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PHENOTYPIC RESPONSES OF RANUNCULUS REPENS POPULATIONS IN GRASSLANDS SUBJECTED TO DIFFERENT MOWING REGIMES

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SUMMARY

Populations of *Ranunculus repens* were studied in grasslands that had been subjected to different hay-making regimes without the application of fertilizers. Cutting for hay occurred either in July, or in July and September, or in September only over a period of nine years that followed a prolonged period of hay-pasture. For comparison, spring and autumn seedlings derived from field populations, also subjected to the different hay-making regimes, were grown under standard conditions in an experimental garden. The differences observed in the field were not retained by the experimental populations with the exception of the seed weight in the spring seedling population. In contrast, differences found in the experimental populations between spring and autumn seedling populations indicated the importance of the time of emergence of seedling cohorts. Populations cut in July seemed to maintain themselves by sexual reproduction. Seedling emergence and survival was higher with the July than the September hay-making regime. The population cut in September maintained itself mainly by vegetative multiplication, since daughter-plants from stolons are independent from the end of August.

1. INTRODUCTION

Population differentiation as a response to man-management of grassland communities has been recorded in several species: *Rhinanthus angustifolius* (TER BORG 1972), *Achillea millefolium, Bellis perennis, Plantago lanceolata, P. major, Prunella vulgaris* (WARWICK & BRIGGS 1979), *Cynosurus cristatus* (LODGE 1964), *Dactylis glomerata, Phleum pratense* (VAN DIJK 1955; CHARLES 1964), *Poa annua* (WARWICK & BRIGGS 1978a, 1978b), *Lolium perenne* (CHARLES 1964).

Selection pressure on grassland plants can operate in the long run, but may begin to operate also after relatively short periods. SNAYDON & DAVIES (1972) found that populations of *Anthoxanthum odoratum* from limed and unlimed plots in the Rothamsted Park Grass Experiment in the United Kingdom, appeared to be both morphologically and physiologically adapted to the specific environmental conditions of their source site within 50 years. There is now evidence that populations of *Anthoxanthum odoratum* diverge within six years (SNAYDON & DAVIES 1982).

The present study deals with the possibility of rapid population differentiation in *Ranunculus repens*. It is a short-lived perennial which flowers between May

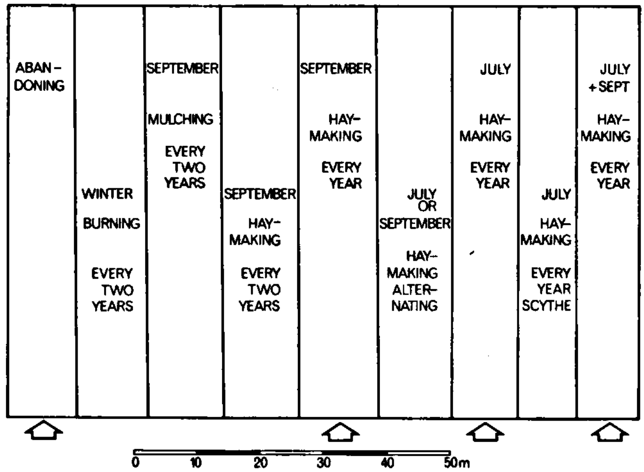


Fig. 1. Plan of the study area, indicating the plots with hay-making in July, July + September and September without application of fertilizers.

and July. The flowering period for each flower is seven days. Pollination may be by insects and/or by rain drops. Both sexual reproduction and vegetative multiplication are found. *Ranunculus repens* produces stolons from the end of May until the mid of July. Daughter-rosettes develop from the stolons. These daughters become independent from the parent-plant during September, having developed their own root system (SARUKHÁN 1974). The life expectancy of a plant arising from seed is about half a year, of a ramet one to two years (SARUKHÁN & HARPER 1973). Population differentiation in *Ranunculus repens* is described with respect to dry matter allocation, which occurred within about twelve years (LOVETT DOUST 1981).

