ANALYSIS OF ROOT ACTIVITY PATTERNS OF TWO COEXISTING ANNUAL SPECIES (*DIMORPHOTHECA PLUVIALIS* AND *SENECIO LITTOREUS*) USING LITHIUM AS A NON-RADIOACTIVE TRACER.

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Botany Honours
Physiology project
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1993

ABSTRACT

The relative root activity and below-ground competitive abilities of two coexisting species, *Dimorphotheca pluvialis* and *Senecio littoreus* were examined using lithium chloride as a root tracer. Samples were taken from plants growing in relative isolation and in pairs of both species. Small sample size prevented statistically significant results, however trends indicate that although both plants have similar habits and synchronised life histories, there are differences in tracer uptake in the soil profile. This may suggest different competitive strategies. The potential of the application of lithium as a non-radioactive tracer is discussed.

INTRODUCTION

Explanations for coexistence within densely populated plant communities are various and have been reviewed by Grubb (1977). Where there is little evidence for either separation along above-ground activity or phenological niches, there has been directed interest toward the role of below ground activity in the coexistence of species, especially in homogenous soil environments (Veresoglou and Fitter, 1984).

The role of spatial arrangement of root systems and their relationship with water and solute uptake ability, has been difficult to quantify. Data on whole root system behaviour in the field, have been derived almost entirely from destructive excavation or soil coring, and thus interpretation has been somewhat subjective (Fitter and Strickland, 1992). Alternatively, tracers can be been used to study both the distribution and activity of roots through injection into the soil or plant tissue. Lithium as a non-radioactive tracer conforms to the tracer requirements in that it is taken up easily and relatively easy to detect (Martin et al., 1982). The technique involves inferring rooting patterns and activity in three dimensions by injection

into the soil profile of a small amount of tracer solution in an array around individual plants.
Expand

Examination of spatial patterns of competition for water and solutes between two coexisting species may assist development of theories of below ground competition and coexistence. Because of the quantitative methods employed (eg. soil coring), and subject of study (eg. interspecific variation in root architecture), studies have been mainly conducted on perennial shrubs (Eissenstat and Caldwell, 1988; Manning and Barbour, 1988). Consequently little field experimentation has been carried out on fast growing annuals.

The phenomenon of intense competition amongst herbaceous annuals is of particular importance where plant community formations are in a state of climax or plagioclimax. For example, due to frequent disturbance or a limited to a short growing season. Such conditions are found throughout the strandveld and succulent karoo regions (Acocks, 1974) of the Cape province. These areas, while dominated by semi-arid grasses and shrubs and succulents throughout most of the year, experience a bloom of annuals, notably members of the Asteraceae, during the spring rainfall period. Many of these ephemerals growing in high density populations have essentially similar life-forms and synchronised life histories and are often subject to intensive inter- and intra-specific competition during the short growing period. It is therefore hypothesised that species growing in close proximity to each other, experience either a degree of below ground niche differentiation and/or inter and intraspecific competition. This study examines root uptake ability for two such existing species using a solution of Lithium chloride as a non-radioactive tracer.

This project poses three questions:

1. Does lithium serve as a suitable tracer method of assessing spatial patterns of root uptake activity in ephemeral plants?

2. Can such a derived root spatial pattern help explain the underlying reasons for the coexistence of annual plants?

3. Do the root uptake patterns of *D. pluvialis* and *S. littoreus* suggest niche differentiation or do they actively compete for the same below ground resources?

The utilisation of lithium chloride as a root tracer.

It is suggested that the use of lithium chloride as a tracer is a practical method of indirectly studying root activity. Such a method is based on the principle of determining changes in water or nutrient uptake in different soil layers between successive sampling points in a soil profile and indirectly inferring from these data spatial rooting activity (Bohm, 1979).

Compared to the above ground parts, the study of plant roots is problematic due to the difficulty of direct observation of physiological processes. While rooting patterns can be described generally (eg. Cannon, 1911), there is very little quantitative assessment of actual rooting activity. The root distribution patterns vary considerably in the soil profile but description of these does not necessarily provide evidence for rooting activity. Simple measures of rooting distribution do not necessarily provide evidence of root activity, because this may vary with respect to both age and physiological status of the root and soil conditions (Tofinga and Snaydon, 1992). For these reasons tracers are being more widely used to examine root activity. Radioactive and stable isotopes have been used to examine root distributions (eg. Ellis and Barnes, 1973; Russell and Ellis, 1968) and activity (eg. Newbould et al., 1971), but these methods are expensive and require sophisticated skill and apparatus. Non-radioactive tracers, for example, strontium and boron, have the advantages of being less expensive, easier to handle and not subject to radioactive decay, and have been used with some success for several studies (eg. Pinkerton and Simpson, 1982; Fox and Lipps, 1964). Martin et al. (1982) suggest several criteria for suitability of a non-radioactive tracer:

