *etc.*). This thesis reports on the allocation of water, phosphorus and photoassimilate using clonal fragments consisting of pairs of connected ramets of *A. stolonifera* to reduce the effect of the age of ramets and to remove many competing sinks from the system.

What are the effects of different phosphorus allocation between pairs of ramets?

Results of experiments described in Chapter 2 show that the effects of shortage of P were dose-dependent and evident after 14 d, in pairs of ramets of *A. stolonifera* and resulted in lower dry weight, fewer leaves, branches and basal tillers, more rapid senescence of leaves and reddening of stems due to production of anthocyanin. These effects have been reported by many workers (Burd, 1947; Asher & Loneragan, 1967; Milthorpe & Moorby, 1969; Clarkson *et al.,* 1978; Ericsson & Ingestad, 1988; Marschner, 1995). Where pairs of ramets were given heterogeneous treatments their combined dry weight was intermediate between that of those given homogeneous high or low P. The combined dry weight of pairs given +P -P was halfway between that of +P +P and -P -P pairs whilst the combined dry weight of -P +P ramets was halfway between the +P -P and the +P +P pairs. The pairs given -P +P consistently had a greater dry weight than those given the reciprocal treatment mainly due to the weight of the +P R2 ramet which had a dry weight similar to, or even greater than, that of the +P +P ramets. Thus the -P ramets were supported by the +P ramets as reported by Pitelka & Ashmun (1985) and Marshall (1990). It is also in agreement with reports that plants grown in a patchy environment may be heavier than those grown in a homogeneous nutrient-rich environment, as shown in *Glechoma hederacea* (Birch & Hutchings, 1994) by enhancing the rate of uptake of

localised Pi (Raghothama, 1999). Fig. 2.30 shows that the total P content of -P -P ramets was approximately 11% that of the +P -P ramets although the dry weight was about 50%. This is in agreement with previous findings, that low P plants make use of phosphorus more efficiently than do high P plants (Qiu & Israel, 1992; Marschner, 1995; Mimura, 1995; Raghothama, 1999; Rao *et al.*, 1999). Support for a P-deprived R2 ramet resulted in a lower dry weight in the donor R1 ramet as found by Salzman & Parker (1985) and by Caraco & Kelly (1991). ^{32}P experiments show that phosphate is transported acropetally from a proximal P-sufficient to a distal P-deprived ramet and also, but to a less extent, basipetally, in agreement with Anderson-Taylor (1982).

Marschner *et al.* (1996) state that because of the gradients in water potential of the various shoot organs, backflow of solutes in the xylem from these shoot organs in a basal direction rarely occurs, except in fleshy fruits. However this is probably only true for non-clonal plants. Basipetal and acropetal transfer of resources is confirmed in experiments in which dyes were used to trace the vascular connections between ramets and shows that materials can move in opposite directions in the xylem vessels of separate vascular bundles in the same internode and at the same time. Although d'Hertefeldt & Jonsdottir (1999) showed that both basipetal and acropetal movement of acid fuchsin are possible in *Carex arenaria* they did not report on the bi-directional movement of contrasting dyes.

As reported for a range of plant species (Fredeen *et al.*, 1990; Paul & Stitt, 1993; Cakmak *et al.*, 1994; Jeschke *et al.*, 1997), an inadequate supply of phosphate increased the root:shoot ratio (R:S) of dry weight in *A. stolonifera.* The reason for this is that although both roots and shoots of -P -P ramets had a lower dry weight than +P +P ramets, the effect was greater in the roots than in the shoots (Fig. 7.1) as found by Paul & Stitt (1993) and Cakmak *et al.* (1994). The dry weight of the roots and shoots and the R:S dry weight ratio was found to be correlated most closely with the P concentration in the shoots and least with the P content of the roots so control of R:S ratio in *A. stolonifera* is in the shoots rather

than in the roots. There are no references in the published literature to sensing and control of the R:S dry weight ratio by the shoot but the rate of uptake of phosphate by the roots is controlled by the P-concentration in the shoot (Raghothama, 1999).

