Variation in the responses of infraspecific variants of wet grassland species to manipulated water levels

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ABSTRACT

The effects of water levels on the germination, growth and reproductive effort of three wet grassland taxa were studied using an experimental water table facility. In addition, effects on related taxa including ecotypes and cultivars were examined. There were significant differences in biomass allocation and reproductive effort between infraspecific variants of *Centaurea nigra* and *Lotus* spp. in response to water table level. Variants had higher root and shoot dry-weights, produced more inflorescences and, in some instances, showed greater seedling establishment in the low water table treatments. In addition, significant differences in seedling establishment between infraspecific variants of both *C. nigra* and *Lotus* spp. were apparent. These findings have implications for the success of grassland re-creation schemes, and the selection of seed for such projects, and suggest that detailed research is needed to provide accurate information regarding the contrasting water table requirements of wetland plants, both at the species and subspecies levels.

KEYWORDS: ecotypes, water regime requirements, wetland restoration.

INTRODUCTION

It is estimated that in the United Kingdom agriculturally improved grassland has increased by 90% in the past 50 years (H.M. Government 1995). Some of this increase has been at the expense of floristically diverse wet grassland communities which, in comparison, are of high conservation value. A possible technique for the re-creation of such habitats uses the sowing of seed to encourage the establishment of desirable plant species and communities (Wells, Cox & Frost 1989; Stockey & Hunt 1994). An objective of the present study was to assess whether commercially available seed is suitable for use in wet grassland re-creation projects.

Purchased seed may vary in its provenance and in the accuracy with which species are identified. In many cases the provenance of commercial seed may mean that it is ill-suited to wetter sites: either as it has been taken from plants growing under much drier conditions (and may therefore exhibit ecotypic variation), or it has been specifically bred, in order to increase vigour and agronomic yield, as a cultivar. Material may be accurately named at the species level, but there may be uncertainty as to the precise subspecies involved. This imprecision may be important where two or more subspecies have significantly different responses to environmental variation. A similar problem may arise where the taxonomy of a group is difficult, e.g. where only specialists are able to distinguish microspecies within a complex. For example, seed supplied as *Taraxacum officinale* may include numerous microspecies, some of which differ in their water table requirements.

Concern has also been expressed about the use of non-native genotypes of wildflower species in habitat restoration, and it has been postulated that genes from these non-native cultivars may dilute the gene pool of the native population (Cairns 1993; Akeroyd 1994). Non-native cultivars have often been specifically bred for rapid growth (Bullard & Crawford 1995), and may effectively out-compete and eliminate their native counterparts. If the restoration of wet grassland ecosystems is to be reliable and successful in the longer term, it is important to test the response of native and non-native genotypes to differing water regimes.

The experiment described within the present paper set out to examine the effects of water levels on the germination, growth and reproductive effort of native material of three species. These effects were compared with the observed responses to the same treatments of infraspecific variants of these species including cultivars, "ecotypes" and subspecies. The experiment was conducted using

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an experimental water table facility at the Institute of Terrestrial Ecology (ITE) Monks Wood, where allied experiments on a range of native flood meadow species (Walker *et al.* 1997) have been conducted as part of a wetlands research programme.

MATERIALS AND METHODS

SELECTION OF SPECIES

The selection of species was based on their occurrence within lowland wet grassland communities, and in particular their importance as constituents of seed mixtures prescribed for the restoration of such communities. Commercial seed of native provenance of *Lotus corniculatus* (Common Bird's-foot-trefoil), *Rhinanthus minor* (Yellow-rattle) and *Centaurea nigra* (Common Knapweed) were sown. Their performance was compared with hand-collected seed from native populations, a forage variety (commonly used as a fodder crop) and seed of closely related species more frequently associated with wet conditions. The variants selected are listed below, with their provenance and abbreviated codes used to annotate text, tables and figures:

1. *Lotus corniculatus* "commercial" (Lcc) – commercially available seed of English provenance;
2. *Lotus corniculatus* "forage" (Lcf) – commercial forage variety, unknown provenance;
3. *Lotus pedunculatus* (Lp) – seed hand collected from populations in Dorset;
4. *Rhinanthus minor* "commercial" (Rmc) – commercial seed, possibly of ssp. *minor*, which is more associated with drier, often calcareous, soils;
5. *Rhinanthus minor* ssp. *stenophyllus* (Rmw) – seed hand-collected from populations at Wicken Fen National Nature Reserve (NNR) in Cambridgeshire;
6. *Centaurea nigra* "commercial" (Cnc) – commercially available seed of English provenance;
7. *Centaurea nigra* "Wicken" (Cnw) – seed hand collected from Wicken Fen National Nature Reserve.

The seeds utilised in the study were less than a year old and placed in cold storage at $4^{o}C$ prior to use.