1. The element should be present in low concentrations in the soil.

2. It should be taken up freely into the shoot system and if possible, should mimic one of the major nutrients.

3. It should be relatively non-toxic, both to plants and animals.

4. It should be easy to determine quantitatively.

Lithium meets these requirements in that it is usually present in low concentrations in many most soils and is easily absorbed by plants, simulating sodium in the uptake processes (Tofinga and Snaydon, 1992). Lithium (in solution as lithium chloride) is not normally toxic but care has to taken to avoid deleterious effects on the plant at high concentrations (eg. Fox and Lipps, 1964). Lithium has an additional advantage over strontium in that it can be detected at lower concentrations (< 0.1 ppm) by atomic emission spectrometry, and even lower (< 0.01 ppm) by atomic absorption spectrometry (Martin et al., 1982). This study used the previously unrecorded plant tissue lithium detection method of ion chromatography which can detect concentrations below 0.001 ppm.

The disadvantages of utilisation of tracers are that the uptake of a particular ion will not necessarily reflect root system activity patterns of all other ions (Bohm, 1979). Therefore caution must be exercised in making general assumptions and drawing predictive conclusions from resulting data.

Lithium has been used as a non-radioactive tracer to measure root activity on several occasions. Sayre and Morris (1940) injected lithium chloride into the soil between rows of maize and sampled leaves spectrographically for the presence of lithium. More recently, studies by Martin et al. (1982) and Fitter (1986) have used lithium tracer solutions to successfully examine competitive root interaction of coexisting plant species.

MATERIALS AND METHODS.

Pilot study.

To initially assess the suitability of Lithium as a tracer 5 ml of 10% lithium chloride solution was injected into the soil profile of pot grown individuals of *Phasoleus vulgaris*. After 48 hours the plant was harvested, oven dried for 48 hours (80°C) and a 500 mg subsample taken. This was digested in a 10:1:1 (by volume) mixture of HNO_3, H_2SO_4 and $HClO_4$, and heated to remove HNO_3 and $HClO_4$, followed by dilution in water. Lithium concentration was analyzed using a Dionex DX-300 High Pressure Ion chromatograph, utilising a Dionex

CDM-3 Conductivity Detector controlled by Dionex AI-450 Chromatography Software System. This involved running a series of solutions of increasing dilutions through the column, until a sample concentration was reached whereby there is a sufficiently high lithium peak to get an accurate level reading but considerably reduced levels of acid, and particularly, sodium which create 'noise' on the chromatogram or distort the lithium peak itself (Appendix 1a). In addition both blanks and controls (Appendix 1b) were analyzed and calibration curves calculated from known Li Cl solutions. This method was repeated for all subsequent samples in this study.

Field study 1.

The initial selected study area was the western slopes of Signal Hill, Cape Town (latitude: 33°54' and longitude: 18°23'). The grass communities cover large areas creating continuous stands with dimensions of up to 50 m across. In addition there are several Renosterveld and Fynbos shrub species (eg. *Elytropappus rhincerotus, Rhus lucida, Euryops sp.*) and several geophytes and herbs. The experimental plots chosen were stands of single and mixed species of *Hyparrhenia hirta* and *Themedra triandra* which were essentially free of any competitive interference from other species. The soils are fine grained and derived from Malmsbury shales. However due to the angle of slope of the study site (30° - 40°) the top soil is less than a metre deep and contains up to 30% stones <20 cm diameter.

The data collection consisted of two phases. Firstly a determination of the three dimensional rooting activity of the *H. hirta* and *T. triandra* and secondly an analysis of root activity in between coexisting plants.

A two dimensional array of samples was taken within the soil profile for both species. Sample points of 3 replicates, were arranged 5 and 15 cm away from the plant centre and at 5 cm, 10 cm and 15 cm depth in the soil. To assess competitive interactions, equidistant pairs of individuals were chosen and sample points (3 replicates) placed midway between them at 5 cm, 10 cm and 15 cm depths.

diameter ?