The present study concerns hay-fields subjected to different cutting regimes since 1973. Hay was cut in either July or in September, or in both July and September. The goal of the present study was to quantify and analyse phenotypic differences between the three populations of *Ranunculus repens*. Various population characteristics were observed both in the field plots and in plants grown from seedlings under standard garden conditions.

2. THE STUDY AREA

The study area is a grassland lot (0.5 ha) in the nature reserve "Stroomdallandschap Drentsche A" (53°05′ N, 6°40′ E) in The Netherlands. It has a sandy soil rich in organic matter with a ground water level between –20 cm in winter and –100 cm in summer. Application of fertilizers, hay-making and grazing were standard agricultural practices until 1972 when the lot was acquired by the State. Manuring ceased in 1973 and various experiments were set up that year, *viz.*

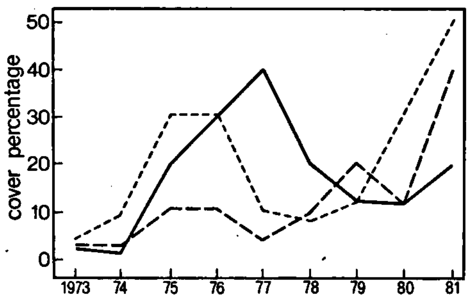


Fig. 2. Cover percentages of *Ranunculus repens* with hay-making in July (dashed line), July + September (dotted line) and September (solid line).

plots for July hay-making, September hay-making and plots for July + September hay-making (each 10 × 60 m) (*fig. 1*).

The vegetational changes and diverging standing crop including litter that resulted from the management practices adapted for the experiment have already been published (Bakker et al. 1980). In 1981 when the populations were studied, the total standing crop including litter just before cutting was 360 g dw.m^{-2} in the July-mown plot, 485 g dw.m^{-2} in the September-mown plot, and 255 + 275 g dw.m^{-2} in the July + September mown plot.

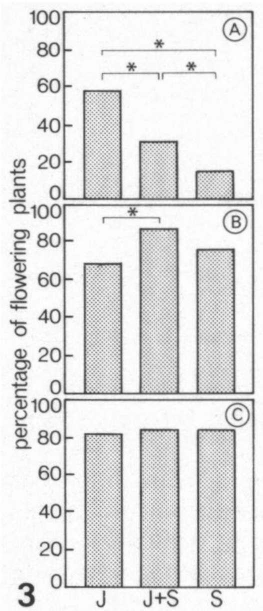
At the start of the experiment, about then rosettes .m^{-2} were found in each population. The reaction to all cutting regimes is illustrated by the cover percentages. These always increased, despite considerable annual fluctuations (*fig. 2*).

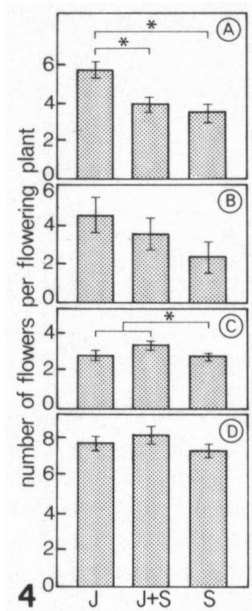
3. METHODS

3.1. Field populations

A hundred plants of *Ranunculus repens,* including ramets and seedlings, were selected at random from randomly selected transects in each plot in May 1981. The distance between the chosen plants exceeded the maximal stolon lenght of 50 cm (Sarukhán & Harper 1973). The ramets and seedlings were marked individually and measured as follows: (i) weekly count of the number of flowers, (ii) number and weight of seeds produced per individual (the inflorescences were caged after fertilization to prevent seedfall), (iii) germination percentage, after 28 days, of 100 randomly collected seeds, sown in Petri-dishes under a 12 hours alternating temperature regime at 25°C in the light and at 15°C in the dark, (iv) germination percentage of 125 randomly collected seeds when sown in the plots in 5 cm diameter rings filled with sterilized soil after hay-making in July, and (v) survival of seedlings marked in May. It was not known whether these seedlings had emerged in spring or in the previous autumn. The weekly count of the number of flowers was repeated in 1982. Nomenclature of plant species follows Van der Meijden et al. (1983).