EXPERIMENTAL DESIGN

The seeds were sown within circular plastic pots, 21 cm deep × 17 cm diameter, filled with approximately eight litres of soil-based compost. The soil had a silty-loam texture, broadly representative in physical characteristics of soils typically found on lowland alluvial grasslands, and was heat sterilised prior to use in order to reduce the number of weed species and pathogens present.

Each pot contained 10 seeds and was labelled to identify the species and seed source. Four replicate pots of each taxon were then placed within large fibreglass tanks, situated outdoors on a gravel bed, in which water levels could be maintained at a constant level. Sixteen fibreglass tanks were used in the study. The four treatments (water table levels) were as follows:

T0 – tanks with the water table at the same height as the soil surface within the pots

T50 – tanks with water table 50 mm below the soil surface within the pots

T100 – tanks with the water table 100 mm below the soil surface within the pots

T150 – tanks with the water table 150 mm below the soil surface within the pots

The four water level treatments were replicated within four blocks. Therefore, 16 pots per seed source were placed at each water level. The placement of pots within tanks, and tanks within blocks, was fully randomised in order to eliminate bias.

Evaporation losses and algal growth were reduced by placing white polypropylene granules on the surface of the water within the tanks.

MONITORING

GERMINATION AND SEEDLING DEMOGRAPHY

The numbers of seedlings in each pot were recorded every seven days from initial sowing in early December 1995 until the end of May 1996. Due to the large number of seedlings present (>5000) it was impractical to follow the fate of individual seedlings. Consequently successive cohorts of individuals were recorded within each pot. On four occasions germination could not be recorded when severe weather resulted in snow and ice covering the soil surface.

BIOMASS ALLOCATION

After approximately six months the seedlings were thinned to a single individual in each pot in order that plants could be grown on for destructive sampling without root competition. These individuals were then left to grow until October 1996, when they were harvested. After being oven dried at 80°C for 24 hours, both root and shoot dry-weights were obtained. It was not possible to obtain dry-weights for either of the *R. minor* variants as there were no surviving seedlings in October 1996.

REPRODUCTIVE EFFORT

At the final harvest the number of inflorescences present was also recorded. For the purposes of recording, the term "inflorescence" was taken to mean the capitulum in *C. nigra* and the cymose heads of the *Lotus* species. All species had seeded by the time of harvesting. Owing to seedling mortalities, it was not possible to gather inflorescence data for the *R. minor* variants.

STATISTICAL ANALYSIS

The preliminary statistical analysis, which was based on tank mean values, involved an examination of the effects of water table on the germination and survival of seeds of individual taxa (species and infraspecific variants). The effects of water table were broken down into constituent linear and quadratic components. It was considered important to make a preliminary analysis of how each taxon was responding to treatments, prior to "ecotypic" comparisons being made between infraspecific taxa of the same species.

For each taxon, the cumulative gains (i.e. germination) and losses (i.e. mortality) of seedlings within each of the four treatments were calculated for four different time periods; i) 49 days, ii) 98 days, iii) 154 days, and iv) 228 days after seed sowing. Over the four periods, a calculation was made of the mean number of seedling gains and losses per treatment for each taxon. Further analysis, using ANOVA, was carried out to examine whether individual taxa were exhibiting a significant response to treatment.

The second stage in the statistical analysis involved an examination of whether closely allied taxa showed any marked differences in their response to the four water level treatments. Using data for the cumulative gains and losses of seedlings, (49, 98, 154 and 228 days after the seeds were sown), species were analysed simultaneously using split-plot ANOVA. Comparisons of the germination and survival of seedlings were made between the following taxa: Cnc and Cnw; Lcc, Lcf and Lp; and Rmc and Rmw.

Further analysis of the variation in the responses of the infraspecific variants to water table depth was carried out using data on the establishment of seedlings, i.e. cumulative seedling germination minus cumulative seedling mortality. Possible differences in the establishment of taxa in the different treatments were examined using ANOVA.

By October 1996, the time of the final harvest, many of the seedlings had died and consequently it was not appropriate to analyse the root, shoot and inflorescence data using ANOVA. An alternative, and more conservative method used regression of the four water table means, weighted by the number of valid observations contributing to those means. The results were shown as an estimate of the slope of the variable on water table (± standard error) and an indication of significance.

TABLE 1. SIGNIFICANCE OF THE LINEAR EFFECT OF WATER TABLE DEPTH ON THE GERMINATION, MORTALITY AND ESTABLISHMENT OF RMC, RMW, CNC, CNW, LCC, LCF AND LP SEEDLINGS OVER A 228 DAY PERIOD.