For each sample, a steel tube was inserted into the ground at the required depth then the tracer solution (10 ml of 10% lithium chloride) directly injected in place into the soil profile. After a period of two weeks the above ground parts of the grasses were harvested and separated into living and non-living tissue both of which were weighed, to give a tracer uptake value in proportion to above ground biomass. To investigate an alternative digestion method, 500 mg subsamples samples were processed utilising water extraction technique as performed by Tofinga and Snaydon (1992). With this technique each sample was dried and milled then a subsample shaken for ten hours in warm water. The solution was then filtered centrifuged and the supernatant used for analysis in the ion chromatograph.

Detection of lithium in these samples was very inconsistent. There was rarely a positive detection of lithium and then only in small quantities (<0.001 ppm). It can only be speculated as to why this experiment failed. It is suggested that one of the problems with the sub-surface growth of grasses is the seasonal growth and death of the root system. In contrast to herbaceous annuals, there is a high probability of injecting a small amount of tracer into a region of dead roots. Alternatively, injection into a particular part of the roots system may not result in translocation of ions into the whole plant, resulting in only a fraction of the aboveground parts containing the tracer. In addition, the heterogeneity of the soil matrix, in particular the presence of large (up to 20 cm diameter) stones, may have resulted in uneven distribution of roots in the soil profile. One final possible error involves the extraction of a subsample. Although the entire living above ground biomass was harvested, it was essential that this sample was well mixed to ensure a representative subsample.

The method of water extraction of lithium was suspected so a further proportion of the samples was taken and acid digested, but without any significant positive results. The further use of water digestion was rejected as an adequate method in this experiment due to the possibility of both inadequate properties of lithium extraction from the plant material, and the likelihood of fungal or bacterial growth in the samples. If not analyzed immediately, any infection, which was observed in a few samples, could result both in lithium removal from the solution and possible damage to the ion-chromatograph column.

As the pilot study conducted with pot grown *P. vulgaris,* where the problems associated with perennial plants are avoided, was successful, a re-run of the experiment was conducted on fast growing annuals

Field study 2.

The second site was an area of perennial and annual grasses and herbs in a cleared area of strandveld dune thicket on the False Bay coast at Muizenberg, Cape Town, latitude 34° 18' South 18° 28' East. The area is predominately thicket species such as *Rhus spp.* and *Chrysanthemoides monilifera* with considerable invasion of species such as *Acacia cyclops*. In several locations there had been considerable man-made site disturbance. In these areas many alien and indigenous ruderal species establish prior to the encroachment of thicket.

Included in these are several members of the Asteraceae normally associated with strandveld. On the chosen site there was a predominance of the two annuals; *Dimorphotheca pluvialis* and *Senecio littoreus,* occurring both in mixed and unmixed stands of several meters area (figures 1 and 2). The soil type was a deep coastal sand (Fernwood form), which presented a more favourable homogeneous soil environment than the previous study site. Both species had similar growth forms and dimensions both above and below ground. Individuals with extreme dimensions were excluded from the study.

Figure 1. *D. pluvialis* community in amongst annual and perennial grasses.

Figure 2. *S. pluvialis* **community in amongst annual and perennial grasses.**

Soil profile arrays followed the same pattern as described for the initial study but with the following exceptions. For the study of the individual species individuals were chosen which were clear of other plants for a 5 cm radius. For study of competitive interactions, pairs of species were selected which were 3.5-5 cm apart (ie. within the observed measured rooting zone) and relatively clear of other individuals around the immediate (5-10 cm radius) area.

An initial pilot study was conducted to ensure success of the lithium application and analysis technique. This was attempted on the *D. pluvialis.* Visual survey of excavation of the respective root systems of both species revealed that the maximum extent of the plant roots were 13 cm depth and 8 cm radius (see figure 4 in results). Injection depths were 3 cm, 6 cm and 12 cm at 2.5 cm and 5 cm horizontal distance from plant stem. The plants were given 2 days to take up the solution, after which the entire plants were harvested and digested using tri-acid as described previously.

Initial chromatography of test samples, although showing the presence of lithium, were insufficient as the high acid content of the samples created too much noise on the chromatograms preventing integration of the lithium peaks. However diluting the samples by factor of 8 resulted in production of measurable data. Following completion of analysis of field samples it was necessary to run a series of calibration samples of lithium concentrations with the chromatograph column under the same conditions. A further problem arising from the test run, was that although the results revealed lithium in all samples, the amount of lithium per unit mass of plant material revealed little variation with respect to injection point. There were several possible reasons for this. Firstly the volume of the tracer was too great (5 ml) which resulted in capillary movement through a large volume of soil thus saturating a large part of the root system. Secondly, the occurrence of rain on the second day following application may have succeeded in spreading lithium down through areas of the upper soil profile. Finally there may have been a wrong assessment of the extent of the root systems, however further field study revealed the initial assessment of root system dimensions was accurate.

