

A review of experiments exploring viability and regeneration within *Rumex obtusifolius* L., *Rumex crispus* L. and the hybrid form *Rumex pratensis*

Heather Moore, University of Aberystwyth, Student Placement at HDRA 2003/2004

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Abstract

The germination of seeds and regeneration of roots in three species of *Rumex*, namely *Rumex obtusifolius* (broad-leaved dock), *Rumex crispus* (curled-leaved dock) and *Rumex pratensis* (hybrid dock) has been studied. Trials were carried out in a glasshouse and outdoors. Seeds were tested for viability and roots were monitored for regeneration. All samples were collected from various locations within two organically managed agriculture sites, HDRA, Ryton, Coventry and Feldon Forest Farm, Frankton, Rugby. Seeds were tested in petri-dishes and monitored for sixteen weeks. The root experiment had two parts: field regeneration and root fragment regeneration (in glasshouse). The field experiment was set up to mimic cultivation depths of 1cm, 5cm, 10cm and 15cm, by cutting dock plants to this level below the surface of the soil. The root fragment experiment was set up to imitate fragments (5cm, 10cm, and 15cm) lying on the surface of the soil after cultivation. Drying treatments of 1, 4 and 8 weeks to reflect a hot dry summer were applied to some roots. All roots were monitored for regeneration. Results suggest that after 16 weeks germination, the hybrid form of dock (*R. pratensis*) has less than half the viability (43%) of *R. crispus* and *R. obtusifolius* in the samples obtained and seed of *R. crispus* proved to be the most viable (100%). Field experiments showed that after 21 weeks, 60% of roots that had been cut at 1cm had regenerated producing shoots at the soil surface, and 25% of roots regenerated at 5cm. Roots cut deeper than this at 10 or 15cm showed no regeneration. Fragment regeneration in the glasshouse with all lengths was also apparent in roots potted immediately and after one-week drying treatments. Roots potted immediately showed an average of 88% regeneration across the two sites in all lengths as opposed to a 21% average regeneration for the roots that underwent a one-week drying treatment. No regeneration occurred from roots that underwent four or eight-week drying treatments. Root hairs developed in 59% roots of the 4-week drying treatment and 45% roots of the 8-week drying treatment, sites combined. All roots (after 4 and 8-week drying treatments) looked dead at the end of the experiments; roots crumbled and contained black fibres.

Introduction

Docks (*Rumex* spp.) are a group of plants that can be found in a variety of habitats and soil types within the UK at altitudes of up to 2000ft. They are common on arable and meadow land, permanent pasture, short term leys and waste ground but do not thrive in acid-rich soils. The highest infestations of docks are in Devon and Sussex (Hagger, 1980), whilst fewer numbers exist in northern Scotland, it is thought that the length of growing season and winter cold are not responsible for this distribution pattern. Mature plants can withstand severe cold and drought but seedlings may be killed. It is unusual to find docks growing in fields cut for hay, grazed by sheep or subject to flooding, but *R. crispus* is able to adapt when flooding occurs by increasing leaf area and developing elongated leaves to account for decreased light levels (Vervuren *et al.*, 1999). Two species in particular are regarded as a major concern in agriculture, namely the broad-leaved dock (*R. obtusifolius* L.) and the curled-dock (*R. crispus* L.) (Holm *et al.*, 1977). The broad-leaved dock is a highly variable perennial species and the curled dock is capable of behaving as an annual, biennial or perennial.

Hybridisation exists between *R. obtusifolius* and *R. crispus* and these hybrid forms are quite common, sometimes occupying entire fields. They exhibit a range of intermediate characteristics but are often more vigorous than the parents although producing few viable seeds. However, the presence of fertile hybrids has been reported and there is evidence that backcrossing has occurred to the extent that some plants of hybrid origin are almost indistinguishable from the parent species (Williams, 1971; Stace, 1997)

Docks can reproduce vegetatively by rhizomes or sexually by seed production. Seed dispersal can occur in a variety of ways. Seeds of these species can float on water for up to 2 days (Carvers & Harper, 1967) and the maritime form of *R. crispus* can float for up to several months. Seed germination and field emergence occur mainly in spring and early summer (Benvenuti *et al.*, 1999). It has been found that dock seeds can remain viable in different substrates. Tests have been carried out with slurry, water immersion, manure, cattle droppings and dung compost. Seeds have remained viable for up to 3 weeks in dung compost and up to 36 months in fresh water storage at depths of between 12 and 48 inches for *R. crispus* seeds (Bruns, 1953).