Seedling survival was also derived from ongoing long term recordings every six to eight weeks (June 1977 until January 1984). Newly emerged seedlings were





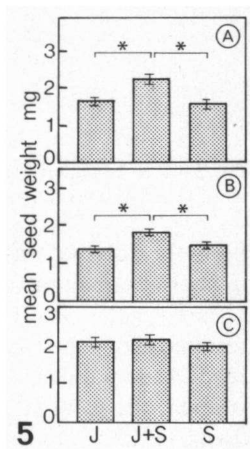


Fig. 3. Percentage of flowering plants of *Ranunculus repens* in (A) field populations with July, July + September and September hay-making, (B) spring seedlings and (C) autumn seedlings taken from these field populations and transplanted to the experimental garden. The asterisk indicates a significance level at $P < 0.05$.

Fig. 4. Number of flowers per flowering plant ($\pm$ S.E.) of *Ranunculus repens* in (A) 1981 and (B) 1982 field populations with July, July + September and September hay-making, (C) spring and (D) autumn seedlings taken from these field populations and transplanted to the experimental garden. The asterisk indicates a significance level at $P < 0.05$.

Fig. 5. Mean seed weight (mg) ($\pm$ S.E.) of *Ranunculus repens* in (A) field populations with July, July + September and September hay-making, (B) spring and (C) autumn seedlings taken from these field populations and transplanted to the experimental garden. The asterisk indicates a significance level at $P < 0.05$.

marked individually and recorded at five replicate plots (10 × 10 cm) (Bakker et al. 1980). This approach remains tentative since seedlings may have emerged and disappeared during the six week interval between two recordings. Seedlings emerged between early spring and July were labelled as spring seedlings and those emerged between hay-making in July and early winter as autumn seedlings, respectively.

3.2. Experimental populations

The garden populations were grown from seedlings collected from the field plots. We assumed that such an experimental population reflects a potential field population. Seedlings were collected in spring and in autumn. We assumed too that the seedlings emerged from recent seeds locally produced, since *Ranunculus repens* is considered a transient seed bank species (Bakker 1983).

Seedlings were collected at random from the three studied plots in spring and

Table 1. Number of seeds (± S.E.) produced per flower of *Ranunculus repens*, spring seedlings and autumn seedlings transplanted to the experimental garden. In the field sites hay-making takes place in July, July + September and September. In addition the total seed production of 100 marked plants in the field populations is represented. The asterisk indicates a significance level at P < 0.05.

Hay-making period	Field population	Experimental populations derived from: Spring seedlings		Experimental populations derived from: Autumn seedlings	Total seed production of 100 marked plants in the field population		
July	25.3 ± 0.8	19.3 ± 1.4	*	20.5 ± 2.0	8420	*	*
July + September	22.4 ± 1.0	25.4 ± 1.5	*	24.3 ± 1.8	3349	*	
September	28.2 ± 2.5	20.8 ± 1.1		20.0 ± 1.1	2028		

autumn of 1982 and transplanted individually into a plastic pot of 18 cm diameter (volume 1.8 l) filled with potting soil (fertilized peat). The pots remained outdoors during winter. Seedling mortality was less than 5%. About 30 plants from spring and autumn seedlings from each plot were available in the spring of 1983. All plants were studied for the same items as the plants selected in the field plots except for the sowing experiment.

Most data were subjected to analyses of variance (CAMPBELL 1967). Differences in percentage of flowering plants and seedling counts were analysed by the χ^2-test. Too few plants grown from autumn seedlings were available to allow statistical analysis.