For purpose of the final run of samples it was decided to use a smaller volume of tracer solution (2.5 ml) in an attempt to reduce capillary migration through the soil.

Results.

Due to the small sample sizes taken in this study, there were few statistically significant differences. It was similarly impossible to test distributions for normality. However, for the purpose of this study it has been assumed that the distributions are indeed normal and that as future studies would be run with larger sample size, analysis of variance (Zar, 1984) and corresponding Tukey multiple range tests were used to test differences. Although there were few significant differences in the data, discussion has been based on the trends expressed in the data.

Table 1. Lithium concentrations in leaf samples of pot grown *Phaseolus vulgaris* with LiCl solution injected at three different soil depths.

Sample	Li conc ppm/g biomass
Control	0
0 cm depth	6.834
2.5 cm depth	0.382
5.0 cm depth	0.040

This is a very strange unit

Concentrations of the tracer (ppm Li/g biomass) in *P. vulgaris* (table 1) and field plants growing in relative isolation from other individuals, varied according to depth implying changes in root activity with respect to utilised depth (figure 3). This is also reflected in the root distribution of *D. pluvialis* and *S. littoreus* (figure 4).

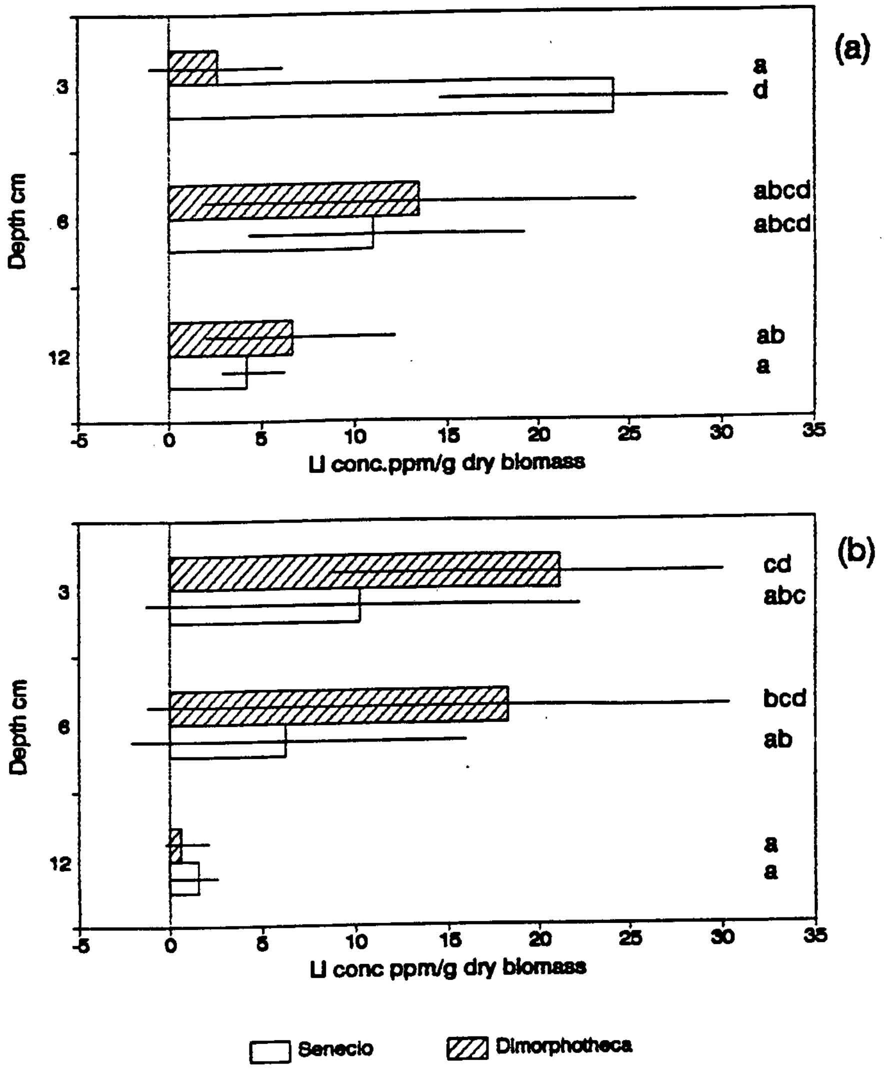

Figure 3. Root activity at three depths (3, 6, 12 cm) and two distances from plant stem (2.5 cm (a) and 5.0 cm (b)), for *D. pluvialis* and *S. littoreus.* Error bars represent S.E. Letters represent homogeneity of groups as determined by Tukey multiple range test ($p=0.05$). Analysis of variance df=11, F-ratio= 1.917, $P<0.1$.

