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Reciprocal transplantations to study local specialisation and the measurement of components of fitness.

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RECIPROCAL TRANSPLANTATION TO STUDY LOCAL SPECIALISATION AND THE MEASUREMENT OF COMPONENTS OF FITNESS

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SUMMARY

Reciprocal transplant experiments have been made to investigate the intra-specific variation in two clonal species, *Primula vulgaris* and *Ranunculus repens*. *Primula* transplants performed best when returned to their native populations, indicating that they were differentiated in response to local conditions. There was marked variation in the degree of local specialisation of plants in different primrose populations and possible causes of this variation are discussed. Although buttercup transplants also showed great variability, there was no evidence that they were specialised, either between, or within, local populations. The lack of genetic specialisation in *Ranunculus repens* may be due to its spreading growth form, widespread distribution and low level of seedling recruitment.

In glasshouse experiments, the presence or absence of neighbours affected many parameters of buttercup growth. Within a genet the effect of edaphic and biotic heterogeneity was integrated, so that ramets in favourable conditions supported interconnected ramets in less favourable sites. Plants of *R. repens* vary phenotypically in different environments but appear to respond to heterogeneous local conditions by phenotypic plasticity of individual ramets rather than genetic specialisation.

The assumption that differences between transplants are solely indicative of genetic specialisation has been questioned. Virus infection was detected in 7 of 14 primrose populations surveyed. Infected plants showed no symptoms of disease, yet they produced significantly fewer but larger leaves than uninfected plants. Differences between transplants which could easily be attributed to genetic variation may be due to differential virus infection. Furthermore, viruses may ultimately contribute to genetic differentiation and have a role as selective forces in the environment. Phenotypic differences between ramets of the same genet of *R. repens* were maintained and even increased after 26 week's growth in a common environment. It is clearly important in transplant experiments to use comparable phenotypes and virus-free plants when determining the role of genotype in the match between organism and environment.

CHAPTER 1

General Introduction

Introduction

Over a 1/4 of a million plant species have been distinguished by taxonomists attempting to order and classify the great variety of living plants. The taxonomists have defined these species on the basis of stable characteristics, mostly of floral morphology, and members of the same species are seen as differing "only in minor details" (Oxford English Dictionary, Concise Edition). Conspicuous morphological and physiological differences between the individuals within a species have frequently been ignored, as they concern taxonomically "bad" characters (see Snaydon, 1973) i.e. they show high genetic variability and are modified by environmental conditions. Yet, it is this variation which is important to the ecologist as it reflects the interaction between the plant and its environment. Characteristics such as: palatibility or toxicity; tall plants or short and different times of germination or flowering are crucial to plant performance. The study of intra-specific variation focusses attention on these ecologically-important differences whereas the taxonomic species may be too broad a category for the ecologist who is concerned with the actual variability expressed in the field.

Darwin (1859) emphasised the importance of intra-specific variation in evolution:

> "No-one supposes that all the individuals of the same species are cast in the very same mould. These individual differences are highly important for us, as they afford materials for natural selection to accumulate."

Phenotypic differences between individual plants are indicative of the <u>proximal</u> effect of the environment on a plant's behaviour whereas, their genetic variability may reflect differential selection which has <u>ultimately</u> occurred in response to different habitat factors. This environmentally-induced genetic variation is frequently based on complex multiple gene

systems (Grant, 1971), unlike the big gene blocks on which the taxonomists' genetically stable characters are based, and alters in time and space with the heterogeneity of many environmental factors. Some genetic differences will also result from the chance fixation of different alleles (drift) and from pre-determined genetic characteristics (e.g. the founder effect). Consequently, it may be impossible to resolve intra-specific variants into discrete, stable units analogous to those of conventional taxonomy. However, by identifying the present pattern of intra-specific variation between and within natural populations and relating this to current environmental factors, we can gain a valuable insight into the forces of natural selection. The locally-differentiated population is a fundamental unit for evolutionary study because it represents the product of evolutionary processes at present operating on individual plants. It is only by the proximal observation of evolution in action that we can hope to understand the mechanisms of natural selection.

Intra-specific variation has been studied on a geographical scale, in plants growing in different populations in the same locality and even within populations. Morphological and physiological variants of the same species have been described and, more recently, differences in plant enzyme systems have been investigated. This variation has been correlated with various climatic, edaphic and biotic aspects of the environment. A number of these studies are reviewed below.

Experimental Studies of Intra-specific Variation in Plants

1. Transplant Studies

Morphological Modifications

Early investigations of intra-specific variation considered the morphological diversity of plants growing in different climatic conditions. Plants were cultivated from seed and grown in experimental gardens at contrasting altitudes: 180m; 569m and 2195m (Kerner, 1895). Individuals of the same species varied in morphological characteristics when grown in the different sites. These intra-specific differences were not transmitted to the progeny but represented temporary (phenotypic) modifications in response to environmental forces. The effects of contrasting environments were compared within a single genet by transplanting clones, or ramets, of the same plant into different altitudinal locations (Bonnier, 1890). A number of modifications were noted, in particular, that lowland plants transplanted to high altitudes showed the dwarf-form characteristic of native alpine plants (Bonnier, 1920).

Ecotypes

The above studies emphasised that the environment could induce morphological variation from a single genotype. The relationship between these observed phenotypic modifications and the underlying genetic variation within natural plant populations was clarified by Turesson (1922a,b). He collected plants from diverse sites, cultivated them in an experimental garden and recorded their physiological and morphological characteristics. By comparing plants growing under uniform conditions he excluded any differential effect of the environment on the phenotype and genetic variation was revealed. As a result of these studies Turesson

recognised genetically differentiated units within the species. These units, ecotypes, were defined as "products arising through the sorting and controlling effect of habitat factors upon the heterogeneous species-population" (Turesson, 1925). Turesson distinguished between climatic ecotypes, in which factors of the climate were important in the differentiation of the species and edaphic ecotypes, which were differentiated in response to soil factors.

(i) Climatic Ecotypes

Climatic ecotypes have been recognised in species occurring along a transect, from sea-level to a height of 3000m, in Central California (Clausen, Keck & Hiesey, 1940). Plants were sampled from diverse locations, cloned and transplanted into experimental sites at 30m, 1400m and 3050m on the transect. Morphological and physiological reactions of the transplants, together with their cytological and genetical characteristics, were used to identify and characterise ecotypes. In widespread species e.g. *Potentilla glandulosa* and *Achillea millefolium* variation was expressed as a series of ecotypes which reflected the range of habitats in which the species occurred.

In assessing intra-specific variation it became "standard procedure" (Stebbins, 1950) to record plant characteristics at a number of transplant stations (e.g. Lawrence, 1945 and Bonde & Foreman, 1973). Gene expression is influenced by environmental factors and in the transplant stations, where plants are subjected to the gross physical factors of their native habitat, gene activity will be more representative of that in the natural site than in the standard garden environment. However, the biotic environment of these transplant sites was often drastically altered by: the removal of natural vegetation; 1-2m spacing between transplants; "frequent

careful weeding to prevent contamination of the cultures" (Hiesey, 1940) and the erection of protective fencing around the site. These practices excluded inter- and intra-specific interactions and larger predators and were likely to lead to mis-representation and especially under-estimation, of intra-specific differences in the transplant sites (Snaydon, 1970).

Reciprocal Transplants

An exact reversal of environmental conditions was attained by reciprocal transplantation, in which pairs of individuals were sampled from contrasting environments and each plant placed in the hole left by the other member of the pair (Clements & Hall, 1918). Such transplants tended to be scattered, their protection and recording was costly and laborious and mortality was high (Clausen, Keck & Hiesey, 1940). However, these transplants were remarkable in being truly reciprocal and thus exposing the plants to all the vicissitudes of their natural environment, both the physical and biotic factors. In subsequent studies, plants have usually been re-planted in a specific study site rather than dispersed throughout their precise sampling locations. Consequently, as with the transplant stations of Clausen *et al.*, cultivation and fencing of the transplant areas has frequently greatly limited biotic interactions (e.g. McMillan, 1957 and Mark, 1965). The importance of reciprocal transplantation into the natural community has only recently been realised and, 60 years after the pioneer experiments of Clements and Hall, true reciprocal transplants have again been used (Turkington & Harper, 1979b).

Ecoclines

The recognition of ecotypes within species implies that genetically discrete units are formed as a result of intra-specific differentiation. Continuous, rather than merely discontinuous intra-specific variation was

recognised by Huxley (1938), who realised that a regular gradation in variation may be more common than the formation of discrete units. He introduced the term cline, "a gradation in measured characters" and advocated its adoption as a supplementary principle in taxonomy.

A clinal pattern of variation was recognised when *Plantago maritima* was sampled from a series of populations (Gregor, 1939). Morphological characters, such as spike-density and growth-habit, showed a continual gradation, or ecocline, which followed an ecological gradient in the availability of water. Similarly, in *Agrostis stolonifera*, the length of stolons varied in relation to an environmental factor, the degree of exposure in the plant's original habitat. Plants from populations sampled in a continuous series showed a gradual, or clinal, variation whereas, plants from a transect with sudden environmental changes varied abruptly, with distinct differences between adjacent populations (Aston & Bradshaw, 1966).

In a less extreme environment the situation is frequently more complex, with individual populations at different points on many environmental clines. The differentiation of 30 populations of *Agrostis tenuis* in Central Wales showed no overall clinal regularity but formed a "graded patchwork" pattern in response to a number of habitat factors. (Bradshaw, 1959a). This fine-scale differentiation focusses attention on the edaphic and biotic factors of the environment which vary within a geographic region.

(ii) Edaphic Ecotypes

Natural populations of many species may encounter a wide range of edaphic factors. *Festuca ovina* plants, which experienced contrasting soil conditions within a distance of one mile, differed in their response to

calcium in culture solutions (Snaydon & Bradshaw, 1961). Chemical analysis revealed physiological variation in the plants' ability to absorb cations. This variation was correlated with the soil-type of the original habitat: plants from the acidic soils showed more effective uptake of calcium than those from calcareous sites. These local populations of *F. ovina* were differentiated in response to edaphic factors and termed edaphic ecotypes.

Edaphic ecotypes have also been identified in the vicinity of old mine-workings (e.g. Antonovics, Bradshaw & Turner, 1971). Across the boundary of a mine there is often a sharp transition in the amount of toxic elements in the soil. This was reflected in different levels of lead tolerance in populations of *Agrostis tenuis* growing within 20m of each other but on either side of the boundary of a lead mine. A similar pattern of differentiation in *Anthoxanthum odoratum* was correlated with the distribution of zinc in soil near a zinc mine.

In the Park Grass experiment at Rothamsted, fertiliser and liming treatments have caused large differences in chemical composition of plots less than 30m apart. The role of specific nutrients e.g. calcium and phosphate, in the differentiation of *Anthoxanthum odoratum* growing in these plots was assessed using the sand-culture technique (e.g. Davies & Snaydon, 1974). Plant growth in culture solution was strongly correlated with the concentration of individual soil factors in the original plot and edaphic ecotypes were recognised. However, plant characteristics expressed in a sand-culture are not necessarily representative of those in the natural environment. Pairs of plants were reciprocally transplanted between contrasting plots to reveal variation which was relevant to the growth of plants in their natural environment (Davies & Snaydon, 1976). Transplants survived longer and produced more dry matter in their native environment than when transplanted into an alien plot. Soil-type, the height of

surrounding vegetation and corresponding light intensity have all been implicated as factors in the population differentiation of *Anthoxanthum odoratum* (Snaydon & Davies, 1972).

(iii) Biotic Ecotypes

The majority of ecotypic studies have sought correlations between intra-specific variation and the physical environment. Wallace emphasised the physical components of the environment, together with predators, as the major forces of natural selection (Darwin & Wallace, 1858). Experiments designed to investigate populations in contrasting climates, or on diverse soil-types, have only attributed differentiation to the differences in climate and soils and identified climatic and edaphic ecotypes. These experiments have frequently been conducted in a "garden" or "culture" environment from which biotic interactions, with neighbours and herbivores, were excluded. Yet, Darwin stressed the importance of inter- and intra-specific interactions and the "struggle" of individuals "with other members of the same or different species" (Darwin & Wallace, 1858). Darwin emphasised the role of biotic forces in the processes of natural selection but few studies have demonstrated their influence in intra-specific differentiation.

(a) Interactions with Neighbouring Plants

Biotic ecotypes were first recognised in *Dactylis glomerata* (Stapledon, 1928). *D. glomerata* is a widespread and variable species which grows in agronomic habitats, where diverse management regimes can result in major floristic differences within climatically similar regions. Correlations between the morphological characteristics of *D. glomerata* and the type of habitat in which it was growing were maintained during cultivation in a common environment and, more important, were also observed in seed progeny.

This showed that there was truly genetic differentiation in response to the contrasting environments experienced prior to sampling. The original habitats differed primarily in biotic factors but because of their wide geographic separation, over three continents, climatic and edaphic variation could also have contributed to the differentiation.

Sinskaja (1931) defined the synecotype as the product of the selective action of different neighbouring species. Two morphologically distinct synecotypes of *Brassica nigra* were recognised in Asia Minor. One form of this species always grew in association with *Brassica campestris* whereas, the second occurred exclusively in pure populations of *B. nigra* but specific environmental differences between the populations were not defined.

Potentilla erecta varies considerably in size when growing in association with different species (Watson, 1969). Plants from neighbouring *Molinia*-dominated and pasture (*Agrostis/Fescue*) habitats differed morphologically at the time of sampling and throughout a three-year period of cultivation. Seed progeny were more variable but, in general, *P. erecta* from the pasture sites was smaller in diameter and had shorter stems and basal internodes and a lower root-stock weight than plants from the *Molinietum*. Differentiation of the populations in these adjacent habitats was indicative of the selective effect of the contrasting biotic environments. However, the variation in *Potentilla* could have been a direct result of those same environmental conditions which initially favoured growth of *Molinia* in one habitat and *Agrostis* in the other, rather than the product of habitat differences imposed by the presence of the two species. Furthermore, morphological differences between plants growing in an experimental garden provide an unreliable estimate of the variation expressed in the natural environment.

Inter- and intra-specific interactions between plants exert a selective influence by affecting the quantity and quality of resources available to neighbours. Plants also interact with other biotic components of the environment; the herbivorous animals and plant-pathogenic fungi, bacteria and viruses.

(b) Plant Interactions with Herbivores

The insect herbivore, *Oporinia autumnata*, attacks *Betula pubescens* throughout Finland but plants from different populations vary in the extent to which they are damaged. In reciprocal plantings, trees that were returned to their natural environment suffered less insect-damage than those from alien populations (Haukioja, 1980). This differential response indicates that leaf damage by the herbivore may be a selective factor influencing differentiation within the plant species.

The importance of defoliation by man as a selective factor was investigated in pot and transplant experiments involving *Plantago major* from lawn and roadside populations (Warwick & Briggs, 1980b). Under a regular mowing or clipping regime plants from lawn populations showed a high reproductive success whereas, the more erect roadside plants frequently lost all reproductive structures, or produced very few seeds on damaged inflorescences. Conversely, in plots not subjected to defoliation, roadside plants had a significantly higher reproductive and vegetative dry weight than lawn plants. These observations provided evidence that populations of *P. major* were genetically differentiated in response to defoliation. The relative performance of plants in contrasting management regimes was also used to estimate coefficients of selection (after Jain & Bradshaw, 1966). However, these experimental data do not provide an accurate estimate of the force of selection because the environment of the

pots and the transplant lawn differed from that of the natural habitats. Furthermore, growth of *P. major* from the contrasting populations would have been differentially affected if the environmental conditions in the transplant site were more akin to one of the two habitats sampled.

In natural populations of *Lotus corniculatus* molluscs preferentially feed on acyanogenic plants. This selective grazing, particularly at the seedling stage, is thought to affect the frequency of cyanogenic and acyanogenic morphs in the population (Crawford, 1972). Variation in the level of cyanogenesis polymorphism in local populations has been correlated with differences in the abundance and distribution of molluscs (Ellis *et al.*, 1976). Grazing by molluscs has been shown to result in differential survival of cyanogenic and acyanogenic clover plants, providing definitive evidence of the selective role of a herbivore in *Trifolium repens* (Dirzo & Harper, 1982). Detailed observation of the performance of transplanted individuals in their natural habitat revealed that a number of selective factors interacted with the different morphs. For example, cyanogenic plants were highly susceptible to the systemic rust, *Uromyces trifoli*, which suggests that differential pathogen attack is a further biotic factor influencing the cyanogenesis polymorphism in white clover.

(c) Interactions with Pathogens

There is little evidence concerning the role of pathogens in intra-specific variation in natural populations. Climatic ecotypes of *Deschampsia caespitosa* differed in their susceptibility to rust fungus. However, this differentiation was thought to be a response to the physiological state of the host plant and not an indication of genetic variation in disease resistance (Lawrence, 1945). Similarly, *D. caespitosa* from high-altitude sites showed little resistance to root-rot pathogens

(*Rhizoctonia*); this was attributed to the poor growth of these plants in the low-altitude transplant garden, rather than a high genotypic susceptibility to the pathogen (Pearcy & Ward, 1972).

Agricultural crops have been extensively studied by plant pathologists and the selective effect of pathogens in cultivated species is well documented. Flor studied the genetics of both hosts and pathogens and formulated the gene-for-gene hypothesis to explain their interaction. Cultivated flax (*Linum usitatissimum*) is attacked by the flax-rust *Melampsora lini* but some some varieties of flax were found to be resistant to particular races of the pathogen. Flor concluded that "for each gene conditioning resistance in the host there is a specific gene conditioning pathogenicity in the parasite" (Flor, 1956). The genic systems for the plant host and its pathogen are complementary, with every host gene for pathogen resistance corresponding with a virulence gene expressed in the pathogen. The abundance and distribution of different races of the pathogen are reflected in the variation of its host and determine the genetic variability of the plant species with respect to pathogen resistance. A similar gene-for-gene relationship has been recognised in plant interactions with: viruses; bacteria; fungi; nematodes; insects and angiosperms (van der Plank, 1982).

Local Biotic Differentiation in *Trifolium repens*

The studies cited above indicate the potential influence of the biotic environment on intra-specific variation. Conclusive evidence of the action of biotic factors in genetic differentiation has been accumulated in studies of *Trifolium repens*. In natural populations, *T. repens* typically has a high genetic diversity (Cahn & Harper, 1976) and experiences predominantly inter-specific contacts in association with a variety of

neighbours (Turkington & Harper, 1979a). A series of transplant experiments was designed to investigate the relationship between the genetic variation of *T. repens* and various neighbouring grass species (Turkington & Harper, 1979b).