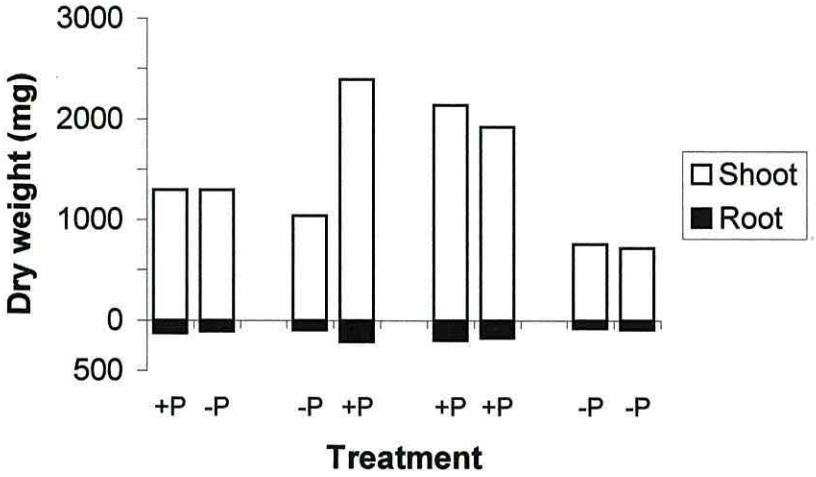


Fig 7.1 *The mean dry weights of roots and shoots of each of paired ramets given the contrasting phosphate treatments shown. +P = 1.33 mmol dm^{-3} phosphate; -P = 0.007 mmol dm^{-3} phosphate*

Resources are allocated by non-clonal plants to the plant organ which is undergoing shortage of that resource (Thornley & Johnson, 1990; Minchin *et al.*, 1994; Thornley, 1995). Thus a low R:S ratio of dry weight would lead to an increase in the allocation of P to the roots to increase their growth so that more phosphate could be taken up. This is specialisation for shortage. A high R:S ratio, conversely, would lead to greater allocation to the shoot to allow increased growth, photosynthesis and production of carbohydrate. There is evidence that in a heterogeneous environment some clonal plants specialise for abundance. Clonal fragments of four ramets of *Trifolium repens* were grown with two ramets given high light and low water and the other two the reciprocal treatment. The ramets given high light produced heavier shoots and the ramets given high water developed heavier roots (Stuefer *et al.*, 1996). Bieleski (1973), Marschner (1995) reviewing work with *Glycine max*, Jeschke *et al.* (1997) in *Ricinus communis* and Raghothama (1999) reporting on potato (*Solanum tuberosum)* show that low P plants retain more phosphorus than high P plants in their roots

which would lead to an increase in R:S ratio of dry weights. This was probably so for *A. stolonifera* since the R:S ratio of P content was highest in low P ramets (Fig. 2.17) although the amount of P in the roots was greater in +P than in -P ramets (Figs. 2.13, 2.14, 4.15, 4.16; Tables 5.11 and 5.12).

Does the rate of transpiration determine the rate and direction of movement of resources through the xylem?

In Chapter 3 it was shown that basic fuchsin supplied to the roots of a parent ramet moved acropetally more rapidly in the presence of the unrooted part of the main stolon than when this was removed. Examination of the stained stolons showed that the dye not only moved through the xylem but that the rate of movement depended on transpiration from the ramets distal to the point of application of the dye. Where contrasting dyes were supplied to the roots of adjacent ramets, bi-directional flow was observed, showing that reciprocal exchange of resources is possible. Contrasting dyes appeared on opposite sides of stolons where adjacent ramets had their roots immersed in either basic fuchsin or crystal violet. The presence of basic fuchsin in the xylem on one side and crystal violet on the other side of the main stolon shows that the vascular connections between the root and the shoot are sectorial. This would be an advantage in clonal plants in allowing nodal roots to send resources to several of the many ramets on a stolon and thus supporting stressed parts of the clone. Thus different xylem vessels, originating in different roots supply separate parts of the clone. Marshall (1996) pointed out that such sectoriality, as well as determining the pathway of translocation of assimilate will also determine the distribution of metabolites, minerals and viruses present in the phloem. Price *et al.* (1996) traced the pattern of distribution in the xylem of *Glechoma hederacea* using acid fuchsin, and of photoassimilate in the phloem using ^{14}C, and showed that sectoriality can restrict the transfer of toxic material, such as zinc, from one IPU to another. Marshall & Price (1997) showed that whereas for a non-clonal plant sectoriality prevents support of the different branch IPUs which make up the