Figure 4. Representative samples of *S. littoreus* (left) and *D. pluvialis* (right).

Generally for both species in the field experiment concentrations decreased with depth at both distances from plant axis, *D. pluvialis* showed low tracer uptake adjacent to the plant near the surface (figure 3a). *D. pluvialis* also demonstrated an increase in uptake 5 cm horizontally from the stem at 3 cm 6 cm depths, whereas *S. littoreus* showed a trend to decrease uptake with respect to both increasing soil depth and distance from plant. These differences were tested using a three way ANOVA (table 2), which only emphasises the significant difference between depth concentrations for both species at depth 12 cm.

Under conditions of single species and both species couplets *D. pluvialis* showed significant
relative to single individual, Fig 3, I presume
decrease in average tracer uptake at 3 cm and 6 cm (figure 5a, table 3.). Evidence of reduction in uptake in *S. littoreus* was negligible (figure 5c, table 4). Tracer uptake by plant couplets of both species indicated reduction in *D. pluvialis* and *S. littoreus* but higher uptake by *D. pluvialis* compared with *S. littoreus* (figure 5b tables 3 and 4).

Table 2. Multiple range analysis for three-way (depth, distance and species) Anova for both *D. pluvialis* and *S. littoreus* growing as isolated individuals. Letter superscript denote multiple range test significant differences.

Factor	ANOVA
Depth 3 cm ^{a} Depth 6 cm ^{b} Depth 12 cm ^{b}	df= 2 F=3.801 Sig lev= 0.0334
Distance 2.5 ^{b} Distance 5.0 ^{b}	df=1 F=0.038 Sig lev= 0.8489
D. pluvialis ^{b} *S. littoreus* ^{b}	df=1 F=0.66 Sig lev= 0.8017

Table 3. Multiple range analysis for lithium concentrations for *D.pluvialis* growing both individually and as pairs with *D. pluvialis* or *S. littoreus*.

	D. pluvialis grown with *D. pluvialis*	*D. pluvialis* grown with *S. littoreus*
Single, depth 3 cm	b	b
Single, depth 6 cm	ab	ab
Single, depth 12 cm	a	a
Paired, depth 3 cm	ab	ab
Paired, depth 6 cm	ab	ab
Paired, depth 12 cm	ab	ab
ANOVA	df=5 F-ratio=1.22 Sig.lev. =0.0336	df=5 F-ratio=1.33 Sig.lev = 0.315