Four sites, each dominated by a different species of grass, were selected within a one hectare field of permanent grassland. There were no major climatic or edaphic differences between the sites. Cuttings of *T. repens* were taken from each field site and cloned to produce many ramets, enabling every genet to be represented and replicated in all transplant sites. Ramets which were transplanted into denuded plots in the field differed significantly in dry weight, demonstrating the presence of genetic variation in *T. repens*. Ecologically relevant differences within the species were revealed by reciprocally transplanting ramets into the natural vegetation. Ramets were transplanted such that every genet was represented in its original, "native", site and in each of the three alien sites. The ramets which were re-planted in their native site made significantly more growth than those in alien sites. This indicated that genetic differentiation had occurred in response to the different environmental conditions in the contrasting sites.

The specific role of the different grasses in the differentiation of *T. repens* was demonstrated by transplanting ramets into pure swards of each of the four grass species in John Innes compost in the glasshouse. Yield was highest when the ramets were transplanted into a sward of the same species which dominated their original site. As a result of these studies, Turkington and Harper concluded that fine-scale genetic differentiation of *T. repens* was a micro-evolutionary response to the selection pressure exerted by different neighbouring species of grass.

2. Electrophoresis

Since the advent of starch-gel electrophoresis it has been possible to obtain a direct indication of genetic variation, principally in the enzyme system, in plant species. Many of the enzymes in plant cells are polymorphic, consisting of a number of related structures or isoenzymes. These isoenzymes differ in mass and net charge and show differential mobility when placed in a gel across which an electric field is generated. Variation in the genes coding for specific enzymes is recorded as different banding patterns, which mark the position of different isoenzymes present in the gel. Electrophoresis has been used in many studies of intra-specific variation (e.g. Clegg & Allard, 1972 and Baker *et al.*, 1975) and yet this technique fails to detect a considerable proportion of the genetic variation present.

Differences in the conveniently assayed products of a few enzyme loci are revealed by electrophoresis but there is no indication of the influence of this variation in net charge of enzymes on the phenotype, or of the overall variability of genes coding for other proteins in the plant. In order to obtain a realistic assessment of the ecologically relevant variation within a plant species it is necessary to characterise the actual differences exhibited between plants in natural populations, rather than merely their primary gene products.

In an attempt to relate variation in the pattern of heterozygosity at a number of enzyme loci to differences in plant growth, plants of *Liatris cylindracea* were grown in uniform conditions in an experimental garden (Schaal & Levin, 1976). Reproductive potential and biomass were positively correlated with heterozygosity but the experimental data may have under-estimated this relationship because it was based on plants growing in a

glasshouse. Differences in the growth and phenology of *Chenopodium album* plants, sampled from and transplanted into different natural populations, were also found to correspond with their pattern of electrophoretic variation. Individuals which were susceptible to atrazine were very variable, both between and within populations and this was associated with the high polymorphism of enzymes in these plants. In contrast, atrazine-resistant plants showed a narrower range of plant characteristics and correspondingly greater enzyme homogeneity (Warwick & Marriage, 1982). However, although returned to their natural physical environment, these plants were transplanted into garden plots at each site and therefore, not subjected to natural biotic factors. The expression of plant characters is dependent upon their environment and the fitness traits exhibited in the atypical physical and/or biotic conditions of a glasshouse or garden plot, even if this is situated adjacent to the natural population, will not necessarily be those expressed by, or relevant to, plants growing within their native site.

More direct evidence of the relationship between the selective effect of local environmental conditions and enzyme polymorphism is found in the differential survival of electrophoretically-distinct seedlings (Ennos, 1981) and transplants (Trathan, 1983) of *Trifolium repens* introduced into natural populations. It is only when the results of an electrophoretic analysis are considered in conjunction with demographic data on the performance of plants in their native field site that we can assess the relevant variation in enzyme systems and begin to speculate about the mechanisms of evolution in natural plant populations.

The aim of the present study was to examine the genetic specialisation, or fine-scale evolutionary differentiation, within and between local populations of two contrasting species:

Ranunculus repens, which shows extensive clonal growth dispersing many ramets along a number of stolons up to one metre long within a season and

Primula vulgaris, with a more limited and slower rhizomatous growth of only a few centimetres per annum. The current season's leaves are borne at the tip of the rhizome, usually as a single ramet, though the rhizome may fragment to produce a small number of ramets.

The fundamental procedure in the study of intra-specific variation is the recording of differences between individuals which:

i) have a genetic basis

ii) are relevant to the survival of plants in their natural environment

iii) can be correlated with environmental variables

thereby identifying differentiation which is due to the action of ecological forces in natural selection. Transplant experiments provide a powerful technique for characterising this variation in plant species.

An attempt has been made to correlate the observed intra-specific differentiation with the diverse biotic factors experienced both between and within local populations. The precise association of transplant characteristics with genetic variation will not be possible if pathogen infection or physiological differences are carried over from the previous environment and contribute to the phenotypic variability of individuals within the transplant site. The potential effects of differential pathogen infection and carry-over of phenotypic differences on individual transplant performance have been assessed and the possible mis-interpretation of experimental results is discussed.

CHAPTER 2

Reciprocal Transplant Experiments with 2 Contrasting Species

Introduction

In a transplant experiment genetic differences are revealed by growing material from diverse sources in the same environment. The variation between individuals which persists under common cultivation conditions is assumed to reflect genotypic differences and may be correlated with the various habitats from which the plants were sampled. If transplants are cultivated in a uniform garden or experimental plot, which is free from native vegetation and partly protected from herbivore attack, they may exhibit characteristics not normally expressed in their native site. In assessing genotypic differences which are relevant to plants in local populations, transplants must be introduced into a natural community in which they interact with both the physical and biotic components of their environment. Furthermore, the comparison between plants from different populations transplanted into a single site, may be misleading if the transplant environment is more similar to that of plants from one particular site, and differentially affects transplant growth. In this study a reciprocal method of transplantation was employed. Plants from a number of different local populations were re-planted in their native site and into all other sites sampled, to achieve a comparable estimate of the ecologically relevant variation between individuals from, and in, different habitats.

Individuals from local populations, which are differentiated through the action of natural selection, should show differences in fitness attributes such that the fitness of plants re-planted in their native environment is greater than that of plants in an alien environment. I have used estimates of relative fitness, obtained by recording parameters of transplant growth and reproduction, to determine whether differences

between: i) primrose and ii) buttercup plants reflect random genetic variation or selective evolutionary specialisation in response to their local environment.

Transplant Experiments in *Primula vulgaris*

The Species

Primula vulgaris is a low growing herbaceous perennial with obovate-spathulate leaves 8-15cm in length, forming a loose rosette typically 5-25cm in diameter. Yellow, or sometimes pink, flowers are borne singly on a long stalk which arises from the base of the plant in March-May and, occasionally, in autumn and winter. The flower stalk curves downwards as the seeds mature and are released from the ovoid capsule.

The primrose has a widespread distribution, from the maritime region of Western Europe to an altitude of 2,500m in Western U.S.S.R. Throughout this extensive range, over 20 sub-species, varieties and forms have been identified on the basis of fluctuations in leaf-shape, pubescence and flower colour. Yet, there is "no significant deviation from what may be termed the normal plant" (Wright-Smith & Fletcher, 1947) and in Great Britain, only one geographical race of *Primula vulgaris* is recognised.

Primroses are found in every region of the British Isles, on a wide range of soil-types, in woods and hedgerows and in open grassy places in the west but not in dense shade.

Reproductive Characteristics

Primrose flowers are predominantly heterostylous and the pin and thrum morphs are self-incompatible as a result of differential pollen-tube growth. In most natural populations, primroses are predominantly out-crossing, although a small proportion (approximately 1/10th.) of pin plants

may self-fertilise (Crosby, 1949). Homostyle plants occur in a few populations in the Chilterns and Somerset and these are highly self-fertile, with an estimated selfing-rate of 0.92 (Piper *et al.*, 1984).

Primroses are insect-pollinated and *Bombylius* species (Weiss, 1903), night-flying moths (Miller-Christy, 1922) and small beetles (Woodell, 1960) have variously been suggested as pollinating agents. However, no single insect, or group of insects, has been identified. It seems likely that many of the insect species which visit primroses (e.g. those listed by Miller-Christy, 1922) are capable of effecting pollination to varying degrees, and in different situations.

Comparatively few primrose flowers set seed and values as low as 1% (French, 1891) and "rarely exceeding 50%" (Highfield, 1916 *in* Miller-Christy, 1922) have been quoted. Many flowers are eaten by rabbits, sheep and slugs, or pecked by sparrows (Keith-Lucas, 1968) and fail to produce seed. The number of seeds produced is apparently related to light intensity, with very few seeds set under a continuous tree canopy but, if light increases, large amounts of seed may be produced by plants which have persisted vegetatively (Helliwell, 1980).

The seeds mature in the shade of the leaves after the capsule has reflexed and fall to the ground close to the parent plant. Ants and wood-mice may disperse primrose seed but many seeds are found within 50cm of the parent plant (Keith-Lucas, 1968).

Vegetative Growth

The current season's leaves grow from the tip of a short, stout rhizome in January or February and continue growth until mid-summer. In late July the terminal bud enters a dormant phase and the leaves present may persist over the winter. In open situations the leaves die in July or August and

may be replaced by a new flush of growth in the autumn.

The rhizome grows only a few centimetres each year and typically bears a single ramet. Leaf-scars and old leaf-bases mark the location of the previous season's leaves. An individual plant may persist for up to ten years, or more, during which time the rhizome can reach a length of 20-30cm. Such large, old plants, particularly in disturbed situations, may fragment and each fragment of rhizome is then capable of producing a new ramet. However, dispersal of these ramets is limited and, in general, the primrose genet, represented by one, or a few, ramets, remains within one or two metres of the site in which it was established.

The Study Sites

5 populations of *Primula vulgaris* were chosen for study. All sites are within 15 miles of Bangor and close to sea-level, with a maximum altitude of 70m. For a description of the sites see Appendix I.

Sampling and Cultivation of Experimental Material

Primula vulgaris plants were sampled from the 5 populations in October 1981. 24 evenly-spaced sampling positions were marked out in a grid-system (in sites 1,2 and 4) or on linear transects (sites 3 and 5). This systematic procedure : i) ensured that plants were sampled from the entire study area in each site and ii) avoided repeated sampling of the same genet. The primrose plant closest to each sampling point was dug up, keeping its rhizome and root system intact.

In the glasshouse, the soil surrounding the plant roots was shaken off and the rhizome divided into 2-3cm pieces using a razor. These ramets were potted in "Plantpax" (7 x 8.5 x 7cm) containing a lime-free John Innes No.

1 compost and carefully labelled to denote their population and parent plant. A total of 120 parental genets were represented by 3-6 ramets, according to the size of the rhizome sampled. At this stage, the number of leaves borne on the ramets varied considerably. The current season's leaves grow from the tip of the rhizome and a few ramets, from this region, had many leaves, whereas most had none.

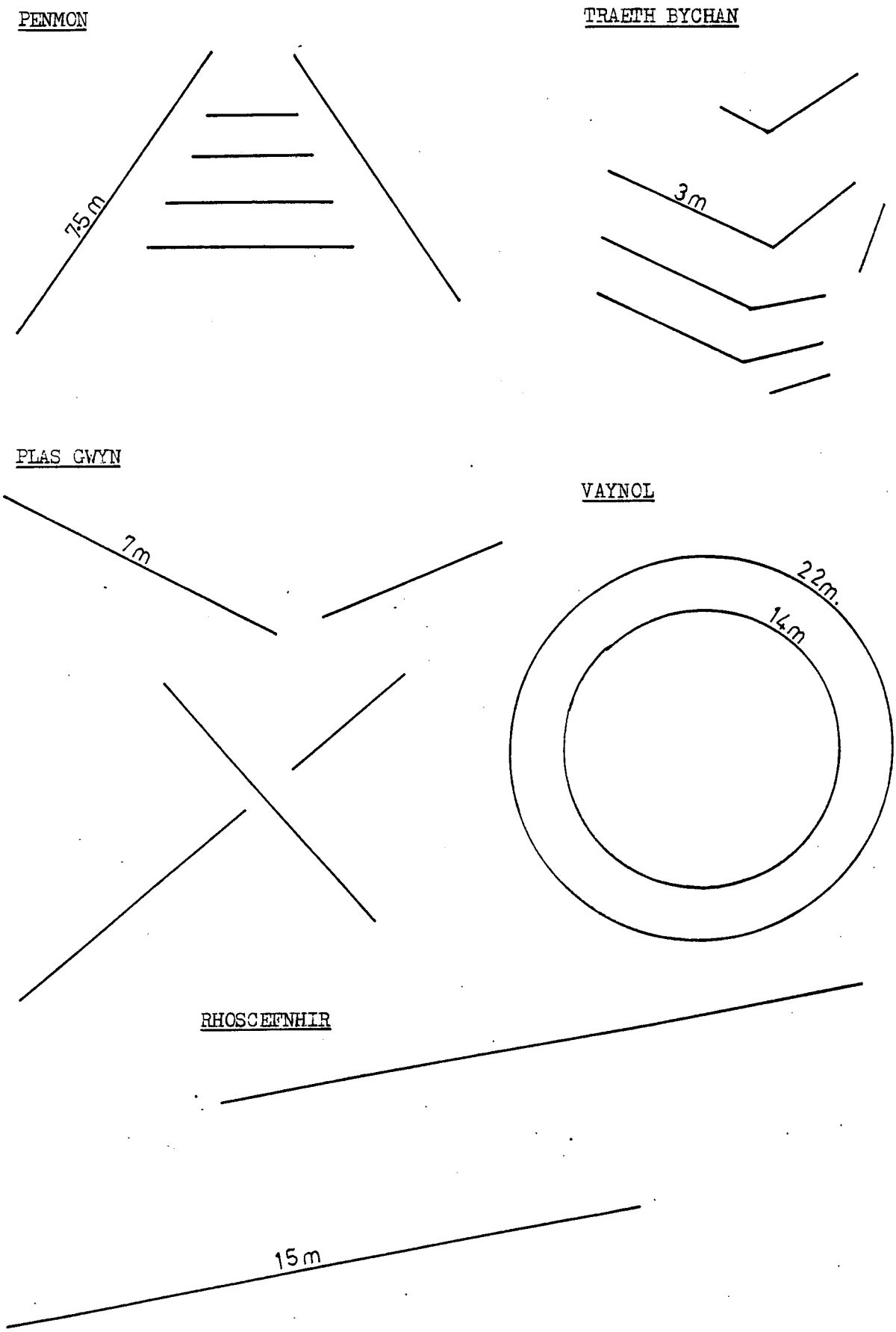
All of the plants were placed in a heated glasshouse (temperature 15-20° C, 16 hour day) and watered regularly for 5 months. Periodically the larger ramets were further sub-divided and re-potted.

Transplanting

In March 1982, ramets were selected for transplanting back into the sites. Only ramets with 6-10 leaves were chosen, to reduce the phenotypic variation of the experimental material at this stage. 60 ramets from each population were available for transplanting; these represented between 20 and 24 genets. The 60 ramets were distributed equally so that 12 ramets per population were transplanted in each of the 5 sites. Plants were allocated to transplant sites at random, except that ramets from the same parental genet were always transplanted into different sites. Therefore, the 60 ramets transplanted within each site represented 60 different genets, 12 from each of the populations originally sampled.

The ramets were randomly assigned to positions in a pre-determined grid pattern. This varied according to the nature of the transplant site (Figure 1) but all ramets were transplanted at least 0.5m apart. Compost was shaken from the roots of the primroses and each transplant was then introduced into a small hole at its transplant location, with minimum disturbance of the native vegetation. This procedure ensured that the

Figure 1. The arrangement of linear transects, along which transplants were distributed at approximately 0·5m intervals, in each transplant site.



transplants experienced the biotic and edaphic conditions appropriate to their new environment. A numbered metal peg was sunk into the ground adjacent to each transplant to mark the position of individual ramets and a separate record was kept relating this position to the transplant's population of origin. In this way any bias in recording the performance of transplants was avoided. All transplants were watered immediately after transplantation.

The numbers of leaves, flowers and seeds produced by transplants were recorded on four occasions during the first 12 months of the experiment and at 3-4 weekly intervals for a further six months. The maximum length and breadth of the lamina of each leaf was measured on two recording dates (July and October, 1982) and the leaf area of transplants estimated. In August 1983 all surviving transplants were assayed for infection with arabis mosaic virus (see Chapter 5). The remaining above-ground parts of the transplants were then harvested and their dry weight determined.

Results

The performance of transplants was compared in a 2-way ANOVA using GENSTAT to estimate the effect of the population (site of origin) and transplant site and their interaction on transplant growth. In studying local specialisation we are particularly interested in comparing the relative performance of plants transplanted back into their original, native, site (and represented on the principal diagonal of the data matrix) with transplants to an alien environment. Plants which are returned to their native population will be growing in the same biotic and physical conditions that they experienced before transplanting and, if locally differentiated, would be expected to make more growth than plants

originating from , and therefore specialised to, a different environment. The value of the principal diagonal as a whole, and the individual elements on it, constitute balanced orthogonal components of the sum of squares of the ANOVA. Therefore, the magnitude and significance of the variation between native and alien transplants was assessed in terms of its specific contribution to the interaction in the analysis: i) over all 5 sites and ii) within each individual site, as an indication of the degree to which plants were specialised to their population of origin. Square root and logarithmic transformations of the data were made where appropriate.

Survivorship

50 plants, 1/6th. of all transplants, died during the 18 months of the reciprocal transplant experiment. The population, or site of origin, of a transplant had no significant effect on its survival but there were differences in the survivorship of plants transplanted into different sites (Table 1). Over all 5 sites there was no evidence for differential survival of transplants in their native sites (Table 2).

At Plas Gwyn, 10 plants died within two weeks of transplantation (Figure 2) when they were uprooted by sheep which strayed from a nearby pasture. Throughout the experiment, grazing was a common, though irregular, occurrence in this site but once their root systems were established the transplants were no longer uprooted by the sheep. The high mortality of transplants in Rhoscefnhir in spring 1983, corresponded with a dry period during which the soil in many parts of this site slumped to the foot of the verge. A number of transplants, which previously had many leaves, disappeared but frequently the metal peg and surrounding vegetation remained. It seems likely that when the substrate became too unstable the larger transplants, particularly from Penmon (with an average of 21.2

Table 1. The survival of *Primula vulgaris* plants from different populations, transplanted into 5 transplant sites. Values are the proportion of transplants surviving after 18 months. Significance of χ^2: ns - not significant and *** $P<0.001$.