the plant, sectoriality allows support of parts of a clone growing in resource-poor patches and restriction of damage brought about by environmental stress.

Birch & Hutchings (1999) demonstrated that sectoriality could result in variation in performance of connected ramets of *Glechoma hederacea* depending on the part of the stolon from which they were cut, and thus on their age. Differences in subsequent ability to produce new ramets also depended on the number of ramets in the fragment. Variation occurred in the connected ramets of *A. stolonifera* in the present study, even where adjacent ramets were given the same treatment. This may be because they have different connections which change the pattern of distribution of resources. Such a strategy would be of value within a clone as a rich resource could be spread throughout the clone and be utilised for growth by ramets separated both temporally and spatially. Amelioration of the effects of environmental heterogeneity by sharing resources could also reduce plasticity within a clone (Alpert, 1999). Sectoriality, by allowing differential sharing, could permit plasticity within parts of the clone not supplied from a particular rich source of resource and result in 'foraging' by unsupported ramets to the advantage of the clone. Sectoriality could also spread the load of transpiration in an environment in which part of the clone became water-stressed.

Application of contrasting dyes to the original nodal roots, and to later-developing roots separated from the original roots by a Blu-tack collar, indicate that the original nodal roots supply the main stolon of a ramet and that the basal tillers and also the main stolon are supplied by later-developing roots. This would tend to supply P preferentially to the shoot at the same node as the roots. The dry weight of roots and shoots of pairs of ramets was variable even between pairs which started off the same size and were given the same treatment. Since there is little correlation between the P content and the dry weight of the roots the P content of the roots could be the source of variation. The barrier between the P in the shoot and the P in the environment is the root, so differences in the vascular connections of the original nodal roots and the later-developing roots which supply both the main stolon and the basal tillers could account for

variation in the supply of P to the shoot and hence variation in the dry weight of the shoot. There was no difference between the rate of movement of dyes in +P and -P ramets.

Although the results of experiments described in Chapter 4 did not support the hypothesis that a low-water ramet would obtain more P from a P-sufficient partner than did a high-water ramet there was a strong correlation ($p<0.05$) between the stomatal conductance of water vapour and the P concentration in ramets given no phosphate but attached to a partner given nutrient solution containing phosphate. From this it is deduced that water and phosphate moved together through the xylem although Tanner & Beevers (1990) and Pedersen & Sand-Jensen (1997) state that transpiration is not responsible for the movement of material in the transpiration stream, only for differences in the rate of flow. As transpiration takes place and as phloem carries water away from the leaves the water potential in the leaf cells decreases; a water potential gradient is set up and water is brought into the leaf and with it, by mass flow, ions. The rate of transport of dye through the xylem of paired ramets and whole stolons of *A. stolonifera* was fastest where there was a greater transpiring surface, thus when the unrooted part of a long stolon was removed and the transpiring surface reduced, the rate of transport of dye slowed down. One of the effects of high phosphate is to increase the number of leaves and hence the area through which transpiration can take place. However the movement of water and dye through the xylem is not necessarily the same as the movement of phosphorus. Comparison of the rate of transpiration in *A. stolonifera* by weight-loss over 6 hours, showed that +P plants lost a greater percentage weight of water than -P plants (data not shown). This is to be expected since the +P plants were larger and leafier. Comparison of stomatal conductance, measured using a porometer or CIRAS, in plants grown hydroponically, showed no difference in the rate of transpiration from the youngest fully expanded leaf of +P and -P ramets whereas, for plants grown in sand, individual leaves of high P plants supplied with adequate water transpired at a faster rate than those of low P plants. Where plants were subjected to drought stress there was a very marked reduction in the