Species	Seedling Germination				Seedling Mortality				Seedling Establishment
	(Days after seeds were sown)				(Days after seeds were sown)				
	49	98	154	228	49	98	154	228	228
Cnc	ns	ns	-	-	ns	ns	ns	ns	-
Cnw	ns	ns	ns	ns	ns	ns	ns	ns	--
Lcf	ns	ns	ns	ns	ns	ns	ns	ns	-
Lcc	ns	ns	ns	ns		ns	ns	+	-
Lp	ns	ns	(-)	(-)	ns	ns	++	++	---
Rmc	ns	ns	++	+	ns	ns	+	+++	ns
Rmw	ns	ns	ns	ns	ns	ns	ns	++	ns

Significantly higher rates of seedling germination, mortality and establishment in the highest water table levels are shown by: (+) = $p<0{\cdot}10$; + = $p<0{\cdot}05$; ++ = $p<0{\cdot}01$; +++ = $p<0{\cdot}001$. Significantly higher rates of seedling germination, mortality and establishment in the lowest water table levels are shown by: (-) = $p<0{\cdot}10$; - = $p<0{\cdot}05$; -- = $p<0{\cdot}01$; --- = $p<0{\cdot}001$. (ns = not significant).

RESULTS

EFFECTS OF WATER TABLE LEVELS UPON SEED GERMINATION, MORTALITY AND ESTABLISHMENT

Table 1 shows the significance of the linear effects of water table depth on the germination, mortality and establishment of Rmc, Rmw, Cnc, Cnw, Lcc, Lcf and Lp over a 228 day period.

Rhinanthus minor Wicken (Rmw) and *Rhinanthus minor* commercial (Rmc)

Seed of both taxa appeared to germinate more readily in the higher water table tanks. Germination was greatest 98–140 days after the seeds had been sown, after which time the rate of seedling mortality began to exceed germination, and hence net establishment of seedlings declined. After 228 days seedling establishment (Rmw and Rmc) had fallen to virtually zero in all four treatments.

Cumulative germination of Rmc was found to be significantly larger in the higher water table treatments at two intervals: 154 days ($p = 0{\cdot}006$) and 228 days ($p = 0{\cdot}010$) after sowing. Cumulative germination of Rmw was not found to vary significantly between treatments.

Cumulative seedling mortality after 228 days was, for both variants, significantly greater in the higher water table treatments. This offset the greater rate of germination in these tanks and consequently the overall establishment of Rmc and Rmw was not found to vary significantly between the different treatments.

When comparing cumulative germination and cumulative seedling mortality, a marginally significant difference ($p = 0{\cdot}096$) between the response of the two *Rhinanthus* taxa was noted 98 days after the seeds were sown. The germination of Rmc seed was found to be slightly greater than that of seed collected from Wicken Fen. This result may be explained by the negligible germination of Rmw seeds in the first fourteen weeks after sowing. In the final two recording periods, in which germination of both Rmc and Rmw increased, the difference in the cumulative germination of commercial and Wicken material was not found to be significant.

Centaurea nigra commercial (Cnc) and *Centaurea nigra* Wicken (Cnw)

In contrast to the two *R. minor* variants, the seeds of Cnc and Cnw were found to have higher cumulative germination levels in the lower water table treatments. This higher germination in the lower water table tanks was found to be significant for Cnc but not the Wicken variant. Cumulative germination and mortality of Cnc, in all four treatments, is shown in Figure 1.

The final establishment of seedlings (i.e. cumulative germination minus cumulative mortality

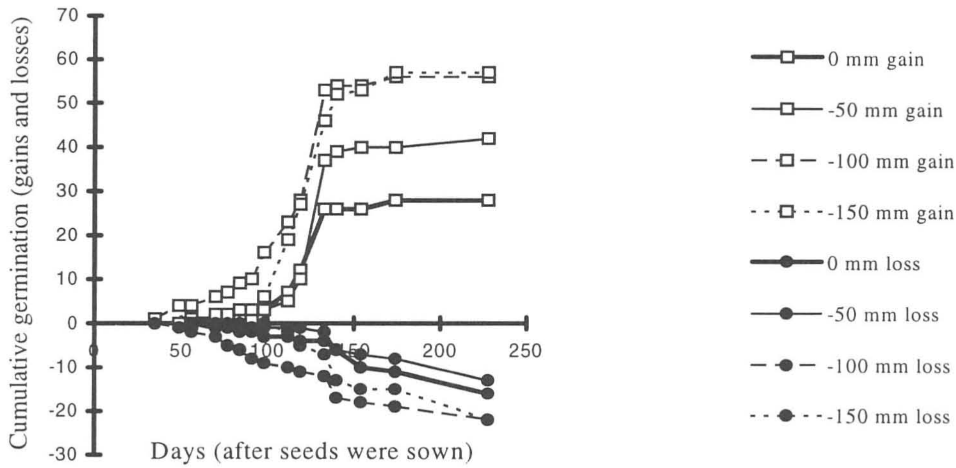


FIGURE 1. Cumulative germination and mortality of *Centaurea nigra* commercial seedlings at four water table levels over a 228 day period. 0 mm = water table at soil surface level, –150 mm = water table 150 mm below soil surface.

after 228 days) was significantly higher in the low water table treatments for both Cnc and Cnw (Fig. 2). However, a highly significant difference ($p = < 0{\cdot}001$) was observed between the final establishment of Cnc and Cnw with a greater establishment of Wicken seedlings in three of the four treatments (Fig. 2).