No correlation has been found between maturity of the seed when removed and subsequent germination behaviour (Cavers & Harper, 1966). It has been recorded however that seeds of *R. crispus* collected from the parent plants at different stages of maturity exhibit a progressive increase in germination with increased maturity (Maun, 1974).

Many scientists have studied the germination and re-generation patterns of docks. The results of these experiments differ between researchers and variations in methods. There are many factors that affect the germination of dock seeds and regeneration of dock roots, these include, temperature (constant or alternating), light, darkness, burial depths, water immersion and viability.

Most researchers investigate the effect of alternating or constant temperatures or light on the germination of dock seeds, these mimic the conditions found at or near the surface of the soil. It has been found that under alternating light and temperature conditions, germination was faster and more complete than with constant light and temperature (Vincent & Cavers, 1977). Other experiments into seed germination have been carried out involving seed from different habitats, different plants within the same site, from different panicles on the same plant and from different positions within the same panicle. These studies provide few consistent results as seeds had different germination requirements due to size of seed or time of harvest.

Experiments exploring regeneration in the roots of dock plants have been carried out in glasshouses investigating the effects of burial growth, root fragment length and position on root that the fragment came from. Other factors explored were presence of secondary taproots, increase in biomass and signs of break up or necrosis. It has been suggested that only fragments above the root collar are able to produce new individuals (Hughes, 1985). Taproots increased in biomass, necrosis was visible and the number of secondary taproots also increased on studying the regenerative capacity of different structural parts. It was apparent that all roots regenerated successfully but growth only arose from the proximal part of the taproot close to the root collar.

Aspects of dock physiology considered in this report are seed viability, the ability for dock roots to re-generate after drying out upon removal from the ground and regeneration of plants from roots cut by a specified length below the surface of the soil.

Study Outline - Experiments exploring Seed Viability and Regeneration in Docks – Jan-Apr 2004

Part 1 - **Seed Viability**

- Germination experiments to be carried out in glasshouse in Petri-dishes on moist filter paper.
- 20 seeds in Petri-dishes
- 4 replicates per sample bag, therefore 80 seeds per bag. 28 sample bags (10 broad leaved, 8 curled, 10 hybrid)
- 112 Petri-dishes (found in Research C)
- Evenly spaced seeds, to be checked and counted every morning for germination between 9-10am
- Results recorded with dates included so that timing and possibly vigour can be analysed. Germinated seeds removed.
- Seeds that remain un-germinated will be tested for viability.

Part 2 - **Regeneration**

Part 2.1

- Locate patch of 40 undisturbed dock plants on each site, Frankton and HDRA.
- Cut root of different dock plants to a depth of 1 cm, 5 cm, 10 cm, and 15 cm below surface of soil
- 10 plants for each depth, each site.
- Mark soil with a tag/cane to pinpoint dock depth, position and species.
- Check weekly for signs of regenerative growth

Part 2.2

- Cut 240 extra plant roots, 120 each site. 2 sites, HDRA & Frankton. 40 roots per length per site. 5 cm, 10 cm, 15 cm. Split sets into 4 treatments as below. 10 roots per treatment.
- From the Part 2.1 cuttings, dispose of plant heads and 20 x 1 cm fragments. 60 roots remaining.
- 300 root fragments: 5 cm (x100) 10 cm (x100) 15 cm (x100)

immediately pot 25 roots
25 roots
13 Frankton, 12 Ryton
Ryton

immediately pot 25 roots
13 Frankton, 12 Ryton

immediately pot
13 Frankton, 12

Part 2.3

- 25x drying treatment 1wk, then pot (13F,12R)
 - 25x drying treatment 4wks, then pot (13F,12R)
 - 25x drying treatment 8wks, then pot (13F,12R)
- 25x drying treatment 1wk, then pot (13F,12R)
 - 25x drying treatment 4wks, then pot (13F,12R)
 - 25x drying treatment 8wks, then pot (13F,12R)
- 25x drying treatment 1wk, then pot (13F,12R)
 - 25x drying treatment 4wks, then pot (13F,12R)
 - 25x drying treatment 8wks, then pot (13F,12R)

Drying treatments carried out in 20°C research oven 2. Potting in washed trays with sieved HDRA field soil. Trays remain in glasshouse and watered approx. every 2days (keeping soil moist). Results recorded.