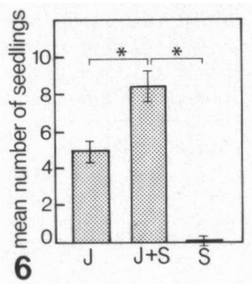
4. RESULTS

4.1. Field population

Flowering plants were found in the field populations from the beginning of May until the end of June. No flowers were generated after cutting. The flowering period was not different for the three management regimes. Hay-making in September resulted in the lowest overall percentage of flowering plants (*fig. 3A*),

Table 2. Germination percentage (± S.E.) of seeds of *Ranunculus repens* after 28 days in a germination chamber at a day and night temperature of 25 °C and 15 °C, respectively. The seeds have been derived either from field populations or from spring and autumn seedlings transplanted to the experimental garden from plots with hay-making in July, July + September and September. The asterisk indicates a significance level at P < 0.05.

Hay-making period	Field population		Experimental populations derived from: Spring seedlings			Experimental populations derived from: Autumn seedlings
July	71.6 ± 3.6	*	61.9 ± 5.5	*	*	85.5 ± 4.1
July + September	57.8 ± 4.6	*	86.2 ± 2.2			77.8 ± 5.3
September	68.2 ± 7.3		84.6 ± 1.9			78.4 ± 3.7



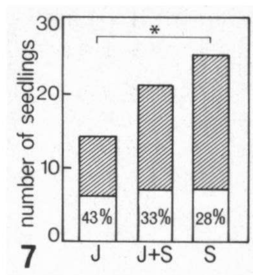


Fig. 6. Mean number of seedlings (± S.E.) emerged from 25 seeds of *Ranunculus repens* sown in July (five replicas), in the period July 1981 – spring 1982, with July, July + September and September hay-making.

Fig. 7. Number of spontaneously emerged seedlings of *Ranunculus repens* in May 1981, number of seedling percentage still surviving in November 1981 (white) and number of seedlings that died (black) with July, July + September and September hay-making. Significance bar refers to the number of spontaneously emerged seedlings.

the lowest number of flowers per flowering individual (*fig. 4A-B*), and the lowest number of seeds produced per 100 plants (*table 1*). These differences were significant. Hay-making in July + September revealed the lowest number of seeds produced per flower (*table 1*), but these seeds were significantly heavier than those from the other plots (*fig. 5A*).

Germination percentages after 28 days proved that more than 57% of fresh seeds from the field populations could germinate within this period. The germination percentage of the populations under the July and September hay-making regime was significantly lower than that of populations with one hay-making term (*table 2*).

Most seedlings emerged from the seeds sown in the plots with early hay-making. Hardly any seedlings emerged in the plot with September hay-making (*fig. 6*). From the seedlings present in May the highest percentages survived until November in the early mown plots (*fig. 7*). Long term recording revealed a trend of more spring seedlings passing into the juvenile/adult phase than autumn seedlings (*table 3*).

Table 3. Survival of spring and autumn seedling cohorts of *Ranunculus repens* in five replicate plots of 10 × 10 cm each, recorded from June 1977 until January 1984 in fields with hay-making in July, July + September and September.

	July hay-making		July + September hay-making		September hay-making	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
Total	24	29	6	16	32	7
Dead	21 (87%)	24 (83%)	5 (83%)	15 (94%)	12 (37%)	7 (100%)
Juvenile/adult	3 (13%)	5 (17%)	1 (17%)	1 (6%)	20 (63%)	0 (0%)

4.2. Experimental populations

The total percentage of flowering plants over the whole period did not differ between the various populations (*fig. 3B-C*). It varied between 70% and 88% in the spring seedling populations and between 82% and 84% in the autumn seedling populations.

The number of flowers per flowering plant did not differ between the populations under different hay-making regimes. This held both for the spring seedling populations and the autumn seedling populations. However, a clear difference was found between spring seedling populations varying between 2.8 and 3.4 flowers and autumn seedling populations varying between 7.3 and 8.2 flowers (*fig. 4 C-D*).