Lotus corniculatus commercial (Lcc), *Lotus corniculatus* forage (Lcf) and *Lotus pedunculatus* (Lp)
Over the 228 day recording period, the establishment of Lcc and Lcf seedlings was erratic due to variable rates of seedling germination and mortality. Both seed types had higher germination and establishment rates in the lower water table treatments, but this was not significant at the $p<0{\cdot}05$ level. Cumulative germination in all four treatments was considerably higher from seed of Lcc than from Lcf. The higher rate of Lcc germination was to some extent offset by seedling mortalities, which were higher than those experienced by Lcf. Net establishment of Lcf seedlings was, nevertheless, lower than that of Lcc in all four treatments.

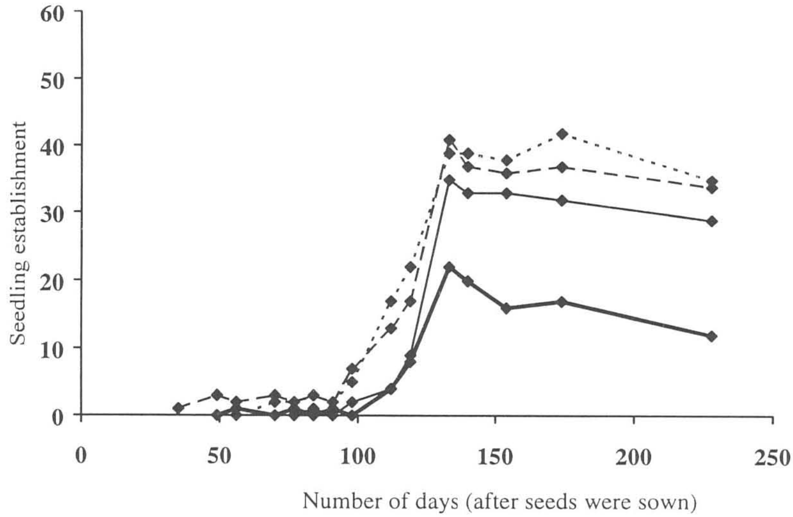
As found with Lcc and Lcf, there was a tendency for higher germination rates of *L. pedunculatus* in the lower water table treatments. This, combined with greater seedling mortality in T0 (the high water table tanks), led to a significantly higher establishment of Lp in the low water table tanks ($p = <0{\cdot}001$).

Highly significant differences ($p = <0{\cdot}001$) in the final establishment of Lcc, Lcf and Lcp were observed 228 days after the seeds were sown. The final establishment of Lp was considerably higher than that of Lcf in all treatments, and also greater than that of Lcc in treatments T100 and T150 (Fig. 3).

EFFECTS OF WATER TABLE LEVELS UPON PLANT GROWTH AND REPRODUCTIVE EFFORT
Tables 2 and 3 show the mean root and shoot dry weights, and mean number of inflorescences for variants of *C. nigra* and *Lotus* spp. at the four different water table levels studied.

Centaurea nigra commercial (Cnc) and *Centaurea nigra* Wicken (Cnw)
Mean root dry-weights of both *C. nigra* variants were significantly higher in the lower water table treatments. In the highest water table treatments, both variants had a mean root dry-weight of approximately 3 g, compared to mean root dry-weight in the lowest water table treatment of c. 56 g for Cnw and c. 64 g for Cnc. Cnw had slightly higher mean root dry-weights than Cnc in T0, T50 and T100.