POPULATION	TRANSPLANT SITE P	PG	R	TB	V
Penmon (P)	1.00	.75	.17	1.00	.92
Plas Gwyn (PG)	.92	.75	.75	1.00	.67
Rhoscefnhir (R)	.83	.75	.67	1.00	.67
Traeth Bychan (TB)	1.00	.83	.58	1.00	1.00
Vaynol (V)	1.00	.83	.75	1.00	1.00

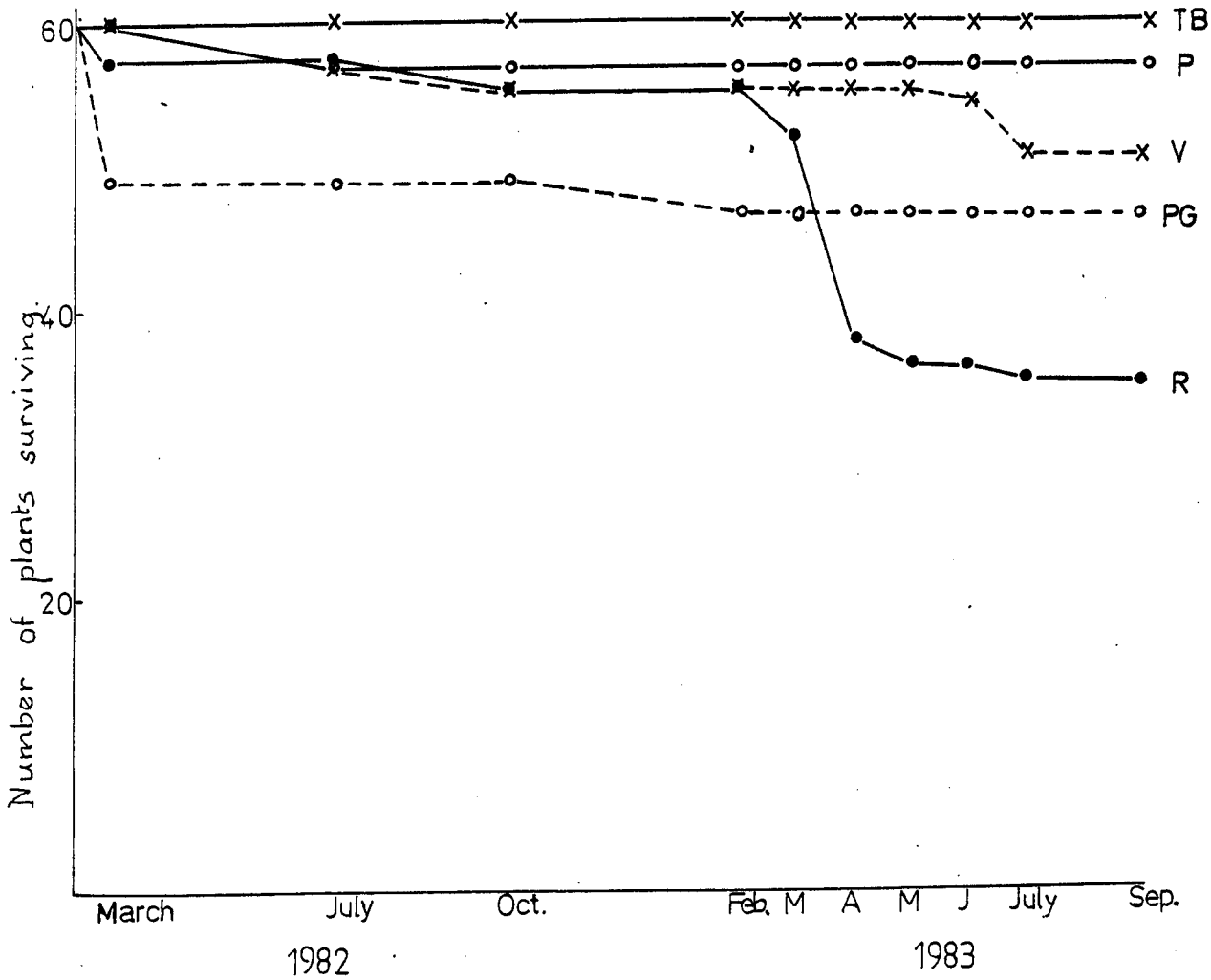
χ^2 Test of Significance

EFFECT	degrees of freedom	χ^2
Population	4	7.2 ns
Transplant site	4	46.07 ***
Population x Transplant site	16	25.92 ns
Total	24	79.19

Table 2. The survival of transplants in native and alien sites, ns - no significant difference between the survivorship of native and alien transplants, using the χ^2 test of significance.

TRANSPLANTS IN:	NUMBER OF PLANTS: SURVIVING	TRANSPLANTED	
Native sites	53	60	$\chi^2 = 1.35$ ns
Alien sites	197	240	

Figure 2. Survivorship of *Primula vulgaris* transplants in 5 transplant sites, with time. Sites: Penmon o——o (P); Plas Gwyn o- - -o (PG); Rhoscefnhir ●——● (R); Traeth Bychan x——x (TB) and Vaynol x——x (V).



leaves in February compared with 11.8 leaves on all other transplants) fell to the road surface and were lost. These two causes of mortality accounted for the majority of transplant deaths and it was decided to excluded dead plants from the analysis and treat them as missing values in the data.

Infection with Arabis Mosaic Virus

In August 1983, 61 transplants were found to be infected with arabis mosaic virus (AMV). These infected plants were omitted from the analysis because AMV affected the number and area of leaves borne on primrose transplants (see Chapter 5, Table 10).

Number of Leaves

The number of leaves borne on each transplant was significantly affected by both its site of origin and the site into which it was transplanted (Table 3). A significant interaction at many of the recording dates showed that these effects were not additive i.e. the growth of plants in different transplant sites was dependent upon their original population. The variation between native and alien transplants accounted for a significant component of this interaction in March, April and September 1983. When the experiment was initiated there was no significant difference between the number of leaves on transplants in their native sites (mean 9.56 ±0.7) and in alien sites (9.34 ±0.35). However, after 8 months, transplants in their native site bore more leaves than alien transplants and this difference was maintained throughout the following year (Figures 3 & 4). A strong principal diagonal effect was evident at site 2, Plas Gwyn, but the difference between native and alien transplants was less marked in other sites, especially at Vaynol (Figure 5).

Table 3. The levels of significance of main effects and interactions on the growth of *P. vulgaris* plants originating from, and transplanted into, 5 different populations. ns - not significant; * $P<0.05$; ** $P<0.01$ and *** $P<0.001$.

VARIABLE	POPULATION	TRANSPLANT SITE	INTERACTION	*PRINCIPAL DIAGONAL	
Number of leaves					
1982: March	ns	**	ns	ns	
July	*	***	ns	ns	
October	**	***	*	ns	D2
1983: February	**	***	ns	ns	
March	**	***	**	*	D2
April	**	***	**	**	D2
May	**	***	**	ns	D2
June	**	***	ns	ns	
July	*	***	ns	ns	
September	*	***	*	**	D1
Area of leaves per plant					
1982: July	*	***	ns	ns	
October	**	***	**	*	D2
Area per leaf					
1982: July	ns	**	ns	ns	
October	ns	***	*	ns	D1
Dry weight - leaves per plant					
1983: September	ns	***	ns	*	D2
Dry weight per leaf					
1983: September	ns	***	ns	ns	D2

* The principal diagonal represents the significance of the comparison between the growth of all primroses returned to their native site with all plants transplanted into an alien site; D1 - D5 denote significantly greater growth of native than of alien transplants within a specific site where: D1 - Penmon; D2 - Plas Gwyn; D3 - Rhoscefnhir; D4 - Traeth Bychan and D5 - Vaynol.

Figure 3. The mean number of leaves per plant in native and alien transplants of P. vulgaris over all 5 sites, with time. ●——● plants re-planted in their native site, x——x plants transplanted to alien sites, ⊥ ± 1 standard error.

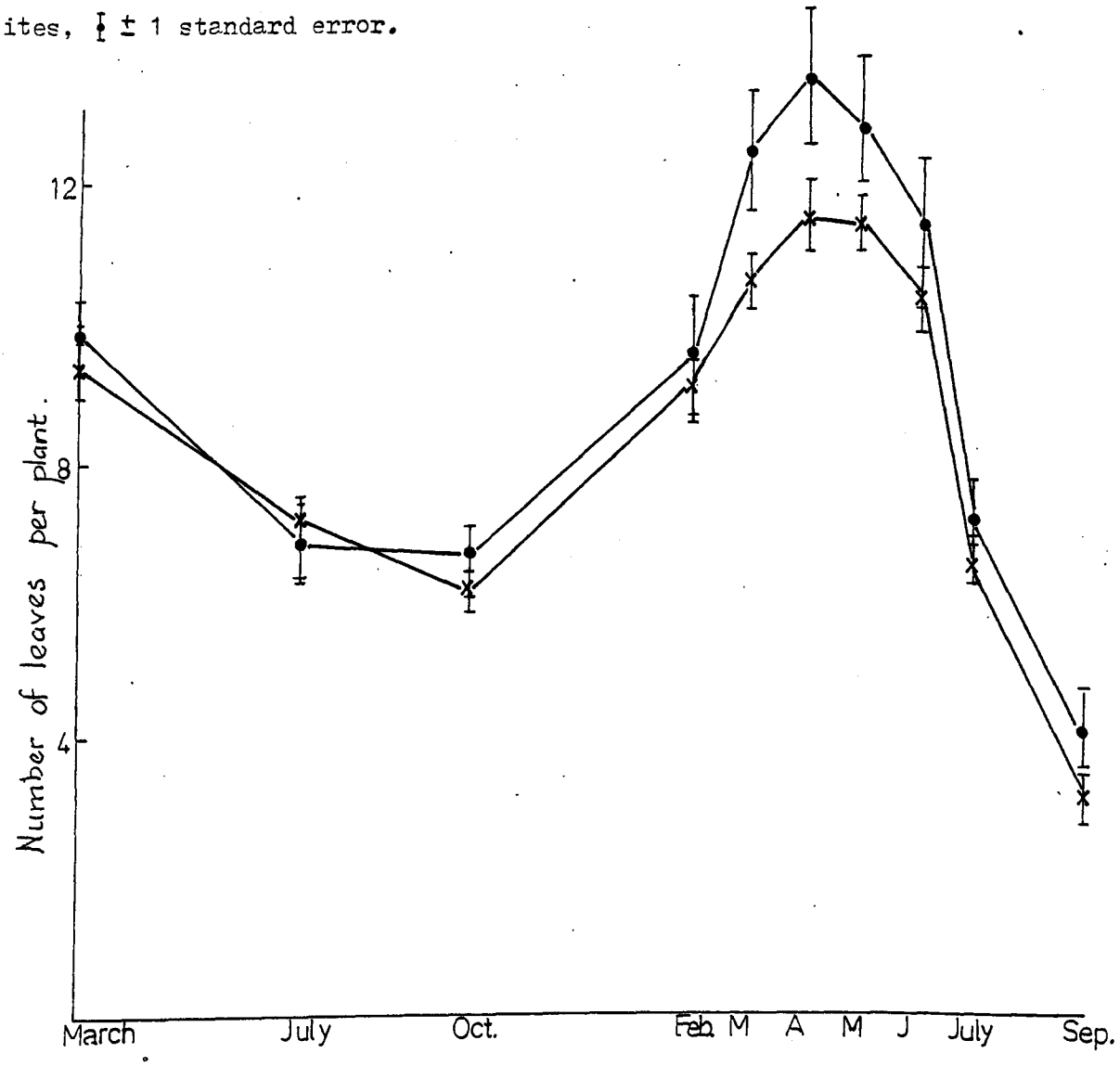


Figure 4. Estimates from the ANOVA of the difference in the number of leaves between native and alien transplants. ⊥ ±1 S.E. of the difference, difference (native - alien) significant at: * P<0·05 and ** P<0·01.

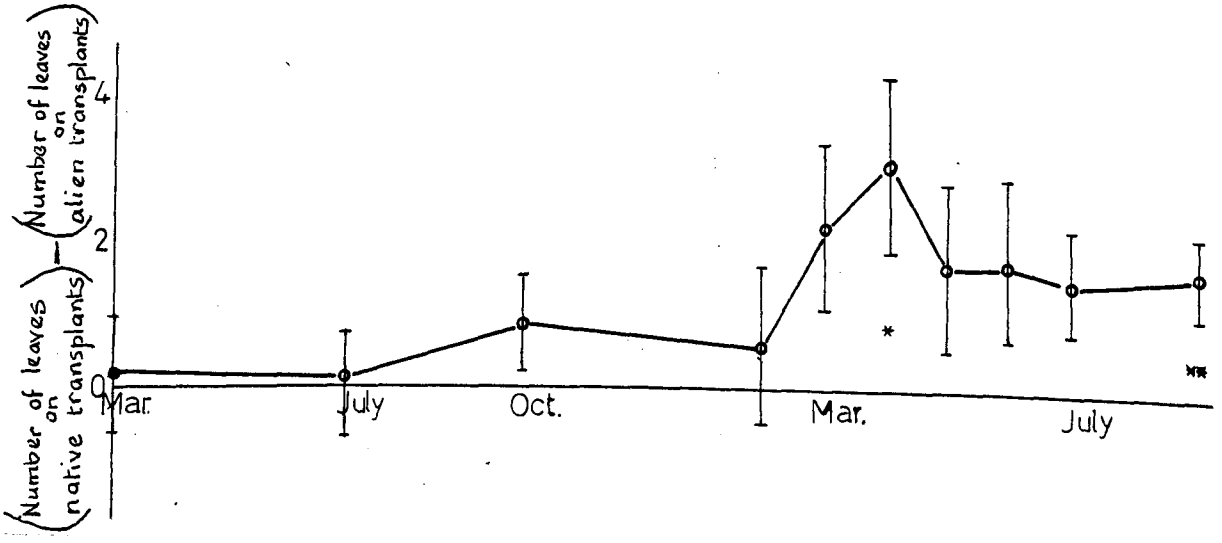


Figure 5. Estimates from the ANOVA of the difference in the number of leaves between native and alien transplants within each transplant site. Difference (native - alien) significant at: * $P<0.05$ and ** $P<0.01$.

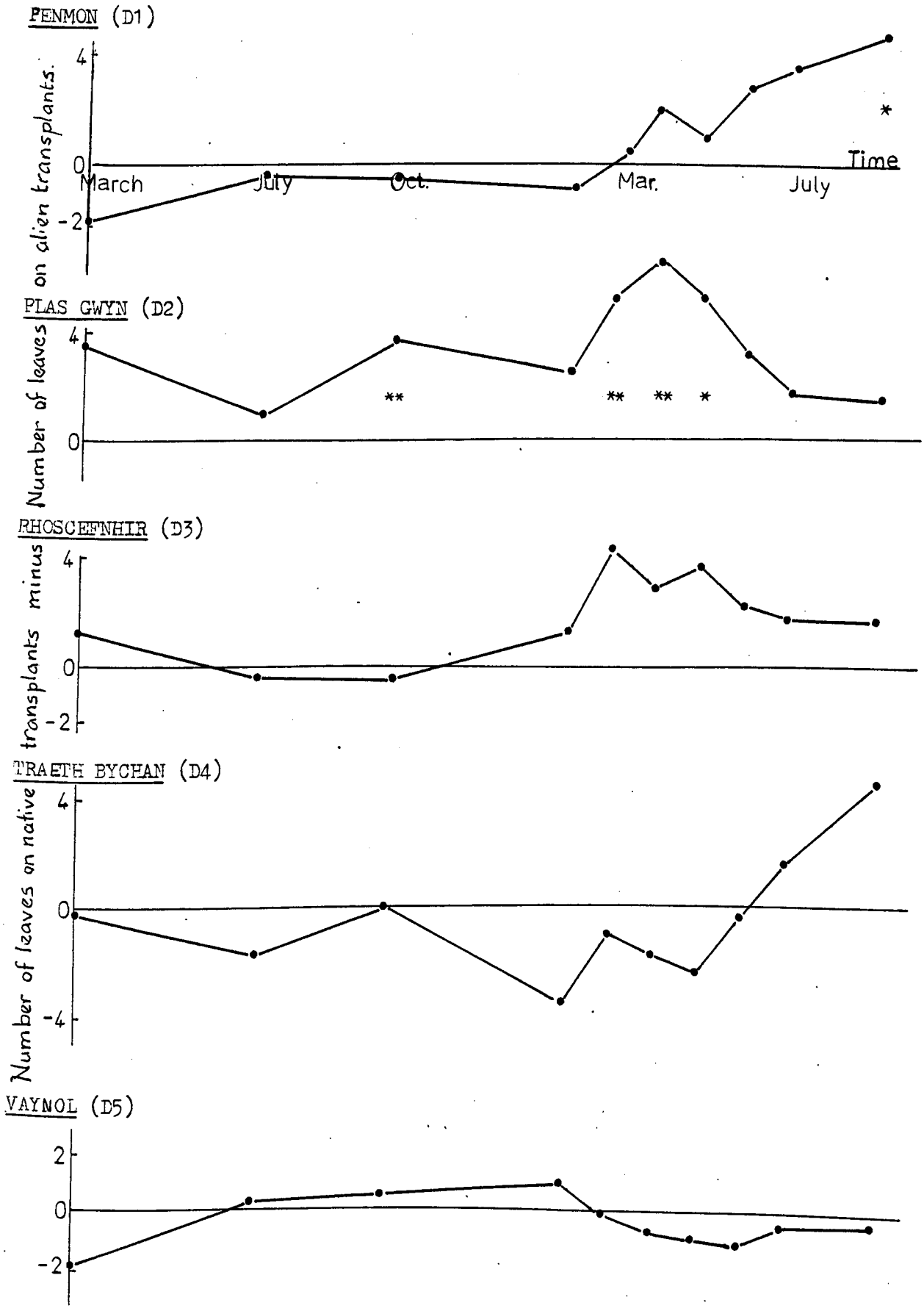


Figure 6. The relationship between the length and breadth of *P. vulgaris* leaves and their area: n = 100; r^2 = .927 and P<.001.