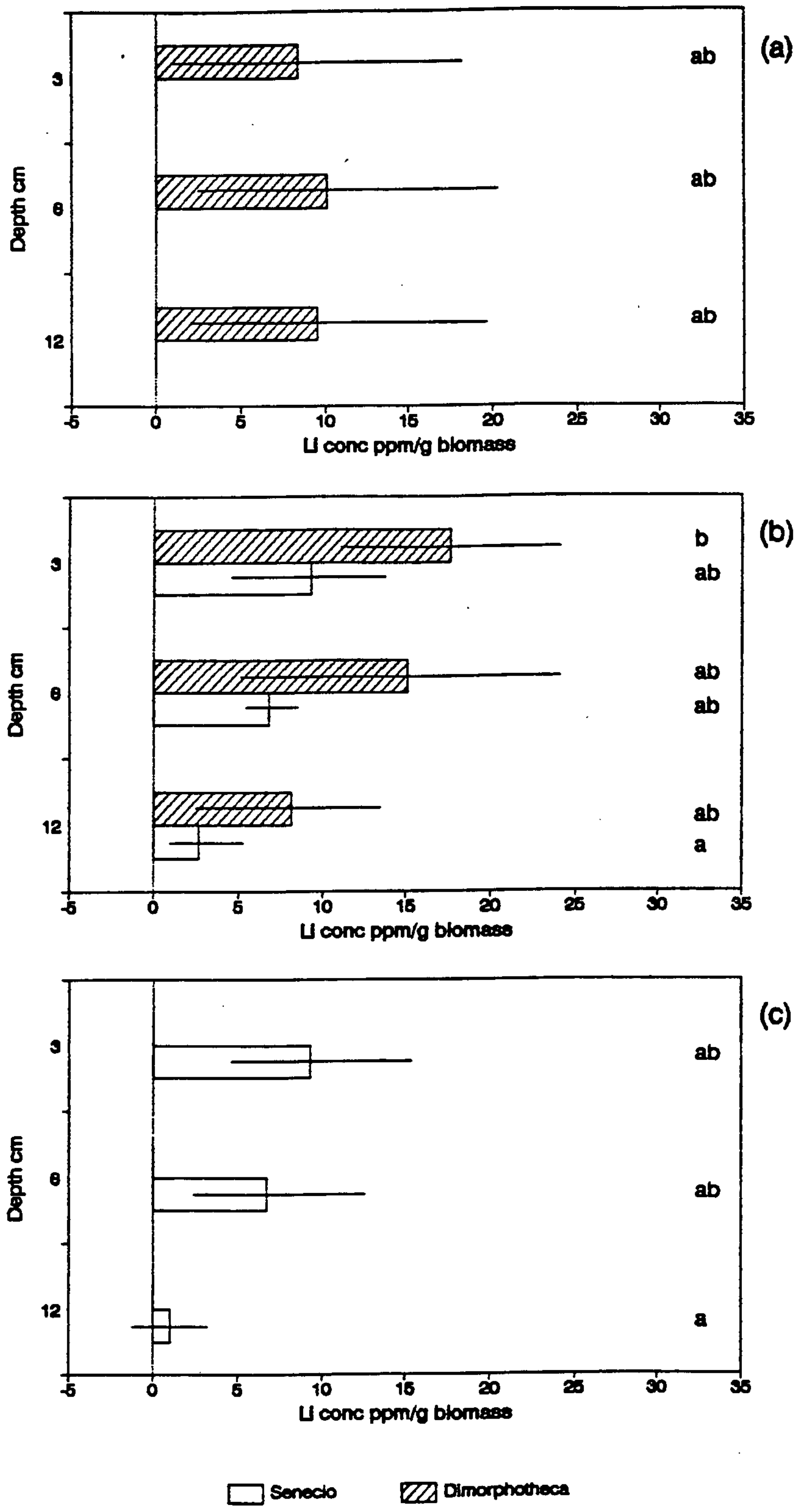

Figure 5. Root activity at three depths (3, 6, 12 cm) for coexisting plant pairs approximately 5 cm apart for *D. pluvialis* x *D. pluvialis* (a) *D. pluvialis* x *S. littoreus* (b) and *S. littoreus* x *S. littoreus*. Error bars represent S.E. Letters represent homogeneity of groups as determined by Tukey multiple range test ($p=0.05$). Analysis of variance df=11, F-ratio= 2.055, $p<0.05$.

Table 4. Multiple range analysis for lithium concentrations of *S. littoreus* growing both individually and as pairs with *S. littoreus* or *D. pluvialis.*

	S. littoreus grown with *S. littoreus.*	*S. littoreus* grown with *D. pluvialis.*
Single, depth 3 cm	a	a
Single, depth 6 cm	a	a
Single, depth 12 cm	ab	a
Paired, depth 3 cm	ab	a
Paired, depth 5 cm	b	a
Paired, depth 12 cm	b	a
ANOVA	df=5 F-ratio= 2.065 Sig.lev. =0.112	df=5 F-ratio= 0.813 Sig.lev. =0.564

DISCUSSION.

Both species demonstrate a reduction in tracer uptake with respect to depth as root density decreases, as found in other tracer studies of root activity patterns (Tofinga and Snaydon, 1992). *D. pluvialis* exhibited reduced uptake immediately adjacent to the stem suggesting an inactive zone of uptake on account of the active rooting zone migrating away from the stem. This was not evident in the *S. littoreus* samples, possibly because the inactive zone lay less than the 2.5 cm from the plant stem, and was consequently undetected. Rates of uptake were generally higher in *D. pluvialis* at all other depths and distances.