Materials and Methods

Part 1. Viability

Pre-experimentation, seeds were chilled in a cold-store. This chilling ensured that if seeds needed a cold spell to break seed dormancy, this would have been achieved. Stratification also leads to loss of dormancy but sometimes induction of secondary dormancy, therefore was not carried out. All seeds were collected in September 2003, seeds from some areas may not have been fully mature due to the red-green colour of

the seed heads. Sample availability was limited by plant availability, however, seeds were taken from similar positions (near the tops) on each plant for consistency.

The viability experiment was set up in the glasshouse. Docks from the research field were used for regeneration experiments. Dock seeds were collected from two sites in Warwickshire, HDRA and a farm in Frankton, Rugby. 28 samples of seed were collected, 10 samples of broad-leaved dock, 8 samples of curled leaf dock and 10 samples of hybrid dock seed. Exact locations were recorded to enable further soil sampling should outlier results occur. The experiment was set up using moist filter paper on 112 petri-dishes. There were 20 evenly spaced seeds on each petri-dish and these were monitored and kept moist when needed. There were 4 replicates per sample. Petri-dishes were monitored every morning between 9am and 10am for germination, results were recorded and germinated seeds were removed. This process took place over a course of sixteen weeks. Daily temperature was recorded at 9am.

After termination of the experiment, seeds were tested for viability using tweezers by applying pressure to the seed coat. If the seed is hard and resists pressure it is considered viable, or if it splits to reveal a firm white embryo the seed it is also considered viable. On collection of all data, ANOVA statistical tests will be carried out, using SYSTAT 8.0. These tests will determine any occurring differences between the replicates, samples and species.

Part 2. Regeneration

Part 2.1

The regeneration experiment was split into 3 parts. The first part involved collecting 80 dock plants that were cut by a specific root length to a certain depth below the surface of the soil. This ground then remained undisturbed and the docks were labelled and monitored for root regeneration in situ. There were 4 different depths, 10 plants for each depth; 1 cm, 5 cm, 10 cm and 15 cm on each site. These areas of ground were monitored for signs of regrowth of the roots. The roots were kept for specific root length regeneration described in part 2.2 and identifiable species were recorded to observe trends that may occur. For example, vigour.

Part 2.2

The root cuttings in Part 2.1 were used in Part 2.2 of the experiment apart from root cuttings of 1 cm length which were discarded. Of the 20 roots collected for each of the three remaining lengths, 5 roots were re-potted the same day, watered and monitored daily for regeneration. These roots were potted in sieved field soil. Using this same method, 240 extra dock roots were cut to make a total of 300 roots to monitor. 120 docks were cut from 2 sites, HDRA and Frankton. Of these 120, 40 docks were cut for each of 3 lengths, 5 cm, 10 cm and 15 cm. These sets provided 10 roots for each of the 4 treatments. 4 treatments: immediate potting, 1 week, 4 week and 8 week drying treatment.

Part 2.3

The remaining 15 roots from Part 2.1 and the 20 extra roots from the two sites for each of the 3 lengths underwent a drying treatment in an oven set at 20°C to see if desiccation had an effect on regeneration. After a week, 25 roots from each length were potted and monitored in the same way as in part 2.2. 13 roots were used from

Frankton and 12 roots from HDRA, Ryton. After 4 weeks, another 25 roots from each length were potted, watered and monitored. After 8 weeks, the final 25 roots from each of the 3 lengths were potted in the sieved field soil and monitored in the glasshouse for regeneration. Results and observations were recorded daily for the whole experiment. All drying treatments were carried out at 20°C and the trays were washed before the potting took place. Trays were used instead of pots to represent the surface lying that occurs following cutting in the field with machinery. An oven was used for a constant 20°C.

Results

On completion of the practical work, all three experimental parts have provided some conclusive results.

Part 1

The germination experiment produced a flush of germination upon watering in all species and seeds continued to germinate continuously for two weeks. *R. obtusifolius* and the hybrid dock had a sudden decrease in germination rate after the initial watering, but *R. crispus* continued at a steady rate for several weeks until it had exceeded the cumulative total of germinated seeds of *R. obtusifolius* at nine weeks. The majority of the *R. obtusifolius* (broad leaved dock) and *R. crispus* (curled leaved dock) seeds germinated with percentage germination at 92% and 99% respectively. As seen from the Figure 1, percentage germination for the hybrid was significantly different at 41%.