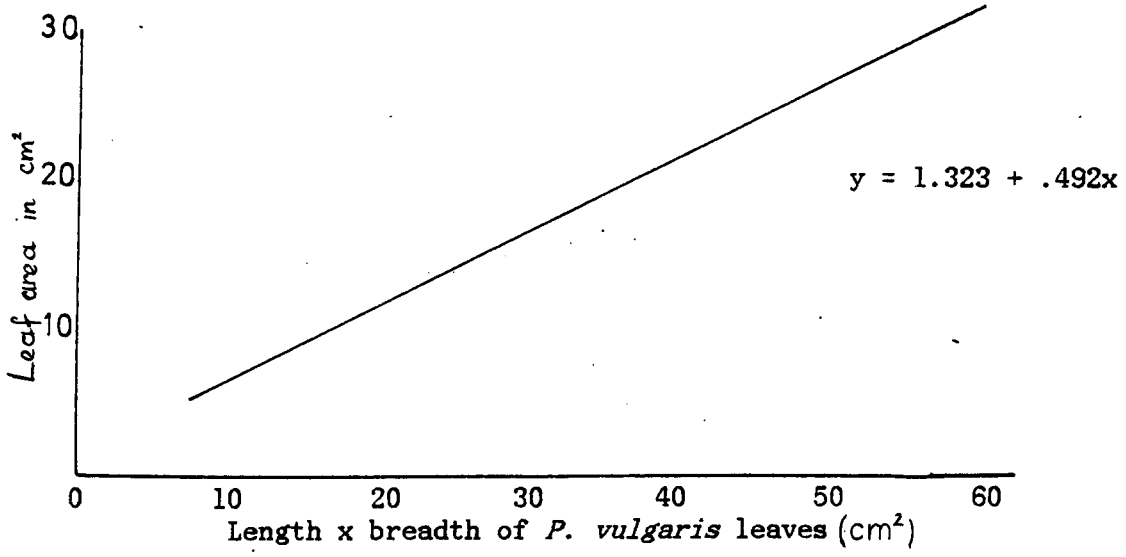


Table 4. Estimates from the ANOVA of the difference in leaf area and dry weight between native and alien transplants. Differences which are significant (P<0.05) are shown in bold type.

	ESTIMATED DIFFERENCE, NATIVE - ALIEN IN:					
VARIABLE	All sites	Penmon	Plas Gwyn	Rhoscefn-hir	Traeth Bychan	Vaynol
Leaf area/plant (cm²)						
1982: July	5.37	7.14	9.17	-25.09	59.36	1.36
October	**16.00**	29.89	**23.37**	-34.07	33.93	8.74
Mean area/leaf (cm²)						
1982: July	0.44	2.11	-0.17	-2.78	5.89	-0.20
October	1.45	5.28	1.45	-4.66	2.58	1.96
Dry weight/plant (g)						
1983: September	**126.15**	972.50	**59.31**	251.52	617.85	1.13
Dry weight/leaf (g)						
1983: September	23.77	34.85	**28.43**	16.01	16.62	1.63

Area of Leaves

In July and October 1982, the area of leaves was estimated from their lengths and breadths using a linear regression equation obtained for 100 leaves sampled from native primrose plants in the 5 study sites. Leaf area, measured with an automatic leaf area meter, was strongly correlated with the product of leaf length and breadth (Figure 6):

$$\text{Leaf Area} = 1.323 + 0.492\ (\text{LENGTH} \times \text{BREADTH})$$

Transplants from different populations showed no apparent differences in the area of individual leaves but the transplant site significantly affected leaf area (Table 3). No principal diagonal effect was identified over all 5 sites but, in October, the native transplants in Penmon (site 1) had significantly larger leaves (+5.28cm^2 ±2.9) than plants originating from alien sites (Table 4).

The total area of leaves per transplant was closely related to the number of leaves and strongly influenced by both site of origin and transplant site. Interaction between these factors was significant in October 1982 and due, in part, to the variation between native and alien transplants. Over all 5 sites, native transplants had a mean total leaf area which was 16.0cm^2 (±9.0) greater than that of alien transplants. This principal diagonal effect was most evident in Plas Gwyn where it accounted for an increase of 23.4cm^2 (±13.2) of leaf tissue per plant (Table 4).

Number of Flowers

Fecundity was estimated as the number of buds, flowers and capsules produced on each plant between March and July 1983. Flower production was not recorded in 1982 because many plants were already flowering at the start of the transplant experiment. At any specific time the majority of inflorescences were at the same stage of development; consequently, the

three variables were combined to give a single value for reproductive output at each recording date.

Reproductive output differed significantly between transplant sites and between plants which originated from different populations (Table 5). The interaction between the effects of transplant site and population of origin was significant in April, May and June. There was no evidence that this interaction was due to variation in the flowering behaviour of native and alien transplants, except in Rhoscefnhir, where primroses re-planted in their native site produced fewer flowers than transplants originating from other sites (Table 6).

Production of Seeds

Capsules containing mature seed were harvested and the seeds counted. However, a quantitative analysis of seed production was not possible because only 52 transplants produced seed and these varied in fecundity from 1 to 288 seeds per plant. No seed was obtained from the remaining 135 surviving transplants (Table 7). Many capsules were empty, the seed having been eaten by animals which crawled into the capsules, made a hole in the capsule wall or removed the entire seed capsule by biting through the stalk 1-2cm above the ground. Over 40% (32) of the transplants bearing capsules in June 1983 were infected with a smut fungus, probably *Urocystis primulicola*. This fungal pathogen inhibits seed production by infecting the anthers and ovaries of its host and filling the seed capsule with a powdery black mass of fungal spores (Reid, 1969).

There were apparent differences between transplants from different populations e.g. plants from Vaynol were particularly susceptible to smut and rarely produced mature seed; and between transplant sites, such as Vaynol and Plas Gwyn where few transplants bore capsules. The fecundity of

Table 5. The levels of significance of main effects and interactions on the fecundity of *P. vulgaris* transplants: ns - not significant; * P<0.05 and ** P<0.01.

VARIABLE	POPULATION	TRANSPLANT SITE	INTERACTION	PRINCIPAL DIAGONAL	
Reproductive output					
1983: March	**	**	ns	ns	
April	ns	**	**	ns	
May	**	**	**	ns	
June	**	**	*	ns	D3
July	**	**	ns	ns	D3

The principal diagonal represents the significance of the comparison between all primroses returned to their native site and all plants transplanted to an alien site; D3 denotes a significant difference between the fecundity of native and alien primroses in site 3, Rhoscefnhir.

Table 6. Estimates from the ANOVA of the difference in fecundity between native and alien transplants. Differences which are significant (P<0.05) are shown in bold type, — no recording made at this date.

	ESTIMATED DIFFERENCE, NATIVE - ALIEN IN:					
VARIABLE	All sites	Penmon	Plas Gwyn	Rhoscefn-hir	Traeth Bychan	Vaynol
Reproductive output						
1983: March	0.74	1.91	0.69	-1.41	-0.46	0.22
April	1.19	4.46	1.04	-6.59	—	2.72
May	0.17	2.02	0.55	-6.32	-0.57	0.96
June	0.53	2.44	0.48	**-6.74**	3.62	0.53
July	-0.22	-0.09	0.24	**-5.62**	5.61	0.31

Table 7. The fecundity of transplants of Primula vulgaris in June 1983: P - Penmon; PG - Plas Gwyn; R - Rhoscefnhir; TB - Traeth Bychan and V - Vaynol.

		TRANSPLANT SITE:					
		P	PG	R	TB	V	Total
Total number of plants							
POPULATION:	P	8	8	1	7	8	32
	PG	10	8	9	8	6	41
	R	10	8	7	12	8	45
	TB	6	5	3	4	6	24
	V	10	8	7	10	10	45
	Total	44	37	27	47	38	187
Number of plants with capsules							
POPULATION:	P	5	1	1	6	1	14
	PG	1	1	7	2	2	13
	R	8	1	5	3	2	19
	TB	2	3	2	3	3	13
	V	5	2	6	6	1	20
	Total	21	8	21	20	9	79
Number of plants with mature seed							
POPULATION:	P	5	0	1	6	1	13
	PG	1	0	5	2	1	9
	R	7	1	5	3	2	18
	TB	2	0	1	1	3	7
	V	2	1	0	1	1	5
	Total	17	2	12	13	8	52
Number of plants with smut							
POPULATION:	P	1	0	0	0	0	2
	PG	0	0	5	1	1	7
	R	4	0	3	0	0	7
	TB	1	0	0	0	1	2
	V	3	0	6	5	0	14
	Total	9	0	14	7	2	32

transplants in their native and alien sites did not appear to differ (Table 7) but it is risky to conclude much from data based on such small and unequal numbers of observations.

Summary

Primroses in local populations are genetically specialised such that plants tend to produce more leaves and a greater total area of leaf tissue when re-planted in their native site, than when transplanted into an alien site. This difference was consistent over all 5 populations as a whole (Figure 4) but varied between individual populations, indeed in some populations e.g. in Traeth Bychan and Vaynol, native transplants bore fewer leaves. There was no apparent tendency for transplants to produce more flowers in their native site, although both population and transplant site significantly affected reproductive output and showed a significant interaction.

Transplant Experiments in *Ranunculus repens*

The Species

Ranunculus repens is a perennial herb with strong, leafy above-ground stolons which root at the nodes. The basal and lower stem leaves are stalked and triangular-ovate in outline; the upper leaves are sessile with narrow segments. Bright yellow flowers are borne on an erect, furrowed stem from May to September.

Buttercups are generally distributed throughout Europe, as far south as the Moroccan and Algerian Atlas and the Nile Delta, east to Japan but not including North European Russia and to a northerly latitude of 72°. The buttercup has also been introduced and become established in North America,

parts of South America and in New Zealand. Throughout Europe, and even within a locality, *R. repens* exhibits great variation between individual plants. However, few characters show any clear regional differentiation, and there is no apparent justification for defining intra-specific taxa (Coles, 1977).

R. repens is a common weed of grassland, arable land and recently disturbed ground in all parts of Britain. It occurs on a wide range of soil types, especially on heavy, wet soils where drainage is impeded.

Reproductive Characteristics

R. repens is principally cross-pollinated, with only a low level of selfing and no evidence of apomixis (Coles, 1977). The flowering peak occurs in June, when a number of insect species visit the flowers for pollen or nectar (Harper, 1957). Both leaves and flowering heads of *R. repens* are eaten by stock. In a grazed site, only 20% of buttercups flowered; 1/4 of these plants set seed, the majority producing less than 20 achenes per plant (Sarukhán, 1974). However, seed which is ingested from mature fruiting heads can remain viable and may be dispersed in the faeces of the grazing animal. Only a small fraction of the seed germinates in the first year; the remainder, if it survives predation, can acquire enforced dormancy and decays very slowly, persisting in the soil for a number of years (Sarukhán, 1974).

Vegetative Growth

Ranunculus repens plants overwinter as small rosettes with 2-4 leaves. New leaves are formed in spring and stolon growth commences in May or June, with each plant producing between 1 and 5 primary stolons. Nodes occur along the stolons at intervals of 3-15cm; the first internode, produced next to the parent, usually being the shortest. Each node bears 1 or 2

leaves and roots which grow out and anchor the stolon to the ground. The rooted ramet will then produce additional leaves and, late in the season, may flower.

The stolons continue to grow throughout July and August, producing secondary and occasionally tertiary branching. In grassland an individual primary stolon rarely exceeds 50cm (Sarukhán & Harper, 1973) but the extent of stolon growth is very variable and depends upon the nature of the surrounding vegetation (Lovett Doust, 1981). A total stolon length of 92.5 feet, bearing 479 nodes has been recorded for one plant in a single season (Anon, 1958 *in* Coles, 1977).

In late August and September the stolons rot, leaving the individual ramets as independent modules. Many of the parent plants die after flowering and are typically replaced by one, but a maximum of 16, daughter ramets in a season (Sarukhán, 1974). The surviving older plants and newly established daughter ramets change to the rosette habitat and remain in a winter-green condition until the spring.

The Study Sites

The sites selected for study were within a single climatic region but experienced contrasting biotic conditions under different management regimes. All sites were located on the North Wales coast between the Menai Strait and Conwy, within 2 kilometres of the sea and less than 30m above sea-level. 2 fields of pasture, 2 lawns and a fifth site of disturbed arable land were chosen. Each site had been subjected to a consistent management policy for a number of years and this was unlikely to change during the course of the experiment. For a description of the sites see Appendix II.

Selection of Sub-sites

Three discrete sub-sites, each approximately $4m^2$ and dominated by a different species, were chosen in the four grassland sites (sites 1-4). The sub-sites were initially identified by visual inspection of the swards and a length of plastic tubing (approximately 15cm) was sunk into the ground to mark their centre. Subsequently, each sub-site was characterised by identifying the inter-specific contacts of *R. repens* plants growing there. Contact samples were obtained in every sub-site by recording all the different species touching the above-ground parts of 12 *R. repens* plants selected at random (Table 8). In each sub-site *R. repens* made most contacts with the species initially recognised as the dominant though there was often a high frequency of contacts with other species.

No sub-sites were distinguished in Site 5 (Cae Groes), which contained a heterogeneous assemblage of plant species recently established from seed.

Sampling and Cultivation of Experimental Material

In June 1981, *R. repens* plants were sampled from the 5 study sites. 9 plants were taken from every site, 3 from each individual sub-site, 45 plants in all. Within the sub-sites, sampling positions were selected at random but only *R. repens* plants growing adjacent to the dominant species were chosen. Care was also taken to sample plants at not less than 2-3m intervals to avoid repeated sampling of the same genet.

A soil-corer (diameter 5cm) was used to dig up the plants from each site. Soil was cleaned from the roots and each genet divided into separate ramets, using a razor blade to sever the stolons and split the root-stock of the parent rosette. The number of ramets obtained from each genet varied from 2-5, depending on the size of the plant sampled. Individual

Table 8. Neighbour relations of *Ranunculus repens* in the different sub-sites. The table shows the 5 species most often in contact with native *R. repens* plants and, in brackets, the frequency of contacts in a sample size of 12.

SITE	SUB-SITE (dominant species)	NEIGHBOURING SPECIES				
Treborth main lawn	***Cynosurus cristatus***	*C. cristatus* (7)	*L. perenne* (7)	*A. tenuis* (4)	*Prunella vulgaris* (3)	*H. lanatus* (3)
	Holcus lanatus	*H. lanatus* (7)	*L. perenne* (6)	*A. tenuis* (4)	*P. vulgaris* (4)	*Bellis perennis* (2)
	Lolium perenne	*L. perenne* (7)	*H. lanatus* (6)	*A. tenuis* (3)	*Potentilla anserina* (3)	*Cerastium fontanum* (2)
Treborth new lawn	***Trifolium repens***	*T. repens* (7)	*Poa annua* (7)	*B. perennis* (2)	*A. tenuis* (1)	*P. vulgaris* (1)
	Holcus lanatus	*H. lanatus* (7)	*T. repens* (6)	*P. annua* (4)	*L. perenne* (1)	*A. tenuis* (1)
	Agrostis stolonifera	*A. stolonifera* (7)	*H. lanatus* (2)	*T. repens* (2)	*C. fontanum* (1)	*P. annua* (1)
Henfaes	***Trifolium repens***	*T. repens* (8)	*C. cristatus* (8)	*H. lanatus* (2)	*L. perenne* (2)	*A. tenuis* (1)
	Holcus lanatus	*H. lanatus* (7)	*Poa trivialis* (4)	*T. repens* (3)	*A. tenuis* (3)	*Leontodon autumnalis* (1)
	Lolium perenne	*L. perenne* (5)	*A. tenuis* (4)	*H. lanatus* (3)	*T. repens* (3)	*Achillea millefolium* (1)
Cae Llyn	***Cynosurus cristatus***	*C. cristatus* (10)	*L. perenne* (4)	*Juncus effusus* (4)	*H. lanatus* (3)	*T. repens* (2)
	Holcus lanatus	*H. lanatus* *(9)*	*L. perenne* *(9)*	*D. glomerata* *(1)*	*J. effusus* *(1)*	----
	Lolium perenne	*L. perenne* (10)	*P. trivialis* (3)	*R. acris* (2)	*Alopecurus geniculata* (1)	*Rumex acetosa* (1)

ramets were potted in John Innes No. 1 compost in "Plantpax" (7 x 8.5 x 7cm) and labelled with their genet, sub-site and site of origin. The plants were placed in a cold glasshouse with no supplementary lighting and watered regularly for 8 months. Benlate and Demetox were used at intervals to control mildew and insect pests. Periodically, the ramets were further sub-divided and re-potted until each of the original genets sampled was represented by at least 9 ramets, Further growth of stolons was then prevented by cutting off all stolons produced. A single genet, from Cae Llyn, made very little growth and produced only 5 ramets. Extra ramets of the two other genets from the same sub-site were used to make up the population.

Transplant Procedure

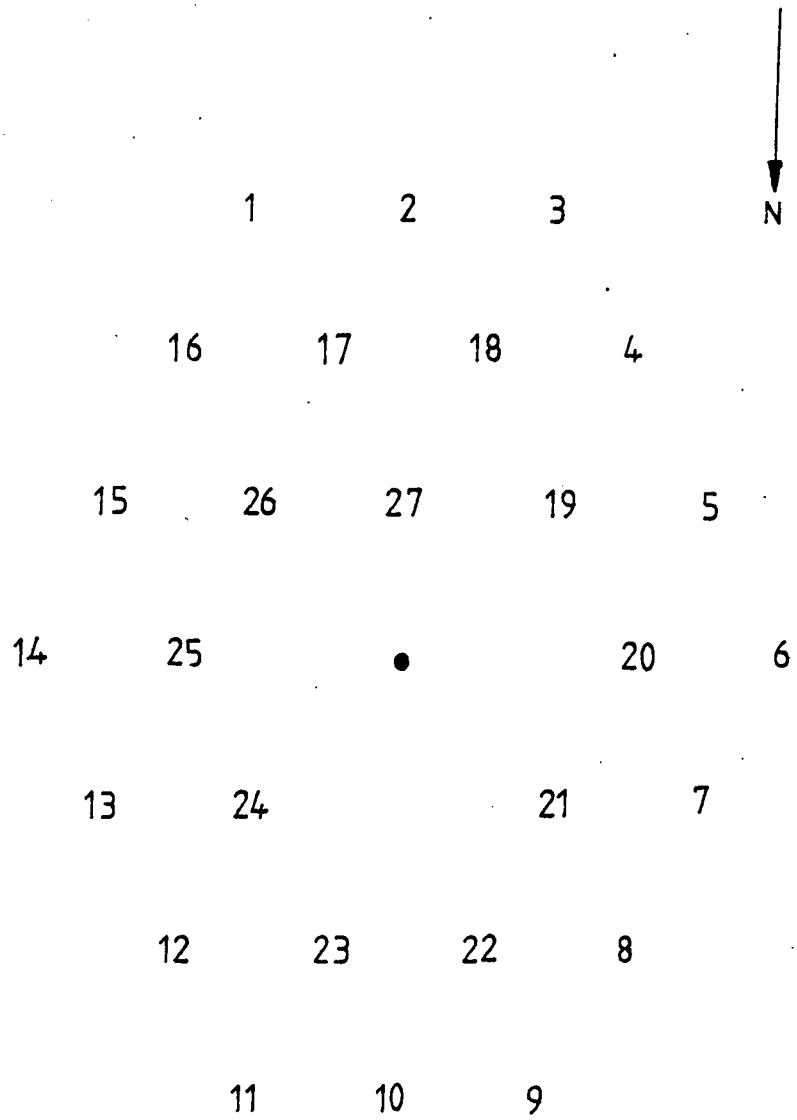
In March 1982, the ramets were transplanted into the 5 study sites so that each genet was represented by 5 ramets in its native site and by 1 ramet in each of the other 4 sites. Within the grassland sites (sites 1-4) each native genet was replicated three times in the sub-site from which it was sampled and once in the 2 alien sub-sites; genets alien to the site were distributed equally between the sub-sites (Table 9). As far as possible, ramets of similar size were transplanted in the same sub-site but within the sub-sites ramets were allocated at random to positions within a grid pattern (Figure 7).