The results from the paired plant data indicate that *D. pluvialis* competes with itself as expected. This result is indicated to a lesser extent with *S. littoreus*. Under conditions of both species growing adjacently, *D. pluvialis* is suppressed by the presence of *S. littorreus*, but maintains an overall higher value. Root uptake activity *S. littoreus* however is not reduced by growth of *D. pluvialis*. It appears therefore that although these two plants of very similar habits react differently under conditions of root interaction. The evidence suggests that the rooting pattern of *D. pluvialis* experiences more interaction. This may not necessarily mean it is the best overall competitor. The fact that this, and many other herbaceous annuals with synchronised life histories, can coexist may suggests that there are many factors influence reproductive success. For example, the stochasticity of reproductive events, self thinning of seedlings or the effects of the regeneration niche.

Given that it has been demonstrated that plants do compete for soil moisture, particularly in water limited environments (Barbour, 1981), and nutrients in most other environmental conditions, there are consequently many possible patterns of distribution of competing plants. Cody (1986) found that there is evidence for contagion in coexisting species and hypothesised that below ground niche separation must be responsible. Experiments with desert shrubs has revealed that coexisting species do have different below ground morphologies (Manning and Barbour, 1988) extracting water from different points in the soil profile. Both *D. pluvialis* and *S. littoreus* exhibit positive and negative associations between species, coexisting in extensive colonies and also as single species cohorts. The latter phenomenon may be the result of other factors such as variations in soil conditions or seed dispersal. Examination of these factors would require further in depth study using pattern and interference analysis and gathering of edaphic data. Accurate analysis of rooting patterns over time, of species grown in laboratory conditions or by neighbour removal would also aid interpretation.

The conclusion from these data suggest that *D. pluvialis* is a potentially more active competitor for water and solutes, which appears that it may enable it to exclude *S. littoreus*. However *S. littoreus* was little affected by location next to *D. pluvialis*, which indicates that *S. littoreus* maybe occupies a different below ground niche. For example, it may exhibit a smaller requirement for nutrients, by utilising a root architecture with a more efficient water or solute uptake facility, thus occupying a smaller area. *D. pluvialis* on the other hand has a more active foraging rooting strategy and increases production according to uptake. This supposition is reflected to some extent by the field observation that there was a considerably larger range of sizes *of D. pluvialis* than *S.littoreus.* This hypothesis would be supported by Grime's (1988) CSR model of coexisting plant strategies, whereby each strategy can be considered to be constructed from combinations of two adaptive characteristics: potential maximum dry matter production and potential maximum size, ie. ruderals (rapid growing, small) competitors (rapid growing, large) and stress-tolerators (slow growing, small/large). Thus *S. littoreus* represents stress tolerator traits and *D. pluvialis* competitive characteristics. The evidence from this study is however, insufficient and further laboratory analysis of physiology of each species would have to be completed to confirm this hypothesis.

In drawing hypothesis regarding root activity based on tissue tracer concentration the basic assumption is made that water and solute uptake is directly proportional to tracer uptake. In addition Fitter (1986) suggests tracer concentration may be complicated by variation in growth rate between individuals and species. With such fast growing annuals flowering and fruiting in such a short growing period, it has been therefore assumed that absolute growth rates of similarly sized individuals within species are approximately equal.

Direct and indirect quantitative study of rooting patterns is difficult in the field. Time consuming methods of excavation and coring do not provide adequate data necessary to assess root activity. The recent usage of radioactive tracers to indirectly infer rooting activity patterns produces more satisfactory data, but these methods are expensive and require a high degree of skill. Non-radioactive tracer techniques, however, afford the properties of being both more economical and simpler to conduct (Martin et al., 1982). Despite the view that these techniques are inadequate due to certain necessary assumptions (Bohm, 1979; Tofinga and Snaydon, 1992), there have been several studies employing non-radioactive tracers for analysis of rooting patterns and activity (eg. Fitter, 1986; Tofinga and Snaydon, 1992; Martin et al., 1982).

There is a clear requirement for improved methods of studying root physiology and ecology in order that more accurate predictions can be made. Further research should include examination of the correlation between living root biomass density and distribution (deduced from direct excavation of roots soil cores of field and laboratory grown plants) and tracer uptake. The applications for this technique could include examination of water/nutrient foraging strategies of coexisting plant species in water limited and agricultural environments, root distribution of trees, etc. Given that the quantitative field study of root physiology is so difficult, lithium and other nonradioactive tracers are cheap and simple to use and serve to provide a sound indirect method of assessing root activity.

ACKNOWLEDGEMENTS.