rate of stomatal conductance of water vapour in high P plants only. Low P droughted plants continued to transpire at the same rate. This effect of high P and low water was noted in barley (Hak & Natr, 1984) in Douglas fir seedlings (Dosskey *et al.*, 1993) and in *Trifolium repens* (Dhananjay *et al.*, 2000). High P, low water ramets of *A. stolonifera* continue to grow as transpiration in other parts of the clone brings a circulation of minerals, including P, into the ramet (Bieleski, 1973) whereas for non-clonal plants closure of the stomata reduces the rate of photosynthesis and thus the rate of growth e.g. of potato (*Solanum tuberosum)* (Cogliatti & Clarkson, 1983) and oil palm (*Elaeis guineensis)* (Smith, 1989).

The pH of the xylem sap of droughted, high P ramets was 7.0 whereas for well watered, high P ramets it was 6.0 In well-watered plants the acidity of the xylem sap inhibits dissociation of abscisic acid (ABAH) in which form it is readily taken up by mesophyll cells. As the pH rises during drought conditions ABAH dissociates to ABA^- which is less readily taken up by mesophyll cells but is taken up by guard cells which therefore close (Taiz & Zieger, 1998). Somehow high P blocks the opening of stomata in droughted plants even though pH rises. If the action were simply due to the buffering capacity of high phosphate the pH would be unchanged.

Do differences in P status lead to differences in the rate of movement of P through ramets?

The vascular connections, both between paired ramets in clonal fragments and along whole stolons of *A. stolonifera,* were apparent when the roots were given contrasting dyes, but these were confined to the xylem. Basic fuchsin supplied to the roots of a parent ramet moved more rapidly acropetally in the presence of the unrooted part of the main stolon than when this was removed. This shows that the dye moved through the xylem and that the rate of movement depended on the rate of transpiration from the ramets distal to the point of application of the dye.

The distribution of P throughout pairs of ramets of *A. stolonifera* was examined using ^{32}P. The ramets in the pairs used in the experiments reported in Chapter 5 were of similar, rather small, size and the effects of pre-treatment with +P and -P were not yet apparent but Radin & Matthews (1989) state that the physiological effects of low P are present in a plant before physical effects become visible. Small ramets were used, to allow ^{32}P to be carried to all parts of the clonal fragment within 24 h and thus to avoid changes in the activity of the isotope due to decay. Despite their similarity in size ^{32}P was found to move at different rates depending on the previous treatment. For pairs given homogeneous high or low P treatment there was little difference in the rate at which the ^{32}P moved from the older to the younger ramet. The greatest difference was seen where the ramets were given heterogeneous treatments. In the +P -P pairs too little was transferred to be detectable by the GM tubes used whereas in the -P +P pairs more than 5 times as much was transferred in the same time. There was no basipetal transfer, detectable by the GM tubes, of ^{32}P from the +P R2 ramets to the R1 -P ramets but ^{32}P was carried from the -P R2 ramets to the +P older sibling. The rate of basipetal transfer from -P to +P was no different from that seen in the homogeneous +P +P and -P -P pairs. Thus whereas in whole stolons the rate of flow of P is probably due to faster transpiration from larger +P ramets since the paired ramets were similar in size, and their rates of transpiration were therefore similar, the direction of flow of ^{32}P in these depends on the P status and possibly reflects the faster growth rate of the +P ramet, the effect being greater in an acropetal direction where there were more extensive vascular connections. Radiolabelled phosphate supplied to the roots of *A. stolonifera* ramets was transported through the xylem to the same shoot and to the shoot of the attached ramet. This was demonstrated by darkening the unfed, monitored shoot and observing the reduction in the flow of labelled phosphate into a monitored leaf as the transpiration rate diminished and restoration of the original rate of flow when the shoot was re-illuminated. From the shoots it was carried in the phloem to the roots as was demonstrated by cooling the main stolon proximal to a fed leaf and observing the decrease in rate of flow until pressure in the phloem built up and forced ^{32}P across the sieve plates (Farrar, 1988).