Of the initial 2240 seeds tested, 503 remained un-germinated by 30 April (22.5%); these consisted of 66 *R. obtusifolius* seeds, 8 *R. crispus* seeds and 429 hybrid seeds. The viable seeds of these remaining, comprised of 2 broad leaf, 6 curled leaf and 15 hybrid. These figures added to the germination values give final viability results of 92.4% *R. obtusifolius*, 99.7% *R. crispus* and 43.3% *R. pratensis*.

On statistical analysis of the data, it is clear that there is no significant difference between the 4 replicates within each sample for each species. There is a significant difference between the 8 samples of each species, as shown by the P values in Figure 2 for each week. A significant difference of results does occur between species as identified above. (Fig. 1).

Figure 1: Differences in cumulative germination between species

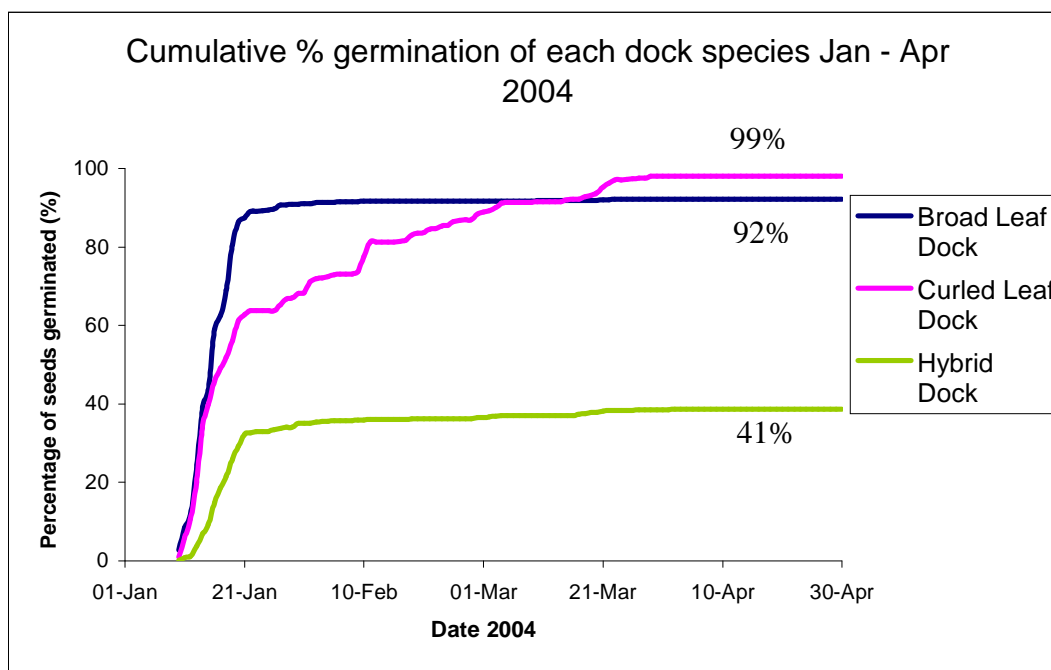


Figure 2: Analysis of Variance between samples and species

Analysis of Variance						
Week	Source	Sum-of-Squares	df	Mean-square	F-ratio	P
1	Sample*Species	314.188	14	22.442	18.004	***
2	Sample*Species	1242.063	14	88.719	18.004	***
3	Sample*Species	1747.104	14	124.793	12.284	***
4	Sample*Species	1830.688	14	130.763	12.896	***
5	Sample*Species	1741.604	14	124.4	14.341	***
6	Sample*Species	1553.813	14	110.987	13.822	***
7	Sample*Species	1497.521	14	106.966	12.53	***
8	Sample*Species	1512.729	14	108.052	13.244	***
9	Sample*Species	1504.312	14	107.451	13.577	***
10	Sample*Species	1492.396	14	106.6	14.632	***
11	Sample*Species	1445.104	14	103.222	14.746	***
12	Sample*Species	1416.229	14	101.159	15.762	***
13	Sample*Species	1417.271	14	101.234	17.201	***
14	Sample*Species	1417.271	14	101.234	17.201	***
15	Sample*Species	1417.271	14	101.234	17.201	***
16	Sample*Species	1417.271	14	101.234	17.201	***
17	Sample*Species	1417.271	14	101.234	17.201	***

Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Part 2.1

The field experiments monitoring roots below the soil surface were carried out for 21 weeks. 60% regeneration was apparent at 1cm depths (60% broad-leaf) and 40% regeneration at 5cm depths (species mixed) at Ryton.