All ramets were removed from the glasshouse and placed, in their pots, in the appropriate transplant site for one week prior to transplantation. Transplanting was done by extracting a soil core and carefully separating the soil and surface vegetation. Compost was shaken from the roots of the chosen ramet and this was introduced into the soil core which was then

Table 9. The reciprocal transplantation of R. repens ramets from 5 sites: TM - Treborth main lawn; TN - Treborth new lawn; H - Henfaes; CL - Cae Llyn and CG - Cae Groes, for a description of the sub-sites see table 8. x represents a single ramet, its position records the genet, a-i, sub-site and site of origin and the sub-site and site into which it was transplanted. ⊗ is a ramet substituted for the genet which produced too few ramets.

POPULATION		TRANSPLANT SITE												
		TM			TN			H			CL			CG
sub-site		1	2	3	1	2	3	1	2	3	1	2	3	
TM	a	xxx	x	x	x					x			x	x
1	b	xxx	x	x			x		x		x			x
	c	xxx	x	x		x		x				x		x
	d	x	xxx	x		x				x			x	x
2	e	x	xxx	x	x				x		x			x
	f	x	xxx	x			x	x				x		x
	g	x	x	xxx			x			x			x	x
3	h	x	x	xxx		x			x		x			x
	i	x	x	xxx	x			x				x		x
TN	a		x		xxx	x	x		x			x		x
1	b			x	xxx	x	x	x					x	x
	c	x			xxx	x	x			x	x			x
	d			x	x	xxx	x		x				x	x
2	e	x			x	xxx	x	x			x			x
	f		x		x	xxx	x			x		x		x
	g			x	x	x	xxx			x			x	x
3	h	x			x	x	xxx		x		x			x
	i		x		x	x	xxx	x				x		x
H	a		x			x		xxx	x	x			x	x
1	b	x					x	xxx	x	x		x		x
	c			x	x			xxx	x	x	x			x
	d	x			x			x	xxx	x		x		x
2	e			x			x	x	xxx	x	x			x
	f		x			x		x	xxx	x			x	x
	g	x					x	x	x	xxx		x		x
3	h		x			x		x	x	xxx			x	x
	i			x	x			x	x	xxx	x			x
CL	a			x			x	x			xxx	x	x	x
1	b		x			x				x	xxx	x	x	x
	c	x			x				x		xxx	x	x	x
	d	x			⊗		x	x	⊗		x	xxx	x	x
2	e										x	xxx	x	
	f		x	⊗		x				x	x	xxx	x	x⊗
	g	x			x				x		x	x	xxx	x
3	h			x			x	x			x	x	xxx	x
	i		x			x				x	x	x	xxx	x
CG	a			x	x			x			x			x x x x x
	b		x			x				x			x	x x x x x
	c	x					x		x			x		x x x x x
	d		x				x			x		x		x x x x x
	e	x			x				x		x			x x x x x
	f			x		x		x					x	x x x x x
	g	x				x			x				x	x x x x x
	h			x			x	x				x		x x x x x
	i		x		x					x	x			x x x x x

Figure 7. The distribution of R. repens transplants in a sub-site.
● marks the centre of the sub-site.



replaced in the ground, surrounded by the soil and natural vegetation of its transplant position. A numbered metal peg was inserted into the ground beside each transplant to enable it to be located and identified. The transplants were watered and all fully-expanded leaves removed. This defoliation was necessary to prevent grazing animals or mowing machines uprooting the transplants before the root system became established.

One month after transplantation, the numbers of leaves and buds and the expansion of stolons were recorded for each surviving transplant. Two further recordings were made by mapping each transplant at intervals of 6-8 weeks. This was done by placing a 1/2m quadrat over the transplant, with the parent ramet mapped in the centre of a co-ordinate grid. The quadrat was aligned from south to north and the co-ordinate positions and numbers of leaves and buds on all ramets along the stolons were recorded.

The above procedure was not possible in the disturbed arable site at Cae Groes and it was necessary to record and analyse the growth of transplants in this site separately. Six weeks after the initial recording, stolon growth was extensive with stolons from adjacent transplants inter-mingled and many unrooted nodes. This prevented the use of a mapping technique and a destructive harvest was thought to be advisable to maintain the integrity of individual transplants. All stolons were excised at this time and their lengths and numbers of nodes, leaves and flowers noted before determining the dry weight. Stolons from these transplants were harvested again after a further six weeks. In September 1982, all remaining transplants were dug up and their total dry weight ascertained.

No final harvest was carried out in the grassland sites because the majority of stolons had decayed and it was impossible to be sure of sampling all ramets representing each transplant.

Results

1. Analysis of Transplants in the Lawn and Field Sites

The data for transplants in site 5, Cae Groes, were excluded from the main analysis because of their atypical growth and consequently, the different recording technique which was used. Plants originating from this site were also omitted in order to leave a balanced data set consisting of plants from, and transplanted into, sites 1-4. This data was analysed by analysis of variance using the regression technique provided by GENSTAT to cope with the unequal numbers of plants at certain levels of the nested design. The analysis was conducted in 2 stages.

(a) To identify the differences between populations

The full data set was analysed to determine the main effects and interactions of the population of origin and the transplant site on transplant performance; variation due to sub-sites was not considered at this stage. Table 10a shows the numbers of genets and ramets in each cell of the data matrix. The contribution to the interaction sum of squares of: i) the principal diagonal as a whole and ii) each individual element on it was calculated to estimate whether there was a significant difference between native and alien transplants over all 4 sites and within each individual site.

(b) To identify the differences within populations

4 sub-sets of the data were analysed; they represented the performance of the 45 plants returned to their native population in: i) Treborth main lawn; ii) Treborth new lawn; iii) Henfaes and iv) Cae Llyn. The ANOVA was conducted as in the previous analysis but estimating the variance between plants originating from, and transplanted into, the three different sub-sites within each of the 4 populations. Differentiation of plants in

Table 10. The distribution and numbers of genets and ramets used to assess: (a) the variation between transplants from, and in, different populations and (b) the variation between transplants from, and in, different sub-sites within individual populations (taken from Table 9). The numbers in bold type denote the total number of transplants and, in brackets, the number of genets x the number of ramets of each genet.

	TRANSPLANT SITE:			
(a) POPULATION:	TM	TN	H	CL
Treborth main lawn (TM)	**45** (9x5)	**9** (9x1)	**9** (9x1)	**9** (9x1)
Treborth new lawn (TN)	**9** (9x1)	**45** (9x5)	**9** (9x1)	**9** (9x1)
Henfaes (H)	**9** (9x1)	**9** (9x1)	**45** (9x5)	**9** (9x1)
Cae Llyn (CL)	**9** (9x1)	**9** (9x1)	**9** (9x1)	**45** (9x5)

	TRANSPLANT SUB-SITE:		
(b) POPULATION SUB-SITE:	1	2	3
1	**9** (3x3)	**3** (3x1)	**3** (3x1)
2	**3** (3x1)	**9** (3x3)	**3** (3x1)
3	**3** (3x1)	**3** (3x1)	**9** (3x3)

response to specific sub-sites was again assessed by calculating the principal diagonal effect over all 3 sub-sites and in each of the individual sub-sites in every population. The numbers of genets and ramets at each level of the analysis are shown in Table 10b.

The performance of transplants from different populations

Survivorship

Only 20 plants had died by the end of the experiment and of these, 14 were growing in their native site. There was no significant difference, $\chi^2 = .516$, between the probability of a transplant surviving in its native site (.922) and that of survival in an alien site (.944).

Transplant Growth

Both the original population and the transplant site accounted for significant variation in all parameters of transplant growth and flowering measured at three recording dates (Table 11). Individual genets from the same population also differed significantly. There was some evidence of interaction between genets and different transplant sites e.g. in the numbers of stolons and their incremental growth, but no indication that this was due to a principal diagonal effect. Indeed there was very little difference between the performance of plants re-planted in their native populations compared with those transplanted to alien populations (Figure 8).

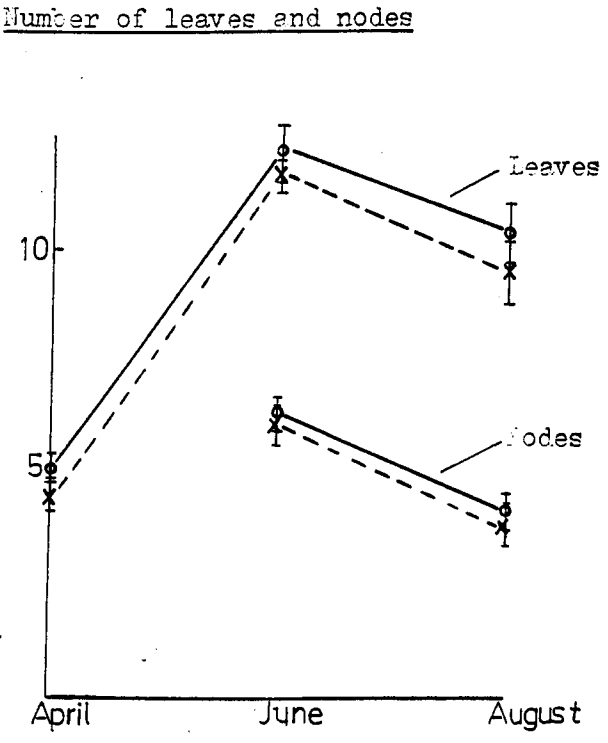
Local specialisation was not particularly apparent in any of the individual sites and, where significant variation was expressed, this did not necessarily reflect greater growth of native transplants. Thus, in June, transplants from Cae Llyn (D3) bore most leaves in their native site but by late August this differential was reversed and these plants had

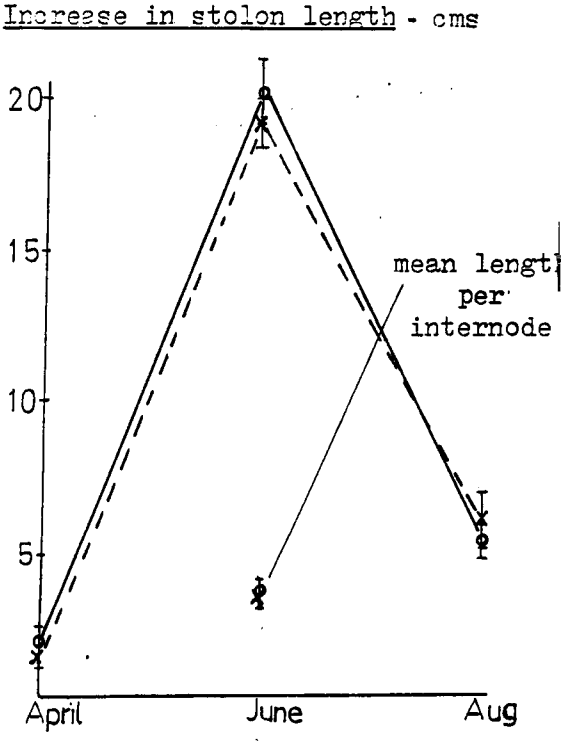
Table 11. The levels of significance of main effects and interactions on the growth of R. repens ramets originating from, and transplanted into, 4 different populations. ns - not significant; * $P<0.05$; ** $P<0.01$ and *** $P<0.001$.

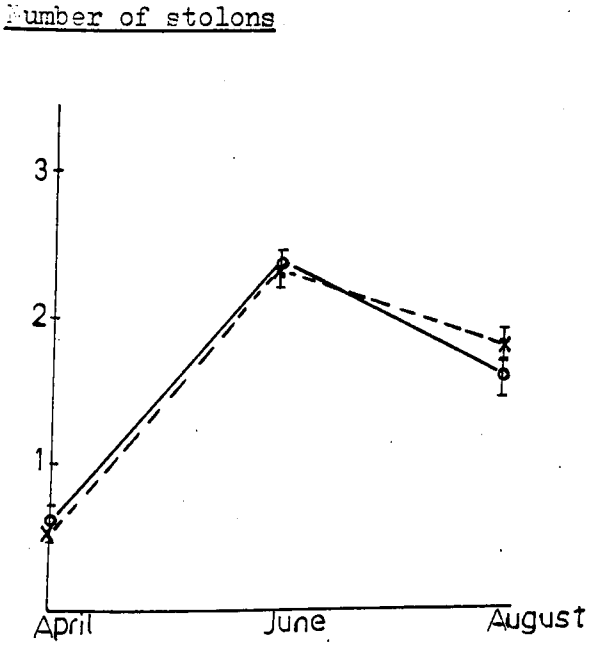
VARIABLE	POPULATION	GENET IN POPULATION	TRANSPLANT SITE	INTERACTION	*PRINCIPAL DIAGONAL
Number of leaves					
April	***	***	***	ns	ns
June	***	***	**	**	ns -D1,D3
August	***	**	***	ns	ns -D3
Number of stolons					
April	***	***	***	*	ns
June	***	***	*	*	ns
August	***	**	***	ns	ns -D2
Increase in stolon length					
April	***	***	***	*	ns
June	***	***	***	*	ns
August	***	***	***	ns	ns
Number of nodes					
June	***	***	***	ns	ns
August	***	***	***	ns	ns
Mean internode length					
June	***	***	***	ns	ns
Number of buds					
April	***	***	***	ns	ns
June	***	***	***	**	ns D1
August	***	ns	***	ns	ns

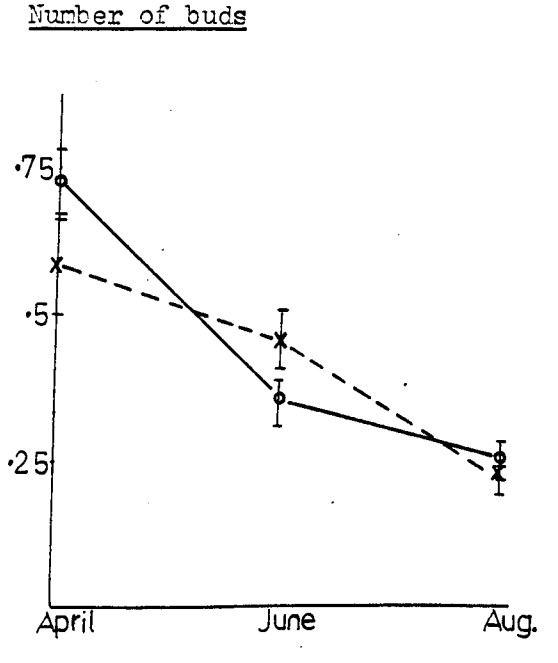
* The principal diagonal represents the significance of the comparison between the growth of all plants returned to their native site with all plants transplanted into an alien site; D1 - D4 denote significantly greater growth of native than of alien transplants within a specific site where: D1 - Treborth main lawn; D2 - Treborth new lawn; D3 - Henfaes and D4 - Cae Llyn. -D signifies a negative difference i.e. native transplants made less growth than alien transplants.

Figure 8. The growth and flowering of R. repens transplants growing in their native population (o——o) and in alien populations (x- - -x); values represent the mean per plant of 180 and 108 transplants respectively, over all 4 populations, I ± 1 standard error.









significantly fewer leaves than plants transplanted from other populations.

The performance of transplants from different sub-sites

In general, transplantation into different sub-sites accounted for little significant variation between transplants in any of the 4 populations (Tables 12-15). The sub-site of origin had some effect on transplant growth e.g. in the new lawn at Treborth (Table 13) and in Cae Llyn (Table 15). There were also some significant differences between the three genets within a sub-site, particularly in the plants from Treborth main lawn (Table 12). However, different genets rarely interacted significantly with the transplant sub-sites and there was very little evidence of any principal diagonal effect.

The principal diagonal was significant at just three recordings. In April, plants which had been returned to their native sub-sites in Treborth main lawn differed significantly from transplants from alien sub-sites but they bore fewer leaves, 7.34 (±.35) per plant compared with 8.78 (±.47) in alien transplants. Similarly, at this date in Cae Llyn, a lower proportion of transplants growing in their native sub-sites had produced stolons, .12 compared with .22 in plants growing in alien sub-sites. This accounted for a significantly lower mean number (.08 ±.04) and length (0.1cm ±.06) of stolons in these transplants than the average of .22 (±.05) stolons, 0.5cm (±.1) long in alien transplants.

Within none of the individual sub-sites was there a consistent difference between the growth of plants originating from that sub-site and transplants from elsewhere in the same population. The only exception was the plants re-planted into sub-site 1 in the main lawn at Treborth but again the native transplants tended to bear fewer leaves.

Table 12. The levels of significance of main effects and interactions on the growth of *R. repens* ramets originating from, and transplanted into, 3 different sub-sites in TREBORTH MAIN LAWN. ns - not significant; * $P<0.05$ and ** $P<0.01$.

VARIABLE	SUB-SITE OF ORIGIN	GENET IN SUB-SITE	TRANSPLANT SUB-SITE	INTERACTION	*PRINCIPAL DIAGONAL
Number of leaves					
April	**	*	**	ns	* -D1
June	ns	*	ns	ns	ns -D1,D2
August	ns	*	ns	ns	ns -D1
Number of stolons					
April	ns	*	ns	ns	ns
June	ns	*	ns	ns	ns
August	ns	ns	ns	ns	ns
Increase in stolon length					
April	*	*	ns	ns	ns
June	*	**	ns	ns	ns
August	ns	ns	ns	ns	ns
Number of nodes					
June	ns	*	ns	ns	ns
August	ns	ns	ns	ns	ns
Mean internode length					
June	**	**	ns	ns	ns
Number of buds					
April	ns	*	ns	ns	ns
June	ns	*	*	ns	ns
August	ns	ns	ns	ns	ns

* The principal diagonal represents the significance of the comparison between the growth of all plants returned to their native **sub-site** with plants transplanted in alien sub-sites; D1 - D3 denote significantly greater growth of native than of alien transplants within a specific sub-site (for detail see Table 8). -D signifies a negative difference i.e. native transplants made less growth than alien transplants.