Centaurea nigra A



Centaurea nigra B

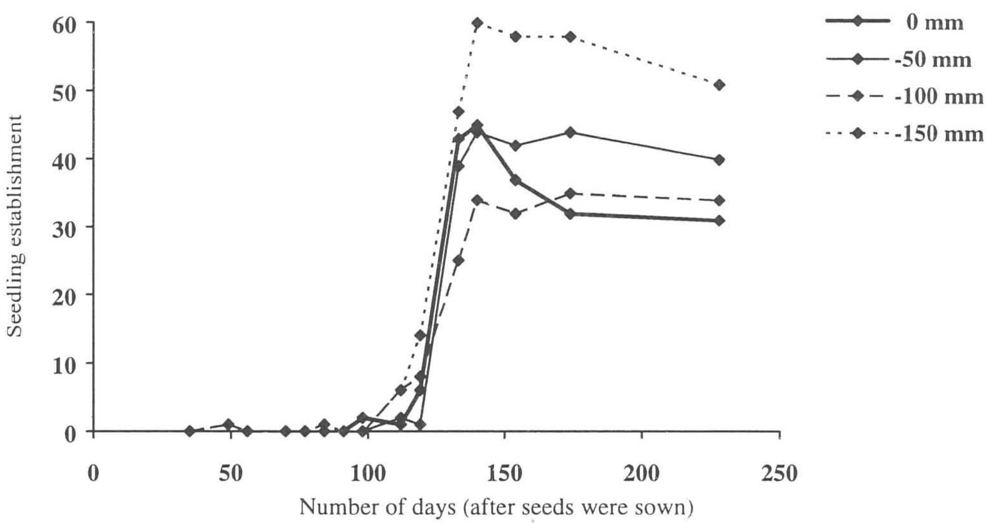


FIGURE 2. Comparison of the establishment (germination minus mortality) of seedlings of *Centaurea nigra* commercial (A) and *Centaurea nigra* Wicken (B) at four water table levels over a 228 day period.

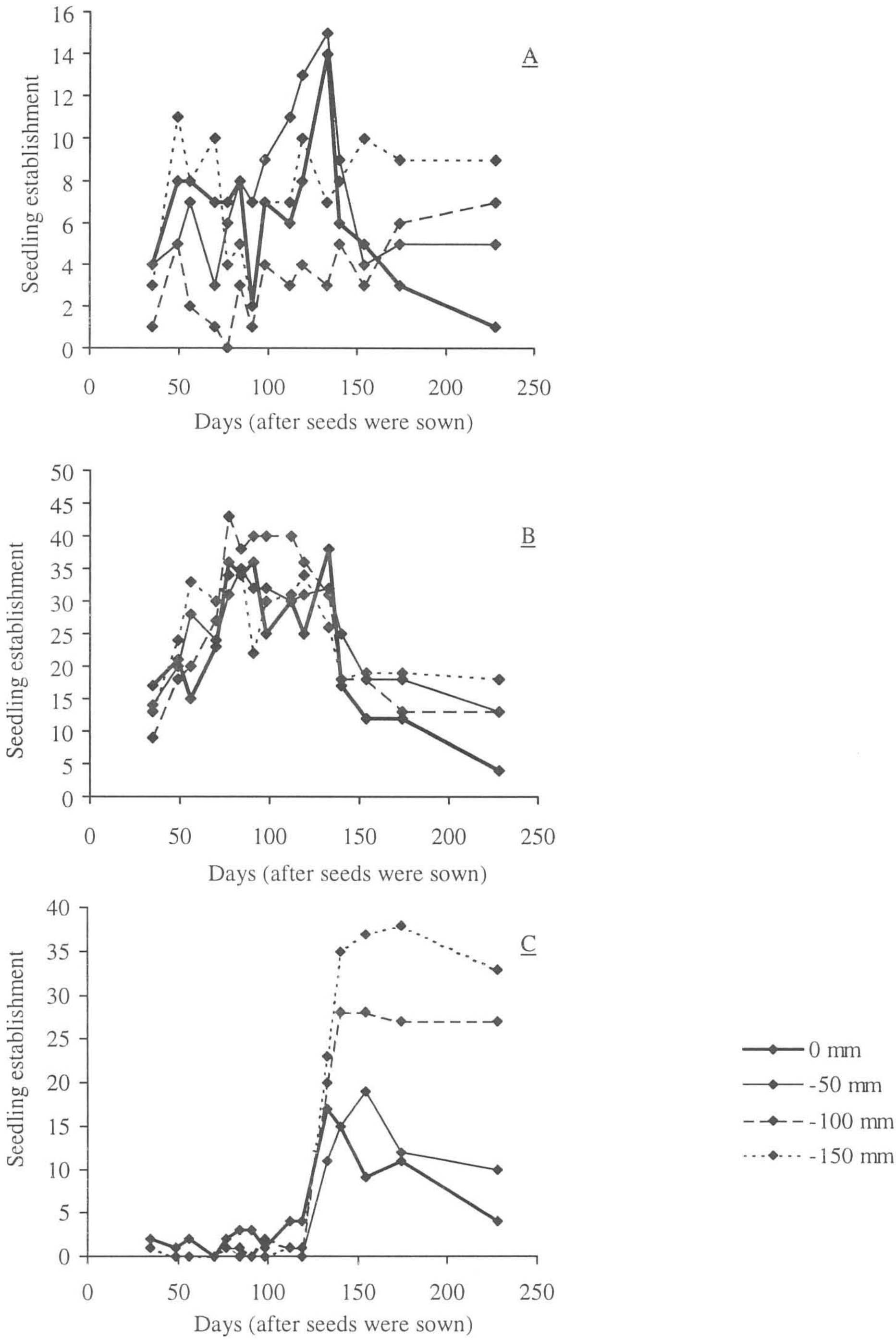


FIGURE 3. Comparison of the establishment (germination minus mortality) of seedlings of *Lotus corniculatus* forage (A), *Lotus corniculatus* commercial (B) and *Lotus pedunculatus* (C) at four water table levels over a 228 day period (note variations in scale).