The numbers of seeds produced per flower were significantly higher in the plants from the spring seedling populations with hay-making in July + September, however not under field conditions (*table 1*). Plants from this regime also produced heaviest seeds under field condition and from the spring seedling population (*fig. 5 A-B*) but not from the autumn seedling population (*fig. 5C*). Again a difference seemed to occur between spring seedling populations varying between 1.4 and 1.8 mg and autumn seedling populations varying between 2.0 and 2.2 mg.

5. DISCUSSION

The field populations of *Ranunculus repens* under three hay-making regimes showed significant differences for a number of parameters. The numbers of flowers per flowering plant and the seed weight were fairly equal in the experimental populations from the hay-making regimes but differed markedly between spring and autumn experimental populations.

Since the experimental spring and autumn seedling populations were derived from the same seed pool in each hay-making regime and differences between field populations were not maintained in both experimental populations we can only conclude to variability among individuals with respect to the studied characters. Further studies should reveal whether the variability can be attributed to plastic response of individual plants or to genetic differences. The seed weight differences in the experimental populations from spring seedlings concord with those found in the field populations and may become important since spring seedling cohorts tend to survive better than autumn seedling cohorts. Although the half-life is comparable to that of *Anthoxanthum odoratum*, averaging two years (DAVIES & SNAYDON 1976) no diverging populations of *Ranunculus repens* were found during nine years of hay-making regimes.

Spring and autumn seedlings taken from the field populations and transplanted to the experimental garden did not concord completely with the measured parameters. This emphasizes the importance of ascertaining the origin of seed samples collected for experiments.

Despite the relatively low germination percentage of fresh seeds from the field plot with July + September hay-making, most seedlings emerging from the seeds sown in the field in July were found in the field plot with July + September hay-making. Many seedlings also emerged with July hay-making, and none at September hay-making, therefore suggesting that the density of the surrounding canopy determines seedling emergence. Most seedlings occurring in May die in the dense canopy of the field plot with September hay-making. A large standing crop generally causes strong light reduction and consequently diminishes the performance of a number of species (HARPER 1977; BLOM 1977; MØLGAARD 1977; BAKKER et al. 1980; SILVERTOWN 1980). The changing red/far-red ratio has the same result (FENNER 1978).

The density of the sward is not the only factor which determines seedling emergence and survival. The heavy seeds of the population in the July + September hay-making plot may also contribute to the performance of that population. GRIME (1979) mentioned that large-seeded species have an advantage over small-seeded species at establishing themselves in closed canopies. Such a relation is found by GOLDBERG & WERNER (1983) for fruits of *Solidago* species and by GROSS & WERNER (1982) for some biennial species. Thus the open sward produced by cutting in July provides good germination sites. This effect is enhanced by the heavy seeds of the July + September hay-making population.

SARUKHÁN (1974, 1976) suggested a trade-off between vegetative multiplication and sexual reproduction. SALISBURY (in ABRAHAMSON 1980) shared this opinion. SARUKHÁN (1974) found that daughter plants of *Ranunculus repens* were independent at the end of September in the United Kingdom. VAN DIJK & KERSSIES (1982) found in grasslands adjacent to our study area that the first stolons developed in mid-May and that the first independent daughter plants can be found at the end of August. It seems that the population with September hay-making maintains itself by both vegetative multiplication and sexual reproduction, while the populations with July or July + September hay-making maintain themselves only sexually since vegetative multiplication is prevented by the summer hay-making. It seems, therefore, to be of ecological significance to produce more and heavier seeds with early hay-making. The vegetative multiplication with early hay-making can be compensated by the ability to produce more flowers per flowering plant resulting in more and/or heavier seeds. These seeds have a higher probability of producing seedlings and mature plants than those from the population under September hay-making, because of the density of the canopy. The heavier seeds produced with early hay-making might also be due to the lack of other sinks for carbohydrates and mineral nutrients.

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