Clarkson & Scattergood (1982) found that phosphorus moved more rapidly through low P tomato and barley plants and Cogliatti & Clarkson (1983) found the same in potato plants when these were switched from high phosphate to low phosphate. Raghothama (1999) also reported higher rates of translocation of Pi through low P plants but Jeschke *et al.* (1997) found that phosphorus moved more rapidly through P-sufficient castor bean plants. The disparity may lie in the amount of Pi moved. The rate of movement of ^{32}P was measured in whole stolons. Small amounts of P were observed to move rapidly along the stolon of a low P ramet whereas a larger amount moved at a slower rate through a similar high P stolon. This was probably the result of the larger size of the strongly-growing ramets along the length of the +P stolon removing P as required and reducing the rate of flow along the stolon. This demonstrates that rather than a low P ramet signalling its shortage, P circulates through the plant and is removed as required by each sink. The fact that the low P ramets along the length of the stolon did not take what they could might be explained by the sink strength of the apex of the main stolon being greater than that of the apices of ramets along the stolon. It was, however, noticeable that whereas xylem sap was easily collected from pairs of ramets given either heterogeneous or homogeneous low P treatments, pairs given homogeneous high P yielded little or no xylem sap. Conversely, Caraval *et al.* (1996) reported that the rate of flow of sap decreased in low P wheat plants and suggested that this might be due to reduction in the activity and abundance of water channels in the roots.

The rates of influx of ^{32}P were calculated for the root and shoot of each ramet in the pairs given each of the phosphate treatments. In the heterogeneous pairs the pool of P was greater in the +P R2 root fed ^{32}P (4638 nmol, Table 5.12) than in the fed +P R1 root (928 nmol, Table 5.11). Nevertheless only 0.1 nmol h^{-1} arrived in the R1 -P roots proximal to the fed ramet whereas 2.59 nmol h^{-1} arrived in the R2 -P distal ramet. In all cases, where a -P root was fed ^{32}P, little arrived in the roots of the attached ramet, but the fed +P R1 ramet in the **+P** +P pairs supplied thirty times as much to the roots of its younger partner than it received from it.

What effect does P status have on the allocation of P to vacuoles and cytoplasm?

Differences in the allocation of P occurred in *A. stolonifera,* between the cytoplasm and the vacuoles of the oldest and youngest leaves as reported for barley leaves by Mimura *et al.* (1996). In the youngest leaves, for both +P and -P ramets, approximately 95% was in the vacuole and 5% in the cytoplasm in agreement with many authors including Farrar & Farrar (1985), Lauer *et al.* (1989), Lee & Ratcliffe (1993) and Marschner (1995). For the oldest leaves on the R1 ramets, where the pair had access to high P the % P in the vacuoles was nearer 86% but the -P -P leaves still had over 90% of their phosphate in the vacuoles. In the R2 ramets those given homogeneous +P +P or -P -P had more that 90% in their vacuoles and the high P R2 ramet had the lowest % P in the vacuoles with only 82%. In the youngest leaves of the R1 ramet the highest cytoplasm:vacuole ratio of phosphorus concentration was in the two sets of -P ramets. This indicates that the vacuoles were releasing P into the cytoplasm to maintain the concentration there. Thus senescence made a difference to the allocation of P between the cytoplasm and the vacuoles (Mimura, 1995). It was hypothesised that to ensure a constant amount of P in the cytoplasm there would be less P in the vacuoles of P-deficient ramets than in P-sufficient, and this proved to be the case (Table 5.6). Paul & Stitt (1993) point out that the vacuole makes up about 95% of the cell volume and the cytoplasm 5%. However if allocation to these two compartments were simply that the available Pi was distributed homogeneously it would be expected (a) that loading into the compartments would overlap – and this does not occur (Fig. 5.5.2) and (b) that as the cell senesced, the C:V ratio of Pi would remain unaltered.