The roots at Frankton also produced 60% regeneration at 1cm depths (30% curled-leaf, 20% hybrid, 10% broad-leaf) and 10% at 5cm depths (curled-dock). No other depths for either site presented signs of regeneration above the ground.

Figure 3: Tables showing recorded results for regeneration. Measurements in millimetres. *=Field cultivated, recorded terminated, v=vigorous, no seedhead.

Field Trials - 1cm

Root	Dates				Dates			
	06/02/04	25/02/04	26/02/04	17/03/04	23/03/04	15/04/04	29/04/04	02/07/04
H1-B								
H2 - B							*	
H3 - B				40		191	*	
H4				6		85	111	
H5 - B				31		157	*	
H6 - B		44	45			227	*	
H7 - B							*	
H8 - B						148	*	
H9 - B		60	60	90		167	*	
H10 - B							*	
F1 - HY								
F2 - C					24			1040
F3 - C					66			450
F4 - C					14			330
F5 - B								
F6								
F7 - HY	185		253		261			800
F8 - HY					49			550
F9 - B					47			
F10								

Field Trials - 5cm

Root	Dates				Dates			
	06/02/04	25/02/04	26/02/04	17/03/04	23/03/04	15/04/04	29/04/04	02/07/04
H1 - C								
H2								
H3								
H4								
H5				59		184	195	
H6			95	81		269	308	
H7 - C				102		225	242	
H8 - C								
H9 - B						139	*	
H10 - B								
F1 - C								
F2 - C								37
F3 - B								
F4 - B								
F5 - C								

F6 - C								
F7 - B								
F8 - HY								
F9 - HY								
F10 - HY								

Part 2.2

The results suggest that regeneration decreased after drying. Roots potted immediately (no drying treatment) for all lengths showed 86% regeneration from Ryton and 90% regeneration from Frankton. 100% of these (regenerated) roots had produced shoots from their root collars, the part cut from the stem at the soil surface.

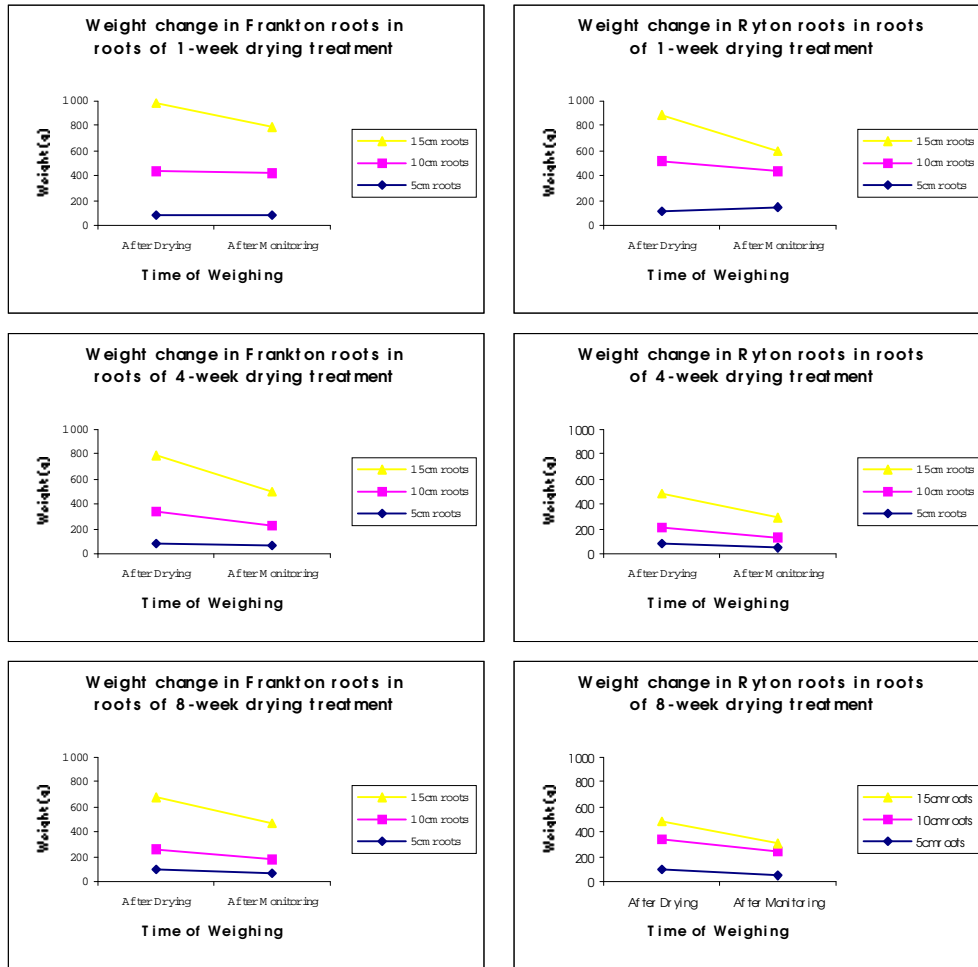
95% of all roots had regenerated root hairs including those that hadn't regenerated new shoots. The root hairs were mainly produced at the ends of existing roots however in some cases the root hairs grew from intermediate root nodes or from the opposite side of the root collar indicating geotropic growth. Root hairs were abundant compared with those in Part 2.3.