TABLE 2. MEAN ROOT AND SHOOT DRY WEIGHTS, AND MEAN NUMBER OF INFLORESCENCES OF *CENTAUREA NIGRA* WICKEN (Cnw) AND *CENTAUREA NIGRA* COMMERCIAL (Cnc) SEEDLINGS AT FOUR DIFFERENT WATER LEVEL TREATMENTS (0, 50, 100 AND 150 MM BELOW THE SOIL SURFACE).

	Mean root dry weights (g)		Mean shoot dry weights (g)		Mean no. of inflorescences	
Treatment	Cnc	Cnw	Cnc	Cnw	Cnc	Cnw
T0	3·07	3·58	2·00	2·62	0·00	0·00
T50	4·98	7·21	3·53	3·80	0·33	0·37
T100	31·08	36·15	11·51	10·54	3·94	3·20
T150	64·29	56·48	15·49	13·54	6·71	5·00

TABLE 3. MEAN ROOT AND SHOOT DRY WEIGHTS, AND MEAN NUMBER OF INFLORESCENCES OF *LOTUS CORNICULATUS* FORAGE (Lcf), *LOTUS CORNICULATUS* COMMERCIAL (Lcc) AND *LOTUS PEDUNCULATUS* (Lp) AT FOUR DIFFERENT WATER LEVELS (0, 50, 100 AND 150 MM BELOW THE SOIL SURFACE).

	Mean root dry weights (g)			Mean shoot dry weights (g)			Mean no. of inflorescences		
Treatment	Lcf	Lcc	Lp	Lcf	Lcc	Lp	Lcf	Lcc	Lp
T0	0·00	1·19	0·65	0·00	1·83	0·70	0·00	14·00	0·00
T50	12·56	1·01	2·47	5·43	1·50	4·44	114·00	37·25	18·33
T100	26·76	7·42	14·87	9·32	6·77	12·85	76·33	63·50	29·44
T150	46·40	28·95	21·77	21·00	21·14	18·16	333·86	142·08	56·19

A similar trend was observed for mean shoot dry-weights, with marginally higher dry-weights from Wicken material in T0 and T50, and higher dry-weights of Cnc in T100 and T150. Again, both *C. nigra* variants had significantly higher shoot dry-weights in the lower water table treatments (Cnc $p = 0{\cdot}028$, Cnw $p = 0{\cdot}034$).

Neither Cnc nor Cnw flowered in the highest water table tanks. The mean number of inflorescences, of both variants, was significantly higher in the lowest water table tanks (Cnc $p = 0{\cdot}029$, Cnw $p = 0{\cdot}032$).

Lotus corniculatus commercial, *Lotus corniculatus* forage and *Lotus pedunculatus*
Mean root dry-weights of the three *Lotus* taxa were significantly higher in the lower water table treatments. The highest mean root dry-weight recorded was 46·40g for Lcf forage in T150. Mean shoot dry weights also increased significantly as water table level decreased, with this trend being particularly notable for Lp ($p = 0{\cdot}008$).

Like the *C. nigra* variants, Lcc and Lp plants produced significantly more inflorescences in the lower water table treatments. Although not found to be significant, the number of Lcf inflorescences was also markedly higher in the lowest water table tanks than in the other three treatments. Indeed, in T150 Lcf produced substantially more inflorescences than the other two *Lotus* strains. Only Lcc flowered in T0, the highest water table treatment.

DISCUSSION

The effects of water stress upon plant physiology and germination have been well documented (e.g. Evans & Etherington 1990; Jackson 1990; Crawford 1996; Olsson *et al.* 1996). A rise in water levels can deprive plants of oxygen, may affect the production and transport of plant hormones and can increase the likelihood of microbial attack (Crawford 1996). Some species have developed adaptations, both morphological and physiological, to help them cope with water table changes (e.g. Voesenek, Blom & Pouwels 1989).

If a plant becomes adapted to suit the specific environmental conditions of its habitat, such that its requirements become markedly different from those of other populations within the same species, ecotypic differentiation has occurred. The ability to select plants that are suited to environmental stresses, such as flooding, drought or even soil contamination, has led to the creation of new plant and crop varieties through plant breeding (e.g. Elias & Chadwick 1979; Yaseen & Al-Omary 1994) and also has large potential for providing guidance when selecting natural species for habitat restoration schemes. For instance, as *L. corniculatus* is a highly variable species (Jones & Turkington 1986) it would seem appropriate to sow the seed of a wetland "ecotype" in sites with higher water table regimes.