Do differences in P status lead to differences in the rate of photosynthesis?

There was no difference in the rate of stomatal conductance of water vapour for either high or low P ramets grown hydroponically. Measurement of the rate of photosynthesis was made on the youngest fully expanded leaf of the main stolon of each ramet of a connected pair grown hydroponically. There was no difference between the rate of carbon assimilation for +P or -P ramets. This is in

agreement with Ciereszko *et al.* (1996) who found no reduction in the rate of photosynthesis in low-P plants whereas Milthorpe & Moorby (1969), Dietz & Foyer (1986), Heineke *et al.* (1989), Rao & Terry (1995) and Jeschke *et al.* (1996) all saw a reduction in the rate of photosynthesis in P-deprived plants. For pairs of ramets of *A. stolonifera* grown in sand, the rate of photosynthesis was slightly reduced by shortage of water but differences in P status caused no change in the rate of photosynthesis. Foyer & Spencer (1986) also found no reduction in the rate of photosynthesis attributable to low P in barley and concluded that differences in photosynthetic rate in response to low P depend on the plant species.

Does the P status alter the allocation of carbohydrate?

The differences in the allocation of labelled carbohydrate were investigated as described in Chapter 6. Photosynthate containing ^{14}C was translocated preferentially to -P roots (acropetally at least) (Cakmak *et al.*,1994; Farrar *et al.*, 1995; Jeschke *et al.*, 1996, 1997). This might be considered a 'functional equilibrium' (Farrar *et al.*, 2000) between carbon and phosphorus so longer roots of -P ramets could scavenge for phosphate. Plant roots proliferate in a band of rich nutrient (Drew & Saker, 1975). It would be of advantage to a clone of ramets if the +P ramets invested in a number of widely spreading branch roots to take up immobile phosphorus from the soil at a one level whereas low P plants, lacking the resources to produce adequate branches, invested in increasing the length of their roots to tap less exploited sources of phosphate from a lower level in the soil This, with increased numbers of P-carriers (Ullrich-Erberius *et al.*, 1984) could increase the uptake of P which would be carried to the shoot but would be diverted to the stronger sink of the +P partner. This in turn would make more carbohydrate available for -P roots to allow increase in growth in length (Thornley, 1972). This may well happen but does not explain why the extra carbohydrate should go to the low P rather than to the high P roots which would be assumed to be stronger sinks. Transfer of material through the xylem appears to be more rapid in low P than in high P ramets and the same might be true for translocation through the phloem.

Traces of labelled carbohydrate moved to all parts of the pairs of ramets of *A. stolonifera* in the phloem, especially into the roots of low P ramets. There is evidence that the movement of sucrose through plants *via* the phloem is sensitive to the sugar status of both source and sink (Farrar *et al.* 2000). P status did not alter photosynthesis so control must be in the root itself. The results demonstrate that vascular connections exist between adjacent ramets through which assimilate can be sent to other ramets along the stolon although the ramets appeared to have separate carbohydrate economies and most assimilate remained in the ramet.

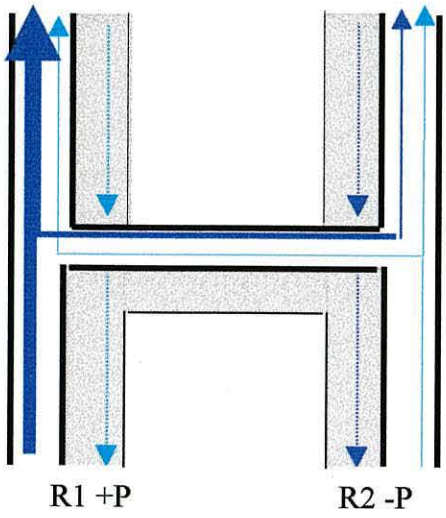
What proportion of the control of the growth of A. stolonifera resides in sinks, what in sources and what in the connections between?