Table 13. The levels of significance of main effects and interactions on the growth of *R. repens* ramets originating from, and transplanted into, 3 different sub-sites in TREBORTH NEW LAWN. ns - not significant; * $P<0.05$; ** $P<0.01$ and *** $P<0.001$.

VARIABLE	SUB-SITE OF ORIGIN	GENET IN SUB-SITE	TRANSPLANT SUB-SITE	INTERACTION	*PRINCIPAL DIAGONAL	
Number of leaves						
April	**	ns	*	ns	ns	
June	**	**	ns	ns	ns	
August	**	ns	ns	ns	ns	
Number of stolons						
April	ns	ns	ns	ns	ns	
June	**	*	ns	ns	ns	
August	**	ns	ns	ns	ns	
Increase in stolon length						
April	ns	ns	*	ns	ns	
June	ns	ns	ns	ns	ns	
August	**	ns	ns	ns	ns	D2
Number of nodes						
June	ns	*	ns	ns	ns	
August	**	ns	ns	ns	ns	
Mean internode length						
June	ns	**	**	ns	ns	
Number of buds						
April	ns	ns	ns	ns	ns	
June	**	**	ns	ns	ns	D1
August	ns	ns	ns	ns	ns	

* The principal diagonal represents the significance of the comparison between the growth of all plants returned to their native sub-site with all plants transplanted into alien sub-sites; D1 - D3 denote significantly greater growth of native than of alien transplants within a specific sub-site (for detail see Table 8).

Table 14. The levels of significance of main effects and interactions on the growth of R. repens ramets originating from, and transplanted into, 3 different sub-sites in HENFAES. ns - not significant; * $P<0.05$; ** $P<0.01$ and — no plants had buds at this recording.

VARIABLE	SUB-SITE OF ORIGIN	GENET IN SUB-SITE	TRANSPLANT SUB-SITE	INTERACTION	*PRINCIPAL DIAGONAL
Number of leaves					
April	ns	ns	ns	ns	ns
June	ns	ns	ns	ns	ns
August	ns	ns	ns	ns	ns
Number of stolons					
April	**	**	ns	ns	ns
June	ns	ns	ns	ns	ns
August	ns	ns	ns	ns	ns
Increase in stolon length					
April	**	**	ns	ns	ns
June	*	*	ns	*	ns
August	ns	ns	ns	ns	ns
Number of nodes					
June	*	ns	ns	*	ns
August	ns	ns	ns	ns	ns
Mean internode length					
June	ns	ns	ns	ns	ns
Number of buds					
April	**	**	ns	ns	ns
June	ns	*	ns	ns	ns
August	—	—	—	—	—

* The principal diagonal represents the significance of the comparison between the growth of all plants returned to their native sub-site with all plants transplanted into alien sub-sites.

Table 15. The levels of significance of main effects and interactions on the growth of *R. repens* ramets originating from, and transplanted into, 3 different sub-sites in CAE LLYN. ns - not significant; * $P<0.05$ and ** $P<0.01$.

VARIABLE	SUB-SITE OF ORIGIN	GENET IN SUB-SITE	TRANSPLANT SUB-SITE	INTERACTION	*PRINCIPAL DIAGONAL	
Number of leaves						
April	*	**	*	ns	ns	
June	**	ns	ns	ns	ns	
August	ns	ns	ns	ns	ns	D2
Number of stolons						
April	*	**	ns	*	*	-D1
June	*	ns	ns	ns	ns	
August	ns	ns	ns	ns	ns	
Increase in stolon length						
April	*	**	ns	**	**	-D1,D3
June	*	ns	ns	ns	ns	
August	ns	ns	ns	ns	ns	
Number of nodes						
June	**	ns	ns	ns	ns	
August	ns	ns	ns	ns	ns	-D2
Mean internode length						
June	ns	ns	*	ns	ns	
Number of buds						
April	*	*	ns	ns	ns	
June	ns	ns	ns	ns	ns	
August	ns	ns	ns	ns	ns	

* The principal diagonal represents the significance of the comparison between the growth of all plants returned to their native sub-site with all plants transplanted into alien sub-sites; D1 - D3 denote significantly greater growth of native than of alien transplants within a specific sub-site (for detail see Table 8). -D signifies a negative difference i.e. native transplants made less growth than alien transplants.

Summary

There was evidence of genetic differentiation in *R. repens* plants from different populations but no indication that this was an evolutionary response to the environment from which the plants originated. Plants from biotically-distinct sub-sites within these populations showed some genetic differences but again these differences were not relevant to their performance when transplanted to different biotic environments.

2. Analysis of Transplants in Cae Groes

The results were analysed by a 1-way nested analysis of variance using the method given by Snedecor & Cochran, 1967 pp.291-294, to cope with the unequal numbers of ramets (Table 16). Using this method, the significance of the variance between genets (σ_G^2) was tested by computing the variance ratio, F (genet mean square/ramet mean square). However, the variance between plants from different populations can not be tested in this way because of the unequal numbers of ramets from different populations. Consequently, the variance component for populations (S_P^2) was estimated from the analysis. This provides a relative measure of the variance between transplants which is due to their different populations of origin. Where the variance ratio for populations is greater than that for genets or ramets the population of origin can be said to have an effect on transplant performance. Conversely, a relatively low value of S_P^2 implies that its population has no effect on a transplant's growth. Logarithmic and square root transformations of the data were made where appropriate.

Sub-sites were not delimited in Cae Groes and the sub-sites of origin of transplants from the other populations were not taken into account in this analysis.

Table 16. The numbers of genets and ramets from different populations, transplanted into Cae Groes.

	TOTAL NUMBER OF:		
POPULATION:	GENETS	RAMETS/GENET	TRANSPLANTS
Cae Groes	9	5	45
Treborth main lawn	9	1	9
Treborth new lawn	9	1	9
Henfaes	9	1	9
Cae Llyn	9	1	9
TOTAL			81

Survivorship

16 transplants died during the course of the experiment, of which 11 originated from Cae Groes. There was no significant difference (χ^2 = 1.406) between the proportion of transplants originating from Cae Groes which survived (.76) and the survivorship of plants originating from other populations (.86).

Transplant Growth

In all recorded parameters of transplant growth, the estimated variance component for plants from different populations was lower than that for ramets and, with one exception (the number of flowers in April), was also less than the variability of individual genets (Table 17). This relatively low variance implies that the different populations of origin had little overall effect on the growth of transplants in Cae Groes. Furthermore, there was no evidence that plants originating from Cae Groes made more growth, or flowered more, than transplants originating from alien populations (Figures 9-12).

In contrast, significant genotypic differences were expressed in all parameters of growth (except the number of stolons) in either April or June. 5 months after transplanting, the variability of individual ramets within a genet had increased (Table 17). In August and September, variation between genets was not significant and the high phenotypic variability (S^2_R) e.g. in stolon growth, dry weight/cm and total dry weight, accounted for much of the variation between transplants.

Summary

Transplants in Cae Groes showed extensive growth, with individual plants producing up to 17 metres of stolons during the first 4 months after

Table 17. Results of the analysis of variance of ramets and genets of R. repens from 5 different populations transplanted into Cae Groes. The significance of F, the variance ratio for genets, is given by: ns - not significant; * $P<0.05$; ** $P<0.01$.

VARIABLE	Estimated variance component for: - RAMETS S_R^2	GENETS S_G^2	POPULATIONS S_P^2	F
Number of leaves				
April	0·16	0·09	0·03	2·02 *
June	21·96	6·07	-0·49	1·50 ns
Number of buds				
April	0·26	-0·05	0·01	0·64 ns
June	0·02	0·03	0·00	3·49 **
Stolon length				
April	0·06	0·19	0·05	6·8 **
June	0·07	0·02	-0·08	1·51 ns
August	0·59	-0·07	-0·03	0·77 ns
Dry weight of stolons				
June	29·65	14·55	0·12	1·88 *
August	0·18	0·00	-0·01	0·97 ns
Dry weight/unit length				
June	12·52	56·98	4·57	9·20 **
August	25·15	9·11	5·95	1·63 ns
Mean internode length				
June	1·66	1·27	0·04	2·37 **
Number of stolons				
June	0·24	0·04	-0·01	1·30 ns
Total dry weight				
September	0·22	0·05	-0·02	1·32 ns

Figure 9. The mean increase in stolon length of R. repens transplants in Cae Groes, originating from different populations: ▽——▽ Cae Groes; x——x Treborth main lawn; •·····• Treborth new lawn; ●——● Henfaes and o---o Cae Llyn. Values are the mean per plant ± 1 standard error.

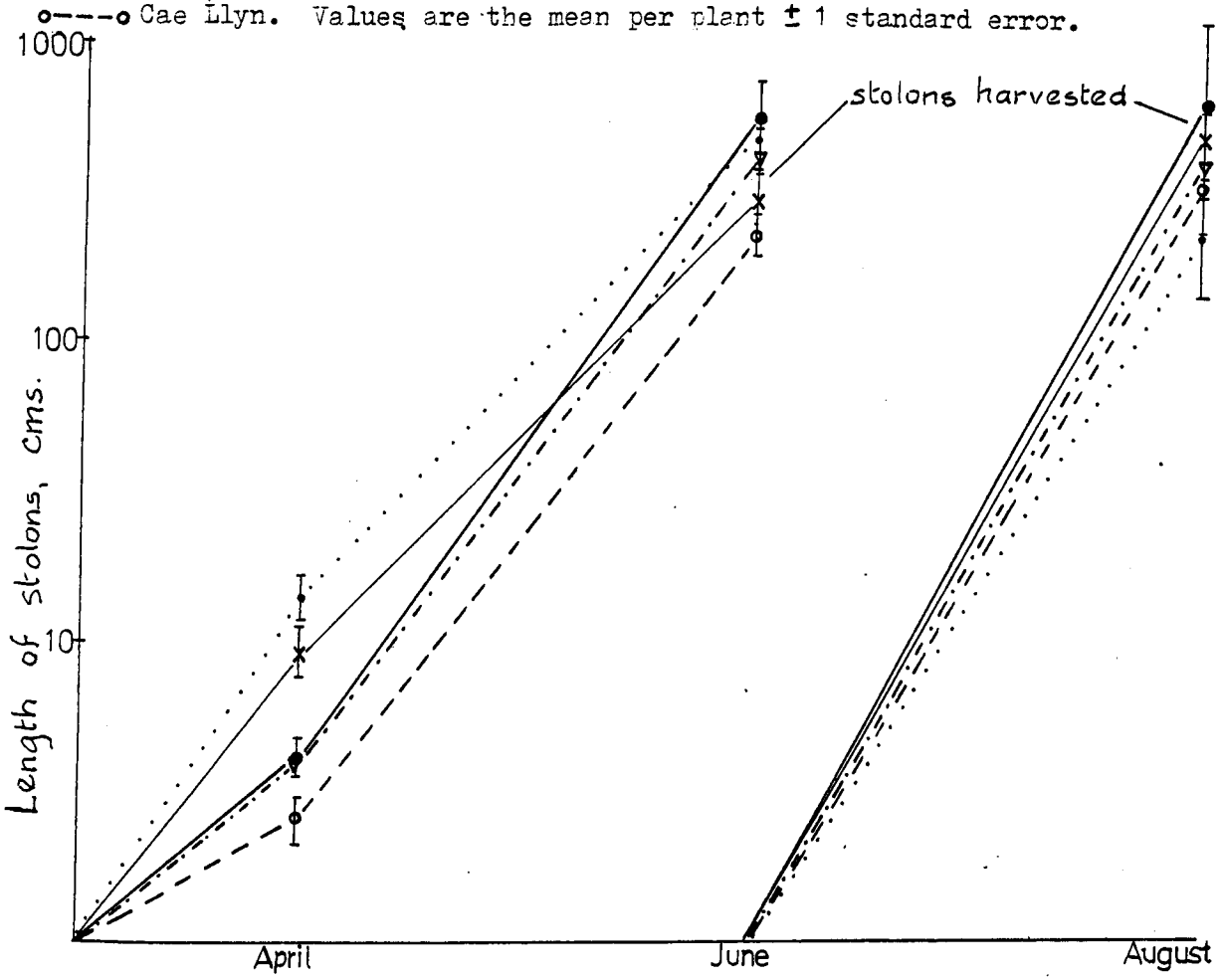


Figure 10. The mean weight of stolons harvested in June and August, from plants from different populations: ▭ Cae Groes; ▨ Treborth main lawn; ▥ Treborth new lawn; ⋰⋱ Henfaes and ▩ Cae Llyn (± 1 SE).

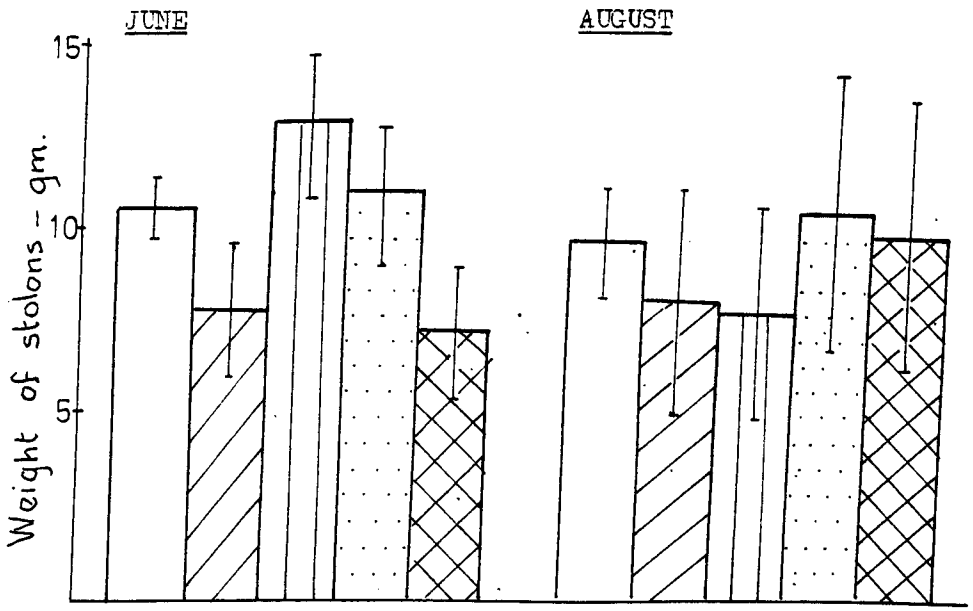


Figure 11. Variation in the growth of *R. repens* transplants at Cae Groes, according to their population of origin: Cae Groes; Treborth main lawn; Treborth new lawn; Henfaes and Cae Llyn. Values are the mean per plant ± 1 standard error.

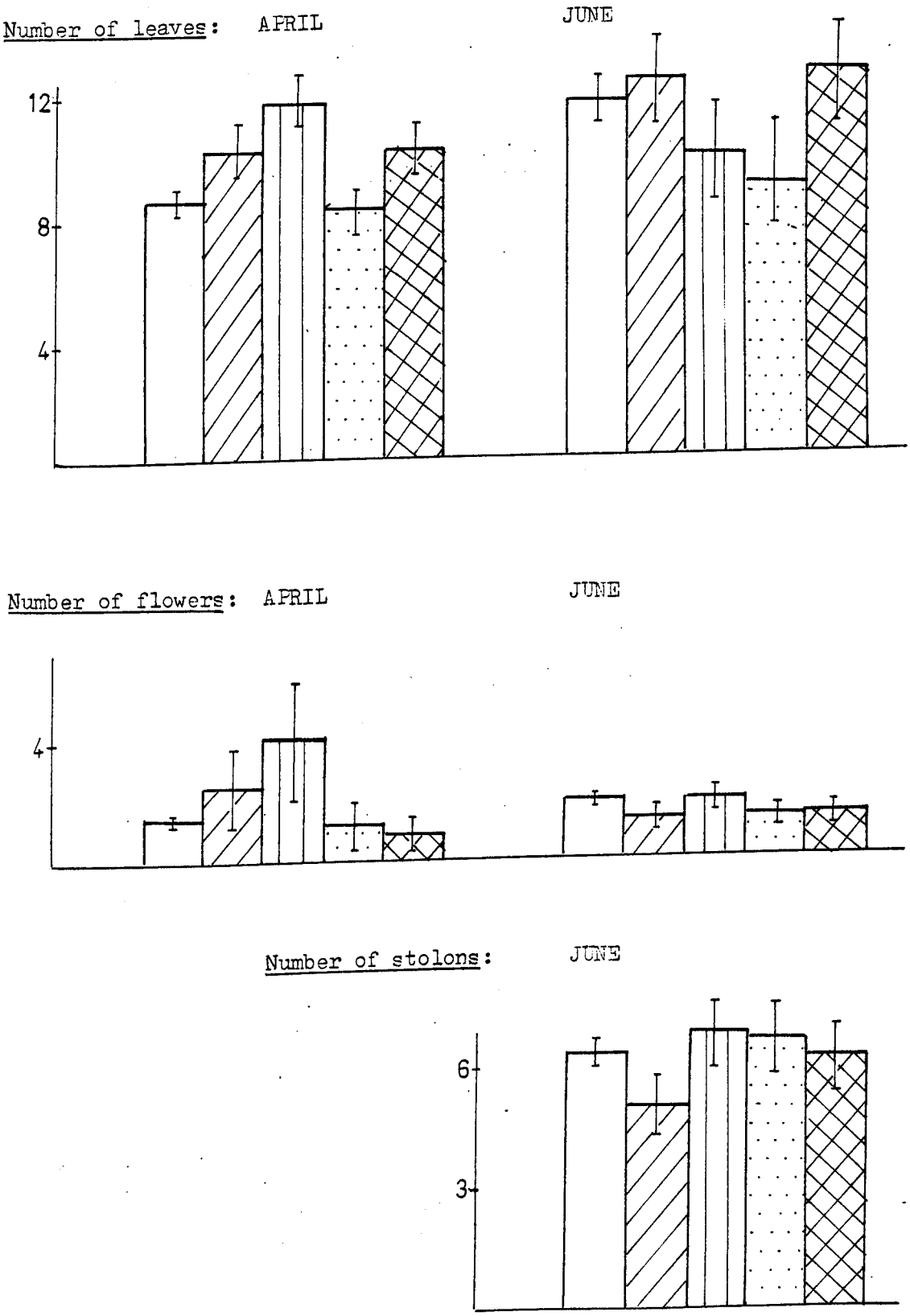
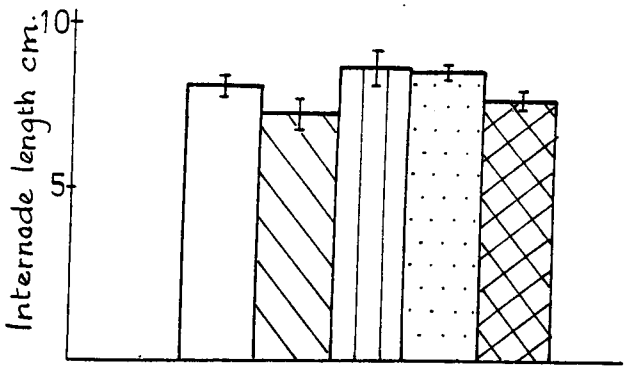


Figure 12. Variation in the stolon characteristics of transplants at Cae Groes, according to their population of origin: ▭ Cae Groes; ▨ Treborth main lawn; ▥ Treborth new lawn; ⋰⋰ Henfaes and ▩ Cae Llyn. Values are the mean per plant ± 1 standard error.