RHINANTHUS SPP.

Ter Borg (1985) studied the population biology of a number of hemi-parasitic Scrophulariaceae, and noted that *Rhinanthus* species often exhibit a wide infraspecific variation. *R. angustifolius*, for example, is thought to have at least eight different subspecies (Oberdorfer 1979). Taxonomic difficulties in *Rhinanthus* have contributed to the concept of seasonal dimorphism whereby species such as *R. minor* have been sub-divided into two taxa; aestival and autumnal, based upon the time of flowering and certain morphological characteristics. In some species of *Rhinanthus*, Soó (1970) has identified not only autumnal and aestival variants, but also montane, alpine and segetal variants (Karlsson 1974). Seasonal dimorphism, as a means of explaining variation in Rhinantheae, is now seen as an oversimplification with some of the apparent differences having been shown to have a genetic basis (Ter Borg 1985). Ecotypic variation may also have been overlooked when attempting to explain the apparent variations in the genus (Karlsson 1974). Grime, Hodgson & Hunt (1988) quote *R. minor* var. *stenophyllus* and *R. minor* var. *minor* as the two commonest *R. minor* ecotypes. *R. minor* var. *minor* is thought to prefer drier sites in southern England with *R. minor* var. *stenophyllus* being more suited to moist grasslands, particularly in the north.

In this study, *R. minor* appeared to germinate more readily in the higher water table tanks, though this trend was only statistically significant for the Rmc (after 154 and 228 days) and not Rmw. Owing to high rates of seedling mortality, no significant differences in the final establishment of Rmc or Rmw could be demonstrated. It is recommended that, for future research, sufficient numbers of plants are established to ensure full representation at each water table depth.

CENTAUREA NIGRA

The findings for *C. nigra* were both fuller and more interesting. Seed of both *Centaurea* variants had higher rates of germination in the lower water table treatments. However, unlike Cnw, the germination of commercial *C. nigra* seed was significantly higher in the low water table tanks, suggesting that germination of Wicken seed may be less dependent upon water table than seed which is commercially available. This supposition is supported by the seedling establishment data, which showed the final establishment of Wicken seedlings to be significantly greater than the establishment of commercial seedlings ($p<0{\cdot}001$). These results imply that Cnw seeds are not only more suited to wetter sites than commercial seed, but also to drier sites as well. The germination of seed originating from Wicken Fen was not significantly affected across the range of water tables studied. Therefore, seed collected from Wicken Fen and similar sites may, in terms of germination and establishment, be more suited for use in wet grassland and other restoration schemes than seed which is commercially available at present.

Both *C. nigra* variants had significantly higher root and shoot dry-weights and number of inflorescences in the low water table tanks. In the T150 tanks, the commercial plants had slightly higher dry-weights, and produced more inflorescences, than the Wicken plants.

C. nigra is, according to Grime *et al.* (1988), a complex group in need of further taxonomic and ecological study. Although botanists have often identified the subspecies *nigra* and *nemoralis*, the distinction between the two is no longer believed to be consistent (Stace 1991). It is likely that the wide distribution of *C. nigra* is, in part, linked to its considerable genetic variation (Grime *et al.* 1988).

LOTUS SPP.

Seed of the two *L. corniculatus* variants showed lower germination rates in the high water table tanks with significantly higher overall establishment of seedlings in the low water table treatments. These results contrast with the findings of Baker (1988), where waterlogging had no effect on the germination of either *L. corniculatus* or *L. pedunculatus*. In the present study, the effect of water table on the establishment of *L. pedunculatus* seedlings was found to be highly significant ($p<0{\cdot}001$), with highest establishment in the low water table treatments. This observation makes interesting comparison with the usually observed habitat preference for *Lotus* spp. where *L. corniculatus* is thought to be extremely tolerant of water deficit (Grime *et al.* 1988; Bullard & Crawford 1996) whilst *L. pedunculatus* tends to grow in damp areas with a higher water table. Nevertheless, the findings do support the hypothesis of Blumenthal, Aston & Pearson (1996) that "variation exists within *Lotus* species for ability to germinate over a range of moisture potentials", implying that the establishment of *Lotus* spp. in wet grassland restoration schemes may be markedly influenced by water table levels.

Highly significant differences between the establishment of the three *Lotus* taxa were revealed. The establishment of Lcf was lower than that of the Lcc and Lp in all four treatments. In the two lowest water table treatments, *L. pedunculatus* also had markedly higher seedling establishment than *L. corniculatus* commercial. These results suggest that, in terms of seedling establishment, seed of Lcf would be less reliable for restoration purposes than Lcc and Lp seed. Sites with relatively low water tables might also benefit if *L. pedunculatus* seed were sown, as it was found to establish better than the commercial seed in treatments T100 and T150.