Low phosphate affects the utilisation of carbohydrate, not the rate of photosynthesis (Paul & Stitt, 1993; Cierezsko *et al.,* 1996). Low phosphate concentration in the ramets of *A. stolonifera* did not affect the rate of photosynthesis but resulted in increased transport of carbohydrate into their roots. The P-status of either the roots or of the phloem must alter the flux of ^{14}C into low P roots. A low concentration of P in the roots resulted in longer roots than those of high P ramets as shortage of a resource often results in increased allocation to the organ responsible for the uptake of that resource (Thornley, 1995; Farrar, 1996a). The R:S ratio of dry weights of P-deprived ramets also increased. However the R:S ratio correlated closely with the P-concentration in the shoots and the shoot dry weight correlated well with the P concentration in the roots. A greater dry weight in shoot was the result of a greater number of leaves and thus the overall ability of the ramet to fix carbon dioxide, although the rate of photosynthesis in individual leaves was unaffected by P-status. Growth thus depends on balanced acquisition and distribution of resources (Farrar, 1996b).

Split root experiments provide two root treatments to a single shoot. Clonal fragments allow two treatments to the roots and the effects can be observed in separate but connected shoots given the same or contrasting treatments. Pairs of ramets given homogeneous +P +P or -P -P treatments distributed labelled

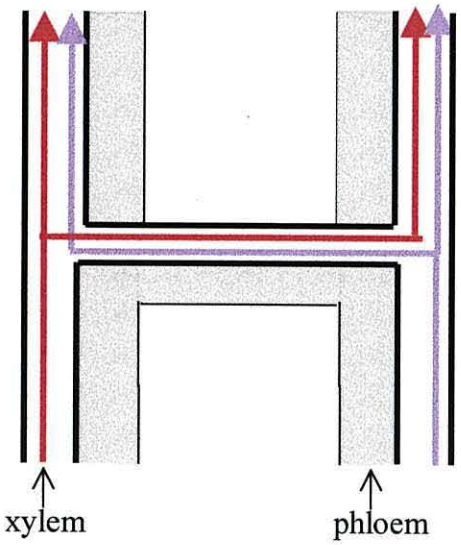
photoassimilates to the roots depending on the dry weight of the roots whereas those given contrasting P-treatments did not. Since a very small amount of carbohydrate was exported from a fed shoot and since different amounts were directed acropetally from the amount directed basipetally the view is supported that such allocation is "source-driven". This is further supported by the observation that the xylem vessels in any vascular bundle supply a particular part of the clone. Such sectoriality must also, presumably, apply to the phloem sieve tubes in those bundles. Dye and phosphorus appear to move readily from one ramet to another and the fact that assimilate appears not to, is a function of the source although Price *et al.* (1996) found that movement of ^{14}C in the phloem in *Glechoma hederacea* did not exhibit such clear sectoriality as movement through the xylem. The P status of ramets of *A. stolonifera* altered the rate at which phosphorus moved through the xylem. It may be that P status also alters the rate of translocation of carbohydrates through the phloem. The pattern of supply of ions to sinks *via* the phloem tends to reflect the relative demand for carbohydrate (Marshall, 1990).

Several mechanisms have been suggested to account for the preferential direction of photoassimilate to the roots of low P plants. The pattern of supply of ions to sinks tends to reflect the relative demand for carbohydrate (Marshall, 1990). Plant growth substances may be involved as where these are locally applied, radio-labelled tracers accumulate (Marshall, 1990). However growth substances might be expected to be more abundant in larger +P roots. A smaller supply of carbohydrate, such as that delivered by plants with fewer photosynthetising leaves, is itself a signal to sinks *via* changes in gene expression (Farrar *et al.*, 2000). The lack of radioactivity in the bathing nutrient solution surrounding the roots suggests that the small amount of labelled assimilate found in the roots of high P ramets was not the result of rapid respiration of imported labelled assimilate and loss of labelled carbon dioxide to the bathing medium, but of lower import into +P roots than into -P roots.