Mean internode length: JUNE



Weight/unit length: JUNE AUGUST

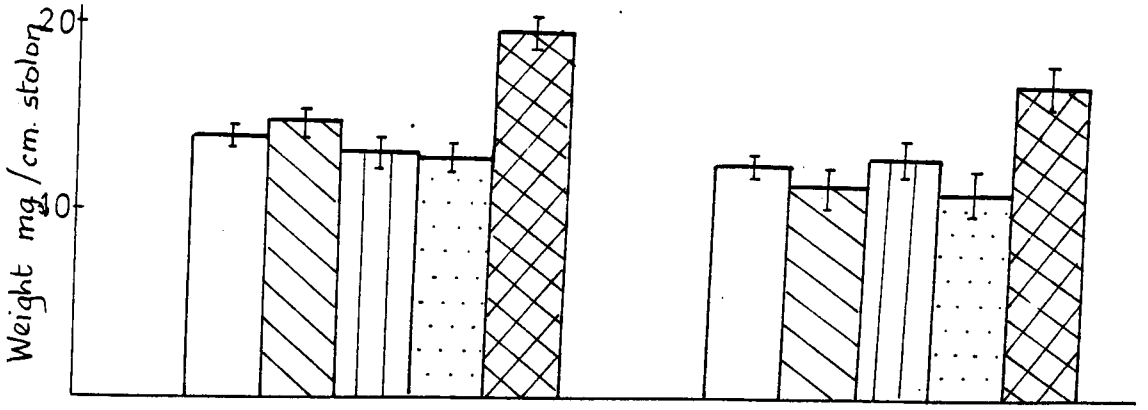
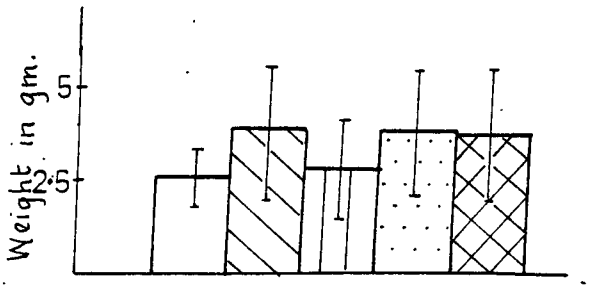


Figure 13. The mean dry weight of plants harvested from Cae Groes in September, according to their original population; the symbols for the different populations are the same as those above (⊺ ± 1 S.E.).



transplanting. There was a high degree of variability between ramets of the same genet and this increased with time as larger transplants pre-empted the resources of their smaller neighbours. Differentiation of plants originating from different populations was not evident in transplants growing at this site, nor was there any indication that plants originally from Cae Groes were specialised in response to their local environment.

Discussion

Local Specialisation: in different species.

In both primroses and buttercups, there were strong differences between the performance of plants originating from different local populations (Tables 3 & 11). It is interesting to note that these differences were not expressed in transplants at Cae Groes (Table 17), where the plants were growing in a garden plot with no neighbours; under conditions similar to those of the common garden and transplant stations used in many studies of ecotypes. Population differences in the remaining sites, which did not diminish during the course of the transplant experiment, were considered to indicate genetic variation (but see Chapters 5 & 6). The transplant site also significantly affected the performance of plants of both species.

Clearly the local environments differentially influenced transplant phenotype and this suggests that selection pressure would differ between the populations. There was however no indication that plants of *R. repens* were specialised to their native population (Figure 8) though primrose transplants did tend to produce more leaves when transplanted back into their original site (Figure 3). Thus, differences between the primrose populations were not random - locally-specialised plants made more growth

when returned to their native, compared to that in alien, environments. This intra-specific variation among the primroses can therefore be attributed to the action of natural selection and contrasts with the apparently random nature of the variation between buttercup populations.

Differences in the growth forms of the two species could, in part, account for these different patterns of intra-specific variation. A buttercup plant may grow over an area of a few square metres and sample different biotic, edaphic and micro-climatic conditions whereas, the primrose grows a short rhizome of only a few centimetres and remains close to the site where it established. A buttercup genet will therefore experience a variety of selective forces in both time and space but can respond to this environmental variation by growing into a different micro-site and so may be much less vigorously selected to respond to local environmental variation (see Chapter 3).

The breeding system also influences the pattern of variation in a species. It is generally considered that inbreeding promotes inter-population differences and outbred species show a higher level of intra-population differentiation (Levin & Wilson, 1976). Both *P. vulgaris* and *R. repens* are insect-pollinated and predominantly outbreeding. Genetic differences between such outbred populations may however be obscured by gene exchange with neighbouring populations. This gene flow is more likely to occur between the studied populations of *R. repens*, which is widespread and common, than in the primroses which grow in discreet and relatively isolated populations. Self-compatible homostyle primroses are found in large numbers in a few natural populations. A comparison of their genetic differentiation with that of outbred primrose populations would provide a valuable test of the above generalisation, though many exceptions may also be found (e.g. those listed in Turkington & Aarssen, 1984).

It is difficult to draw precise comparisons between the species because their contrasting growth forms forced the use of different experimental designs e.g.:

(a) It was not possible to replicate all primrose clones in each transplant site and therefore variation between genets could not be assessed in the same detail as in the buttercup transplant experiment.

(b) Different parameters were measured to describe the growth of transplants of the two species. Local specialisation may not be identified if variation in measured characters is not directly correlated with habitat differences yet, characters other than those considered may show significant differences (Schaal & Levin, 1978). Ideally, the same or similar components of fitness should be recorded in both species.

(c) The buttercup transplants could not be re-located when stolons decayed after six months and the experiment had to be terminated whereas, the primroses were recorded for a further ten months.

Furthermore, different study populations were employed for the two species. Differences in the method of sampling the original plants from these populations could have affected the pattern of intra-specific differentiation identified (Bradshaw, 1962). Population factors may also have influenced genetic differentiation, these are discussed below.

Local Specialisation: differences between populations.

Traditionally, the calculation of the overall principal diagonal forms the end-point of the analysis of a reciprocal transplant experiment but it is possible, and extremely useful, to sub-divide the principal diagonal into separate components, each of which represents the local specialisation within an individual population. Differences in the degree of local

specialisation were evident between the five primrose populations (Figure 5). Indeed, plants from only one population (Plas Gwyn) showed a high degree of specialisation; with little difference between native and alien transplants or even reduced growth of native plants in the remaining populations. There were no obvious differences in the degree of differentiation in the four buttercup populations included in the main analysis, none of which showed significant local specialisation (Tables 12-15).

Both genetic and environmental factors could have accounted for the different levels of specialisation in the primrose populations. The genes available in a population, and their exchange, will be determined by the number and distribution of plants and their proximity to other populations of the same species. In a small, isolated population local specialisation may be limited by the genetic constitution of its founder members. Gene flow is also influenced by environmental conditions which affect the time of flowering, movement of wind-borne pollen and the number and activity of insect pollinators. An investigation of gene exchange has revealed that primrose populations may differ significantly in their genetic structure and organisation (Cahalan, 1983). Such differences could be reflected in the extent of population differentiation and it would be interesting to relate these data to the actual level of specialisation exhibited by plants in a reciprocal transplant experiment.

Highly specialised genotypes are more likely to evolve in a population in which the physical and biotic environment is temporally and spatially homogeneous, or shows a regular change. In such a population, the degree of differentiation may be greater in the older plants, which have survived the selection pressures of the site, and is also likely to increase with time since the population became established.

However, these factors are complex and difficult to evaluate because in a natural population, even within a species, individuals of different age and size will sample their environment and interact with each other and with neighbouring species in a variety of different ways. Thus, whilst it is possible to recognise a higher level of local specialisation in one population than in others, it is difficult to identify the specific factors responsible. Any experimental measures of gene flow or environmental forces will be valid only for the time, conditions and individual plants concerned.

Local Specialisation: differences within populations.

I did not look at variation within the primrose populations because each of the primrose sites was relatively small (<100m^2) and homogeneous and I therefore sampled plants systematically from the entire study area. There was no indication that buttercup plants were specialised to different localities within their native population. Plants originating from subsites dominated by different grasses did show significant differences (e.g. Table 13) but transplants returned to their original site rarely made more growth than those in alien subsites. This contrasts with *Trifolium repens*, in which variability was correlated with the presence of different neighbouring species and plants performed best when returned to their original sub-site (Turkington & Harper, 1979b).

Within many plant populations the dispersal of seed and pollen is severely limited (e.g. Kerster & Levin, 1968 and Bradshaw, 1972) and genes may only be exchanged within an area smaller than that over which selection acts uniformly (Antonovics & Levin, 1980). For example, in natural primrose populations effective population sizes consisted of only 1-38 plants

(Cahalan & Gliddon, 1985). If effective population sizes are so small (less than 200 individuals) genetic drift may occur within interbreeding groups (see Wright, 1946) and random intra-population differentiation will result.

In permanent grassland *R. repens* rarely establishes from seed (Sarukhán & Harper, 1974), reducing the opportunity for genetic specialisation to a changing biotic environment. Despite the infrequent input of new genotypes, a high level of genetic diversity may be maintained by the continuing clonal growth of established ramets within the population (Soane & Watkinson, 1976). The differential growth of these ramets provides a means of phenotypic differentiation to the present environment whilst conserving a spectrum of genetic variability which represents a "memory" of past selection pressures.

Conclusions

1. We might predict that population differentiation would be less likely in *R. repens* than in *P. vulgaris* because of the extensive clonal growth, widespread distribution and low level of seedling recruitment of buttercups. This would seem to be borne out by the results of the transplant experiments, although the experimental procedure for the two species was not directly comparable.

2. Local specialisation varies between populations of the same species. This could be a direct result of different patterns of environmental heterogeneity but probably also reflects variation in pollen and seed dispersal between populations of plants at different densities with individuals of various sizes and ages.

3. Genetic differences were identified within populations of *R. repens* but apparently were not correlated with different biotic environments. This may be because of the wandering growth form of the buttercup and therefore the high variability of biotic forces experienced, or due to genetic drift or phenotypic plasticity (see Chapter 7).

4. However, biotic forces clearly affected the phenotype of buttercup plants as revealed by the atypical growth of those transplants in Cae Groes. This demonstrates the importance of estimating population differences between transplants in a natural population where they are subjected to the totality of environmental forces.

5. The relative performance of transplants varied with time; transplant experiments must therefore be long term to even out the effects of seasonal variation and other temporal heterogeneity.

6. Some questions which have arisen as a result of these transplant studies will be discussed in the following chapters:

a) Are the differences between transplants in the same transplant site solely indicative of genetic differentiation? This assumption is made whenever differences between plants are maintained in a common environment yet, nutrients, hormones and pathogens could be carried over from the original environment and affect growth in the transplant site. In chapters 5 & 6 I consider to what extent: i) differential virus infection and ii) physiological differences could contribute to the variation which is traditionally attributed to genetic factors.

b) Do different species of neighbours differentially affect the growth of plants and therefore, by implication, exert different selection pressures to which plants may become locally specialised?

c) Individual ramets of the same genet of *R. repens* will encounter a

variety of neighbours and edaphic conditions. Are these micro-environmental factors entirely reflected in the growth of the ramet which experiences them or is the performance of a genet which is composed of interconnected ramets an integrated function of all their environmental experiences?

CHAPTER 3

Phytometer Experiments to Investigate the Influence of Environment on the Growth of *R. repens*

General Introduction

The Development of the Phytometer Technique

The influence of climate and altitude upon vegetation, which was identified in early transplant experiments (e.g. Bonnier, 1890 and 1920, Kerner, 1895 and MacDougall, 1906), led to an increasing interest in evaluating environmental factors in terms of plant growth. Thus, a range of temperatures was compared as differences in the "growth velocity" of wheat plants (MacDougall, 1914). The growth of plants and the species present in the natural habitat were considered to be the best "indicators", or measures, of environmental conditions (Clements, 1917). This concept of "indicator plants" was coupled with the transplant technique in the proposal that standard plants or phytometers should be deliberately introduced into a series of habitats. Measurement of the response of these phytometers would provide a comparative estimate of environmental factors in the different habitats (Clements *et al.*, 1918).

A series of experiments was conducted at the Carnegie Institution in Washington to develop the phytometer as an experimental tool (Clements & Goldsmith, 1924). Species chosen as phytometers were hardy and vigorous, ideally with simple entire leaves e.g. *Helianthus annuus*. A battery of 8-10 plants was selected to be as uniform as possible and placed in each habitat which was to be measured. The relative growth, photosynthesis and transpiration of these phytometers was assessed as a comparative measure of the physical factors of the different environments. The results of the phytometer experiments yielded values which represented the "integration of all habitat factors expressed in terms of plant functions". In contrast, instruments were seen as measuring the exact amount of an individual environmental variable; enabling a closer analysis of specific factors but

failing to describe that variation between habitats which was relevant to plant behaviour.

Applications of the Phytometer Method

Phytometers were sown at different densities in containers and placed within a field of wheat to study the effect of surrounding plants on transpiration (Clements, Weaver & Hanson, 1929). Subsequently, the influence of solar radiation on transpiration was measured as the relative transpiration rates of sunflowers growing at a series of different light intensities, under shade-tents and in lath-huts (Clements *et al.*, 1950). Sugar beet phytometers were placed at different heights within a crop, where their net photosynthetic rates provided a direct indication of the quality of the environment at different levels in the crop profile (Leach & Watson, 1968).

In all of these studies, the physiological activity of the phytometer provided a quantitative estimate of environmental factors. Variation in the histological structure of epidermal cell walls has also been proposed as a quantifiable plant feature suitable for use in phytometer experiments (Takenouchi, 1933). Yet, plant growth is the "ultimate expression of the factor-complex" (Phillips, 1925); the relative growth of phytometers will yield an estimate of the environment which is more relevant to plant activity than either individual physiological processes or plant morphology.

Parameters of growth such as: plant height; shoot number; ear length and yield were recorded in phytometers at different positions within a field as an estimate of the effect of slope micro-climate on cereal yield (Radomski, 1977). Oats and ryegrass have been used as phytometers to quantify plant growth potential in comparing various tundra sites (e.g. Lewis & Callaghan, 1971; Bonde *et al.*, 1973 and Walton & Smith, 1976). The

phytometer method has proved a valuable tool in studying primary production in cold climates as it enables the use of relatively fast-growing agronomic species, which are more uniform and easier to process than native tundra plants (Smith & Walton, 1975).

Phytometers have recently been transplanted reciprocally into natural populations such that each plant was returned to its native site and to every other sampling location (Turkington & Harper, 1979b and Antonovics & Primack, 1982). These phytometers were used as instruments to measure the environments of the different transplant sites. In addition, the variation in reproduction and survivorship of phytometers from different localities was indicative of the differential fitness of genotypes which were specialised in response to the contrasting local environments of their original sites. The specific influence of different neighbouring grass species in local differentiation was studied by monitoring the growth and survivorship of phytometers in experimental swards of different grasses (Turkington & Harper, 1979b).

I have used phytometers to assess the response of *Ranunculus repens* to variation in biotic and physical environmental factors, at the level of both the genet and individual ramets.

1. The Influence of Neighbours on the Growth of *R. repens* Genets

Introduction

Ranunculus repens is a clonal perennial which overwinters as a small rosette of 2-4 leaves. Clonal growth occurs in late spring (May/June) when stolons develop from buds in the axils of the rosette leaves. Nodes are formed at intervals of 3-15cm along the stolons. Each node bears a modular unit of 1 or 2 leaves and a pair of roots which anchor the node to the

soil. The rooted module then produces additional leaves and roots to form a daughter ramet similar to the parent rosette.

In a stoloniferous plant such as *R. repens*, the pattern of branching and the lengths of internodes determine the way in which the genet, through its ramets, experiences the environment. In my reciprocal transplant experiment (Chapter 2), transplanted rosettes of *R. repens* developed a maximum of 7 primary stolons, each one up to 1 metre in length. These stolons frequently branched to form secondary and even tertiary stolons, distributing the ramets of a single genet over an area in excess of $3m^2$. This spreading growth form maximises inter-specific contacts. Thus, the leaves of *R. repens,* in both grassland and woodland plants, made many more contacts with leaves of different species than with their own clone or with other clones of *R. repens* (Lovett Doust, 1981). A decrease in life expectancy of *R. repens* ramets has been associated with increased intra-specific interactions (Sarukhán & Harper, 1973); inter-specific contacts, which are relatively more frequent, might also be expected to influence ramet growth.

Ranunculus repens plants were grown as phytometers in experimental swards to determine the effect of three different neighbouring species on the performance of *R. repens* genets.

Method

Rosettes of *R. repens* were sampled from the 1 hectare field of permanent grassland at Henfaes (see Appendix II). In November 1981, single rosettes of *R. repens* were selected from plants at widely separated locations within the field to obtain 12 different genets. Soil was carefully shaken from the roots and the rootstock of each rosette split

into two equal parts, which were labelled and potted in John Innes No. 1 compost in four-inch pots. After cultivation for one month in the glasshouse each of these ramets was again sub-divided and the resulting ramets allowed to grow for a further month. 8 of the original 12 genets, each represented by four equivalent ramets (with 5-7 leaves), were selected for use in the phytometer experiment.