I wish to thank Professor W J Bond for the original project idea, Dr W D Stock for project supervision, Professor A P le Roex, Professor J P Willis and staff of the Department of Geological Sciences, University of Cape Town, for their assistance with chemical analysis.

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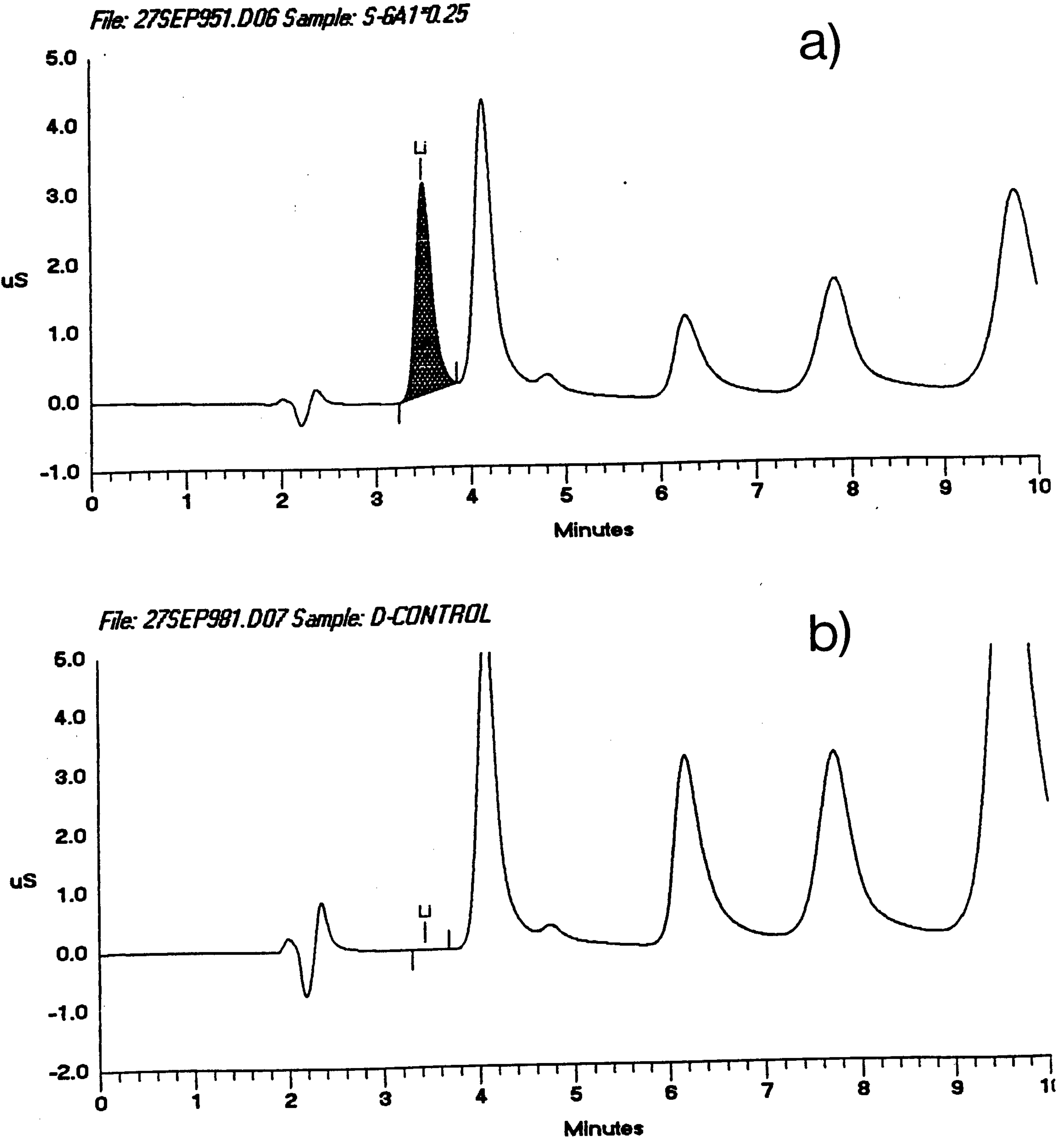

Appendix 1. Representative ion chromatograms for two samples *S. littoreus* (a) and control *D. pluvialis* (b). Lithium peak represents a sample concentration of 1.257 ppm (a) and 0 (b). Note the sodium peak which separates off the column immediately after the lithium peak. The concentration of sample must be adjusted to avoid distortion of the base line thereby deleteriously affecting the accuracy of the integration of the lithium peak.