The biomass data underlined the germination and seedling establishment findings, with all three *Lotus* species performing significantly better (with higher dry-weights) at the lower water table levels. Unsurprisingly, the mean root and shoot dry-weights of the forage variety were markedly higher than those of Lcc and Lp. When examining inflorescence data at the lowest water table treatment, Lcf produced almost six times as many inflorescences as Lp and twice as many inflorescences as Lcc. This highlights the need to consider a range of factors, and not just seedling germination and establishment, when making decisions on the suitability of seed for use within restoration mixtures. Introducing forage and commercial cultivars, which may grow quickly and produce many inflorescences, could put less vigorous native species at a competitive disadvantage.

Grant & Small (1996) have carried out a detailed examination of the ancestry of the *L. corniculatus* complex in which evidence regarding the geography, genetics, chemistry and morphology of the genus was reviewed. On the basis of this they have suggested that *L. corniculatus* may have arisen from a hybrid of *L. glaber* and *L. pedunculatus*. If *L. pedunculatus* is a direct parent of *L. corniculatus* this might partly account for the ability of *L. corniculatus* to germinate and establish in both damp and dry conditions. Following an examination of the response of 91 species to flooding, Justin & Armstrong (1987) classified *L. corniculatus* as a plant of intermediate habitats. Prior to this research, they had assumed it to be a species indicative of non-wetland environments. Such findings further underline the potential variability within this genus, which makes defining the precise water table requirements of any available strain of *L. corniculatus* difficult for restoration purposes. As the present study was based upon seed obtained from a small number of sources, only a small fraction of the genetic variation present within *Lotus* may have been examined.

The present study has shown that the germination and establishment of seeds and seedlings, both between and within species, can vary significantly depending on the source of the seed. Seed collected from "ecotypes", adapted to specific environmental conditions, may have significantly different hydrological requirements to those of seed collected from other populations. A recognition and understanding of the differing autecology of ecotypes, and other closely related species and varieties, is essential if wetland restoration schemes are to achieve their full potential. Seed obtained from commercial sources may, as demonstrated in the present study, perform poorly in comparison to seed obtained from other known populations. In addition, one is often uncertain as to the age of commercially supplied seed and the conditions in which it has been stored. A lack of accurate information regarding the identification (usually at the subspecies level) and precise origin of commercial seed is a further problem.

Further research is required to investigate the effects of different water table regimes on both intra- and inter-specific competition between a range of ecotypes and cultivars of common wild flower species. Such interactions may exert considerable influence on the outcome of secondary succession following the sowing of seed mixtures containing such ecotypes and cultivars. It is likely that competitive interactions will be significantly accentuated under higher water table conditions. Using an experimental water table facility, the effects of water levels on the germination and survival of 23 wet grassland species have been studied (Walker *et al.* 1997). The first species to germinate were productive grasses, which suppressed the establishment of a number of herb species. This suggests that attempts to re-create wet grassland using seed may not always lead to the establishment of those species which germinate late and are subsequently out-competed. In such circumstances, plug plants may be a more effective means of introducing desirable species (Walker *et al.* 1997).

Crawford (1996) has reiterated the need for a holistic approach when studying how plants react to water table fluctuations. Plants may vary in their response to water stress at different stages in their life cycle (Evans & Etherington, 1990; Yaseen & Al-Omary 1994; Ollson *et al.*, 1996) and have to adapt to changing water levels whilst being in competition with other species (Walker *et al.* 1997). The present study was conducted under stable, non-competitive conditions and, as noted by White (1985), "there is doubt about the usefulness of studying the population dynamics of natural plant populations in isolation from their phytosociological environment".

CONCLUSIONS

It is estimated that between 1930 and 1984 semi-natural lowland grassland decreased by 97% in England and Wales (Fuller 1987; H.M. Government 1995). Re-creation of such communities and habitats needs to be underpinned by a scientific understanding of wetland species and ecosystems. This preliminary experiment has demonstrated that the autecological requirements of closely allied taxa and infraspecific variants should not be overlooked. The biomass and reproductive effort of variants of *Centaurea nigra* and *Lotus* spp. were found to be significantly affected by water table depth. Variants had higher root and shoot dry-weights, produced more inflorescences and, in some instances, showed greater seedling establishment in the low water table treatments. In addition, significant differences in seedling establishment between infraspecific variants of both *C. nigra* and *Lotus* spp were apparent. It is probable that such differences also exist between "ecotypes" of other species and research is needed to further our understanding of the water table requirements of the main constituent species and subspecies of wet grassland vegetation (Mountford & Chapman 1993). Habitat re-creation will become more successful if species' requirements are matched to site conditions. At present, the suitability of commercially available seed for wet grassland re-creation schemes is largely unknown and requires further investigation.

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