Part 2.3

Roots that underwent a one-week drying treatment had shown 15% regeneration from Ryton roots (10% 5cm roots and 5% 10cm roots) and 26% regeneration in roots from Frankton (8% 5cm roots, 8% 10cm roots and 10% 15cm roots) after 17 weeks. Six out of 39 roots of Ryton roots (5cm and 10cm lengths) from the one-week drying treatments had developed thick 1cm diameter secondary roots. The same trend occurred in the Frankton roots where four out of 39 roots (10cm and 15cm) produced secondary roots with large diameters also. All other roots for this and other treatments only produced secondary roots up to 2mm in diameter.

The roots potted after 4 week drying treatments did not show any shoot regeneration, however roots had developed root hairs, 78% of Ryton roots and 41% of Frankton roots. Roots were monitored for 15 weeks. Roots potted after 8 weeks drying treatment and monitored for 11 weeks showed one possible root regenerated and 47% root hair development from Ryton roots. It was unclear whether the shoot was from seed or regeneration. No regeneration occurred in Frankton roots but 44% developed root hairs. All roots (after 4 and 8-week drying treatments) appeared dead at the end of the experiments; roots crumbled and contained black fibres. On weighing the roots after the experiment, all roots in collective length groups had decreased in dry weight from their starting weight apart from one result.

Figure 4: Weight change (g) in roots from Frankton and Ryton after, 1,4 and 8 week drying treatments at 20°C.



Discussion

Viability

On analysis of results, data suggest the hypothesis may be supported in that seed viability in the hybrid species is reduced compared with the broad-leaf and curled-leaf dock. Modifications had to be made to ensure consistency in the statistics programme, this resulted in only eight samples being used in analysis instead of the full ten sets of data for *R. obtusifolius* and *R. pratensis*. Although viability results are fairly reliable it was initially difficult to distinguish between hybrid and parental species when collecting seeds for each species. Seeds do not seem to have been affected by dormancy, increased weight or maturity because of the top position they were taken from the plants. The results are reliable; as seed collection was from the same position on each plant as far as was possible.

It is clear that the parental crossing of species does affect the hybrid species' viability. This is observable in the data for cumulative hybrid total germination at 42% compared with parental species at 92% and 99% germination total (Figure 1). According to Foster (1989), hybrids formed between *R. crispus* and *R. obtusifolius* usually have fertility of less than 1%. Although data from this study shows an average of 41% hybrid germination across 8 samples, observation of separate samples indicates much variability. Some samples have a very high germination count (89%) and others a very low count (less than 3%); this trend can be seen in Figure 2. These differences may have been due to misidentification of hybrid plants based on seed appearance due to no leaves being present in winter to aid with identification. Differences in total germination may be due to the differences between hybrid plants, including the amount of parental backcrosses that have occurred previously. Foster (1989), may have used pure hybrids.

A similar trend is visible in *R. obtusifolius* in Figure 2. Samples 2 and 6 present a lower germination count than other samples. This may again be due to misidentification; a *R. obtusifolius* plant may have crossed with a *R. pratensis* plant and given a genetically mild form of hybrid with many broad-leaved dock characteristics.