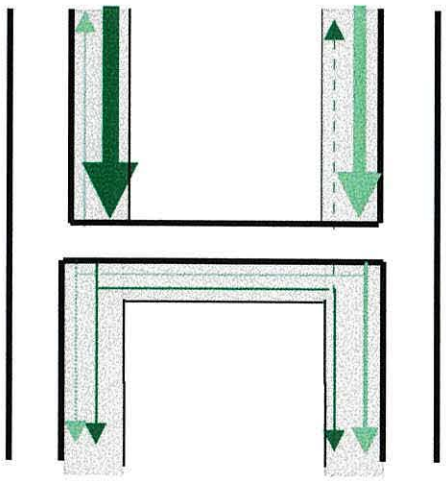


(a) $Pi/^{32}P$ supplied to the roots of the R1 and R2 ramets. Pi is transported from the roots to the shoots in the xylem and circulates back to the roots in the phloem. More is carried from the roots of a +P R1 ramet towards a -P R2 ramet than from a -P R2 ramet basipetally to a +P R1 ramet.

The amounts carried varied with the phosphate treatment given to the roots beforehand.



(b) Basic fuchsin applied to the roots of the R1 ramet and crystal violet to the roots of the R2 ramet. Pre-treatment with high or low phosphate had little effect on the rate of flow of dye. The dye was confined to the xylem, was not seen to enter the phloem and did not enter roots except those where it was applied.



(c) $^{14}CO_2$ provided to the shoots of both ramets. Very little assimilate was translocated from the fed ramet to the partner, but where the partner was pre-treated with low P more ^{14}C was found in its roots.

Fig. 7.2 *Diagrams to show the movement of Pi, dye and carbohydrate through paired, joined ramets of A. stolonifera in which the older R1 ramet was given +P (1.33 mmol dm^{-3}) phosphate and the younger R2 ramet was given low P (0.007 mmol dm^{-3}) phosphate. The thickness of the arrows indicates the relative amounts of material moved but is not proportional. Open bars represent the xylem; shaded bars represent the phloem. Dark colours show movement of resource from R1 to R2; light colour shows movement from R2 to R1.*

How are resources transported through paired ramets?

Fig. 7.2 summarises the pathways of phosphorus, contrasting dyes and photoassimilates through clonal fragments of *A. stolonifera.* The diagrams show the pathways in paired ramets given +P -P treatment – the patterns for ramets given -P +P, +P +P and -P -P are slightly different, except for the passage of dyes where pre-treatment with high or low phosphate had little effect on the distribution.

Further work

Further work would include detailed analysis of the carbohydrates present in the roots of P-sufficient and P-deficient ramets to establish whether the extra carbohydrate in low P roots is part of the metabolic or storage pools. If the former then it can be deduced that the carbohydrate is required immediately and the control is "attraction", and thus control, by low P roots. If the latter then the carbohydrate is directed to these roots from the source in response to a signal from the sink. A study of the rates of respiration in the roots of +P and -P plants would determine the fate of the extra carbohydrate arriving in the -P roots although total respiration is said not to be much altered in P-deficient roots as alternative respiration pathways, not involving P-compounds, are utilised (Theodorou & Plaxton, 1993; Plaxton, 1996). Experiments with labelled carbon dioxide, similar to the "pulse and chase" experiment carried out for ^{32}P in the xylem, would establish how the P-status could alter the rate of delivery of carbohydrate to sinks and elucidate the control exerted by the delivery system between source and sink within the ramets. If sufficient sap could be collected from the stumps of the shoots of paired ramets given contrasting phosphate treatments, analysis of both Pi and Po as well as of carbohydrate could be a novel approach to analysis of inter-ramet relationships.

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