Results for *R. crispus* in Figure 2 show to be fairly consistent, this suggests reliability and unlikelihood of misidentification.

The difference between viability results in seeds of the eight samples could have been due to the positioning of the different samples on the bench. Some were nearer to the heating pipes near the edge of the glasshouse and some may have been subject to more shade than others. A thermometer would need to be kept at the different sample positions to test this and monitor the minimum and maximum temperatures over a 24-hour period, however these factors suggested by ANOVA statistical tests did not affect seeds. Instead, seeds' ability to germinate may have depended on location of plants, for example, level of light gained, disturbance or vigour of plant as well as pureness of specific strain.

Regeneration

Root regeneration occurred in field roots cut at 1cm and 5cm depths but not from 10cm and 15cm depths. 60% regeneration was apparent at 1cm depths (*R. obtusifolius*) and 40% regeneration at 5cm depths (species mixed) at Ryton. Roots at Frankton also produced 60% regeneration at 1cm depths (30% *R. crispus*, 20% *R. pratensis*, 10% *R. obtusifolius*) and 10% at 5cm depths (*R. crispus*). Roots possibly could not regenerate at depths of or lower than 10cm due to starvation of light and surface water or composition, however these roots would need to be removed from the ground to study possible underground regeneration taking place not seen at the soil surface. It is possible that further research could be carried out on 1 cm depths between 5cm and 10cm to find the minimum cultivation depth to stop regeneration of these roots. These findings may coincide with the suggested 7.5cm for *R. obtusifolius* (Holme et al., 1977) and 4cm for *R. crispus* from previous research. Not all roots at 1cm and 5cm regenerated, so different factors may control the regrowth process. This could include thickness of roots, time given for regeneration, or existing underground branching of root.

A problem encountered during the field regeneration experiment was measurement of regeneration during the summer months of monitoring. Overgrown grass made it difficult to find the tagged specimens at Frankton, and at Ryton some specimens could no longer be recorded after cultivation of the area.

Potted roots in the glasshouse only regenerated after immediate potting with no drying treatments or after one-week drying treatments. The size of roots had no affect on ability to regenerate. It is likely that 4 and 8-week drying treatments expelled all water from roots disabling cells to recover cell structure and content on re-hydration.

On weighing the roots after the experiment, all roots in collective length groups had decreased in dry weight from their starting weight (just after drying treatment) apart from one. This could have been due to loss of brittle fragments of roots; the water content may have been the same as after the drying treatments as roots were left to dry out for 3 days before weighing.

Regeneration after drying treatments

The regeneration of roots experiment proved to be fairly reliable. It was expected that after drying treatments, regeneration would decrease. This was true on comparison of heat-treated roots with roots potted immediately after removal from the ground. Roots potted immediately showed regeneration from each length 5, 10 and 15cm, was on average 77, 100 and 85% respectively with all regeneration arising from the upper 2cm of the root, the root collar. Regeneration was reduced to 20, 28 and 12% from 5, 10 and 15 cm respectively after 1 week drying at 20°C. No regeneration occurred in roots that underwent four or eight-week drying treatments, thus suggesting a correlation between length of drying treatment and regeneration.

Root hair regeneration occurred at an average of 60% in the four-week heat-treated roots and at an average of 46% in the eight-week heat-treated roots, a marked decrease. All roots appeared dead and unlikely to regenerate secondary roots. It is may be possible that some root hairs were present before the drying treatment, they

may have less visible if they were not brittle and dried out. In Figure 4, all roots had decreased in weight at the end of the experiment. This may have been due to the drying treatment evaporating all moisture, leaving small possibility for regeneration or utilisation of water. In figure 4, there is an increase in dry weight for 1-week dried 5cm roots from Ryton, it is unclear why this outlier result occurred, however may be due to the amount of shoot regeneration that occurred causing an increase in weight on measurement.

In conclusion, the data suggests reliable results but further research needs to be carried out. The hybrid form of dock produces less viable seed than the parental species and root regeneration does occur. This happens when roots are cut at 5cm or less below the soil surface. Roots can also regenerate from root fragments on the surface of the soil, this is not dependent on root length, however, roots of 10cm length produce more regrowth. Root regeneration will not occur after a drying treatment at a constant 20°C that exceeds four weeks.

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