In January 1982, individual ramets were transplanted as phytometers into experimental swards of different species. The swards had been established for two months in John Innes No. 1 compost in plastic trays (20.5 x 15.5 x 4cm) from plants sampled from Henfaes (rather than from seed) to produce a uniform sward. The three experimental swards were:

i) *Holcus lanatus*, 30 tillers/tray

ii) *Lolium perenne*, 30 tillers/tray

iii) *Ranunculus repens*, 8 plants/tray

iv) Control no plants/tray

There were 8 trays of the four treatments, 32 trays in all. Individual ramets within a genet were allocated at random to different treatments, such that each of the 8 genets of *R. repens* was represented by one ramet in each of the four treatments. The trays were randomly assigned to positions on a bench in a heated glasshouse and watered regularly for five months. In treatments (i) and (ii) the grasses were clipped to 3-5 cm at two-weekly intervals. Plastic rings were placed around a number of leaves on the phytometers in treatment (iii) to distinguish them from the neighbouring *R. repens* plants which made up the sward.

Five months after transplanting, all phytometers were harvested and their vegetative characteristics were recorded. Reproductive performance was not assessed as few phytometers flowered during the experiment.

Results

Overall Growth

There was significant variation in the yield of roots and shoots and the number of leaves on phytometers in the different treatments and in the above-ground growth of the 8 genets (Table 1). In the presence of neighbours *R. repens* made significantly less growth than in the control treatment. However, association with neighbours of different species resulted in no significant variation in dry matter or leaf production (Table 2 and Figure 1). A number of phytometers produced stolons during the course of the experiment but the presence of neighbours did not significantly affect the probability of stolon production (Table 3).

Stolon Growth

Individual genets differed significantly in their performance (Table 1). In assessing the effect of neighbours on growth characteristics of stolons it was therefore necessary to compare phytometers of the same genet, or genets, in different treatments. This presented a problem in that none of the genets produced stolons in all treatments, indeed one showed no stolon growth in any of its four ramets (Table 3). Four genets (1,2,4 and 6) produced stolons in both the control and in association with *R. repens*, treatment (iii). Paired *t* tests were used to compare stolon growth within these four genets and between the two treatments (Table 4). It was not possible to analyse the effects of the different grass species on the growth of *R. repens* because too few phytometers growing in the grass swards produced stolons.

Phytometers grown in the presence of *R. repens* had significantly reduced root and shoot dry weights (Figure 2) fewer leaves (Figure 3a) and produced only one stolon compared with an average of 4.25 stolons per plant

Table 1. Significance of (i) the effect of the presence and species of neighbours and (ii) the difference between 8 different genets on the performance of *R. repens* phytometers, results of a 2-way ANOVA: ns - not significant; * P<0.05; ** P<0.01; *** P<0.001.

VARIABLE:	(i) EFFECT OF NEIGHBOURS	(ii) EFFECT OF GENETS
Dry weight of shoots	**	*
Dry weight of roots	**	ns
Number of leaves	**	*

Table 2. Mean yield (g) and number of leaves on 8 *R. repens* phytometers in the presence of different neighbouring species.

		NEIGHBOURING SPECIES:				
VARIABLE:	CONTROL	*H. lanatus*	*L. perenne*	*R. repens*	D	LSD
Dry weight of shoots	6.64	0.87	1.04	1.02	-5.66 ± 1.0	1.27
Dry weight of roots	9.67	1.76	2.42	1.73	-7.71 ± 2.2	2.72
Number of leaves	80.13	16.38	18.50	21.00	-61.50 ± 18.6	22.81

"D" (±95% confidence limits) estimates the difference due to the presence of neighbours by comparison of the control (no neighbours) with the three treatments.
LSD - the least significant difference, is a comparison of the effects of the three different species of neighbour.

Figure 1. The mean growth (± 1 standard error) made by 8 phytometers representing 8 genets of R. repens grown in the glasshouse in the presence of different species. Phytometers were grown with: no neighbours ▭ ; Holcus lanatus ▨ ; Lolium perenne ⁘ and Ranunculus repens ▦.

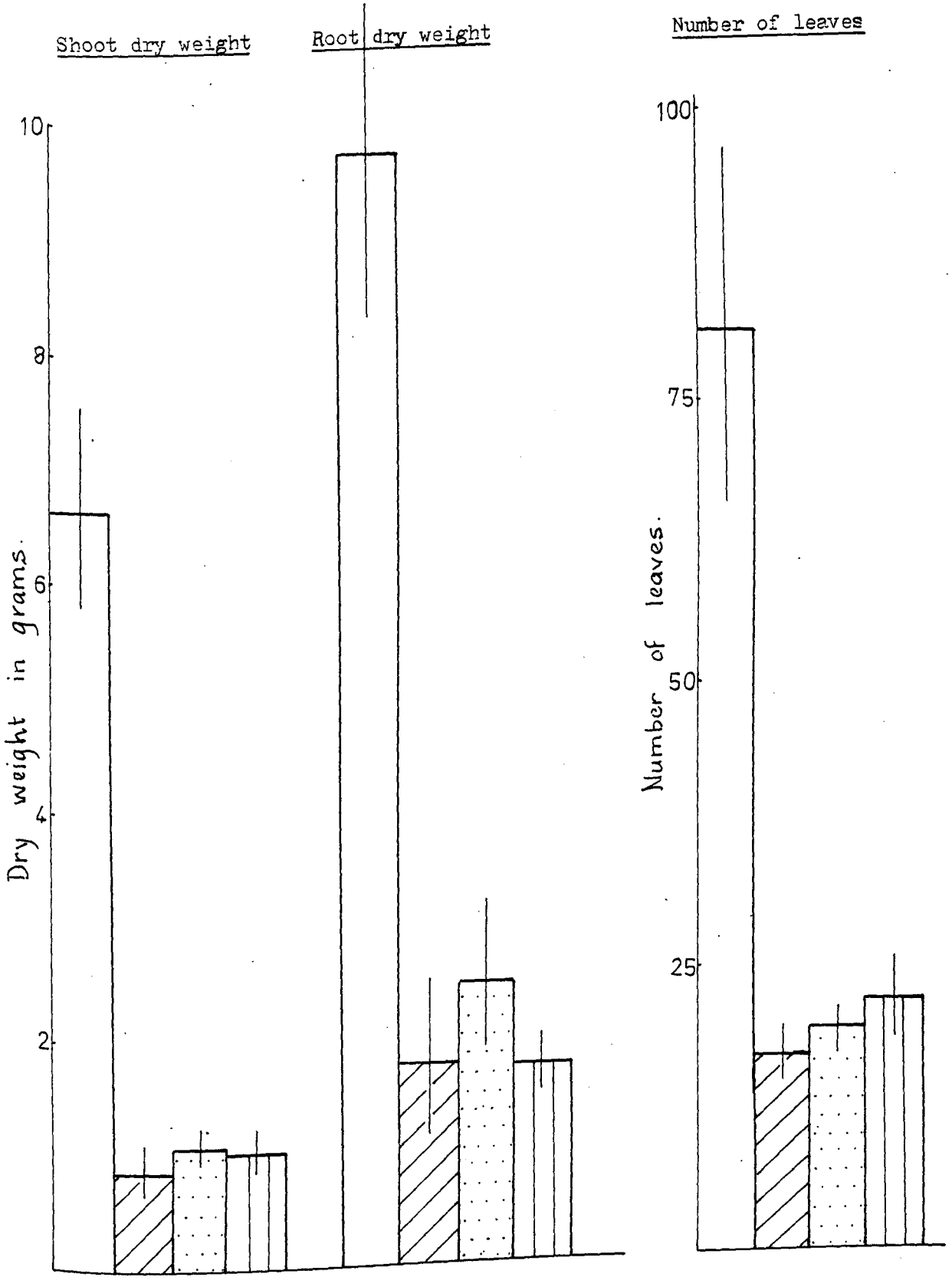


Table 3. The production of stolons by *R. repens* phytometers in the presence of neighbours (+ denotes at least one stolon was produced).

	GENET:							
NEIGHBOURS:	1	2	3	4	5	6	7	8
CONTROL	+	+	–	+	–	+	–	+
H. lanatus	+	–	+	–	–	–	+	–
L. perenne	–	–	–	–	–	+	–	–
R. repens	+	+	+	+	–	+	–	–

	Total number (proportion) of phytometers with stolons:		χ^2	
With no neighbours (8 phytometers)	5	(.625)		
			1.524	not significant
With neighbours (24 phytometers)	9	(.375)		

Table 4. A comparison of the growth of paired ramets of 4 *R. repens* genets; 1 ramet of each genet was growing with no neighbours (control) and the second ramet grew in association with other *R. repens* plants. The significance of the difference between ramets, calculated using a paired *t* test, is also shown; ns - not significant; * P<0.05; ** P<0.01; P<0.001.

	DRY WEIGHT OF:		NUMBER OF:		STOLON
	SHOOTS	ROOTS	LEAVES	STOLONS	LENGTHS
TOTAL/PLANT					
Control	8.05	10.91	105.00	4.25	
	***	*	*	***	
with *R. repens*	1.03	1.48	24.50	1.00	
PARENT ROSETTE					
Control	2.83	5.83	38.75		
	ns	ns	*		
with *R. repens*	0.41	0.76	9.00		
STOLONS					
Control	5.22	5.08	66.25		161.50
	*	ns	ns		ns
with *R. repens*	0.63	0.72	15.50		31.75
MEAN/STOLON					
Control	1.28	1.33	16.63		42.14
	ns	ns	ns		ns
with *R. repens*	0.63	0.72	15.50		31.75
MEAN/NODE (INTERNODE)					
Control			2.48		7.62
			ns		*
with *R. repens*			3.75		4.98

Figure 2. Allocation of dry matter in phytometers of R. repens growing in the absence of neighbours (control) and in the presence of R. repens. * denotes a significant difference between the two treatments.

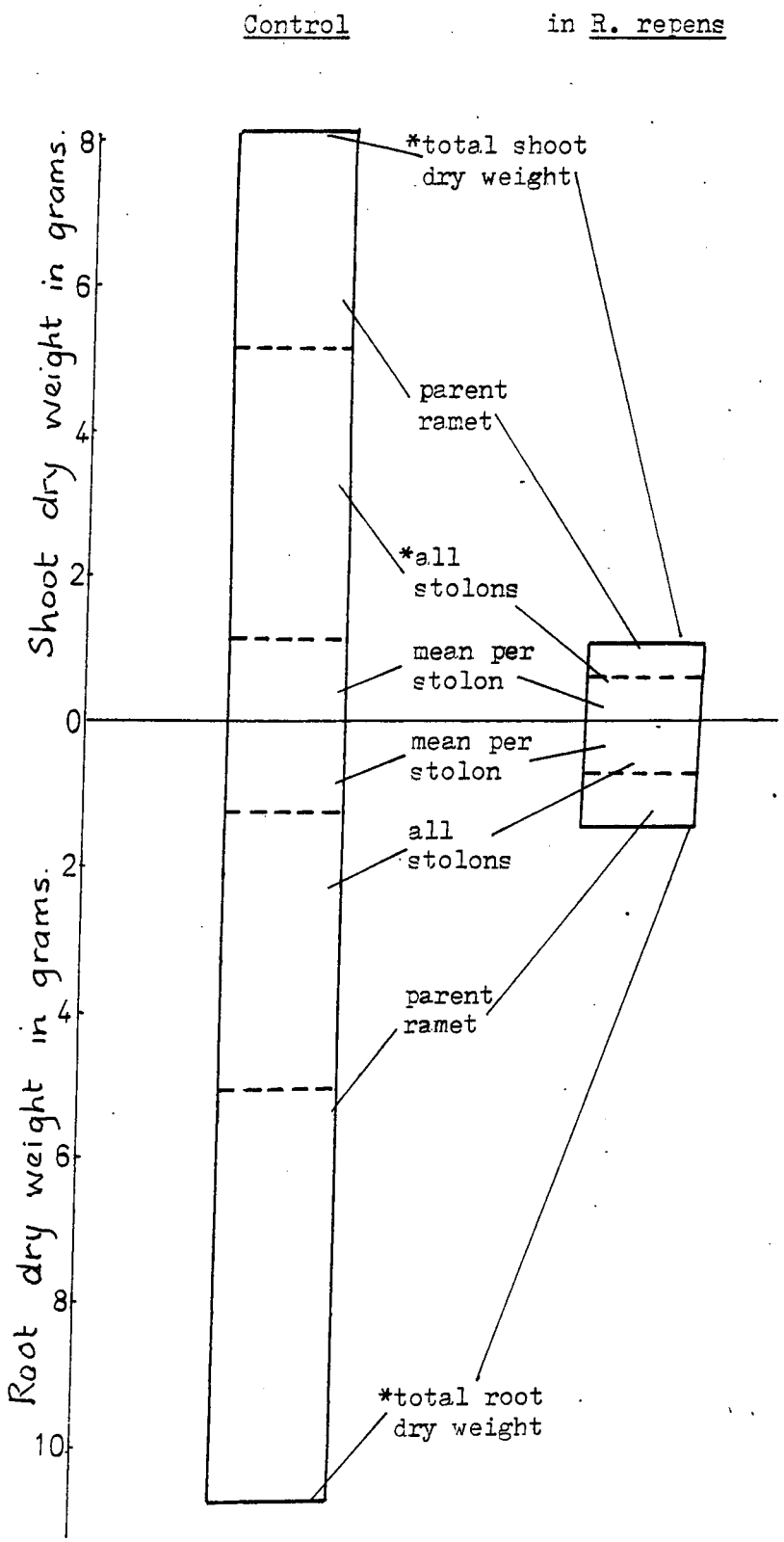
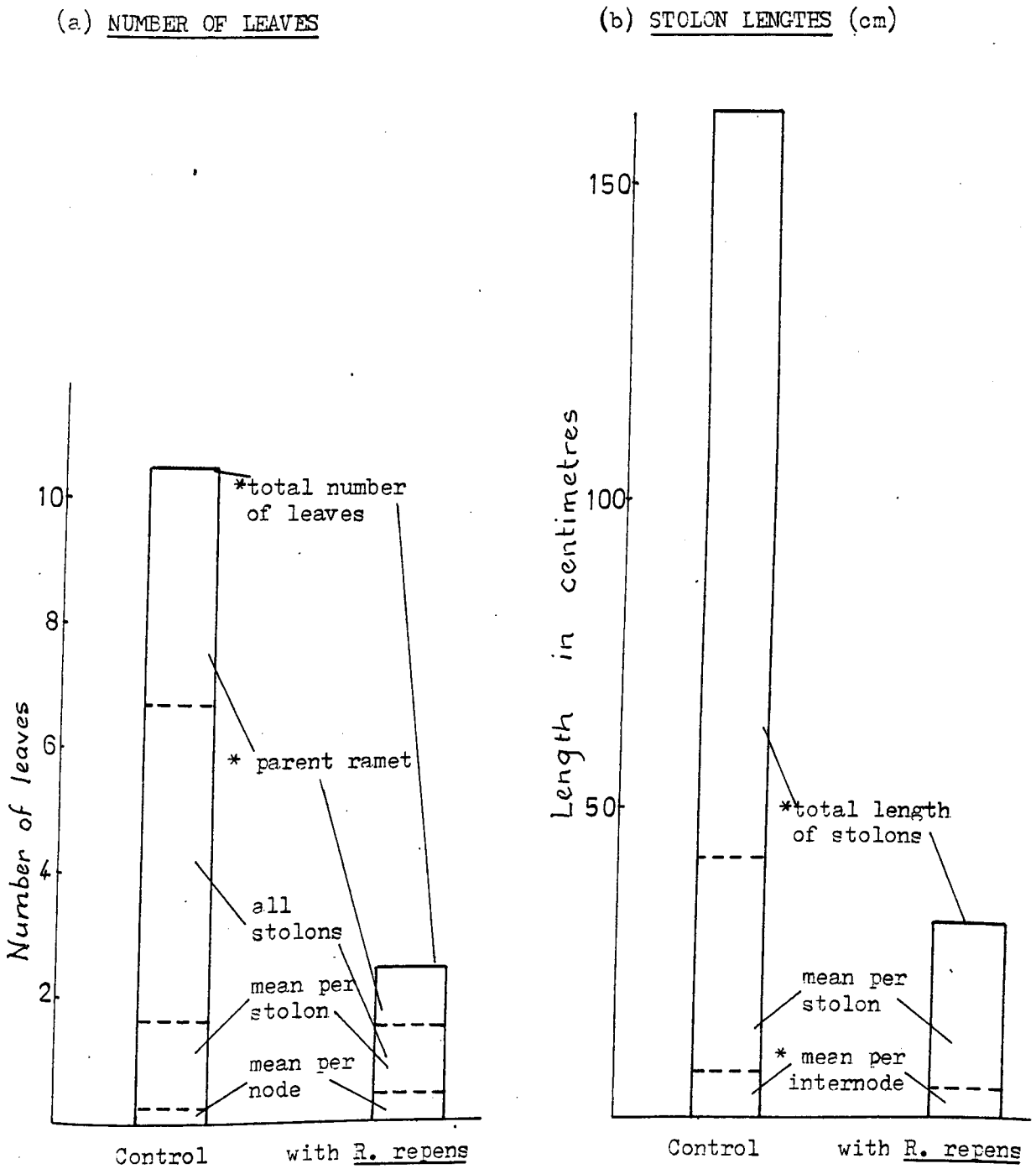


Figure 3. (a) The number and distribution of leaves and (b) the length of stolons on R. repens phytometers growing in the absence of neighbours (control) and in the presence of R. repens. * denotes a significant difference between the two treatments.



in the control trays (Table 4). This single stolon tended to be short, with a low dry weight of roots and shoots but bore more leaves per node than the stolons on control phytometers (Figure 3b), though these differences were not significant. The average internode length of stolons on phytometers growing with *R. repens* was only 4.98 cm compared with the significantly longer internodes of 7.62 cm in the absence of neighbours.

2. The Influence of Small-scale Biotic and Edaphic Variation on the Growth of Individual Ramets

Introduction

Ranunculus repens is a clonal species in which the genet grows by the re-iteration of modules or ramets. Individual modules of growth may be independent, as in a fragmented genet e.g. *Lemna*, or remain, for all or part of their life, physiologically inter-dependent. Stolon connections between ramets of *R. repens* are maintained until early autumn. This retention of stolons affords the opportunity for correlative control of bud growth and the exchange of assimilates between ramets. In *R. repens*, resources have been shown to be mainly transported acropetally, from the parent and older ramets to newly-formed daughter ramets (Ginzo & Lovell, 1973b). In a natural population, the ramets of a single *R. repens* genet will be re-iterated over an area of a few square metres and encounter a variety of spatial and temporal environments. Thus, parts of the same genet may meet different neighbours, explore different patches in a soil mosaic or extend from shaded areas into an area which is fully lit. The number of seeds and possible descendants left by all of these parts determine the fitness of the clonal genet; the fitness of a genet is therefore, an integrated function of the variety of experiences of all the

ramets.

In a locally heterogeneous environment, assimilates may be translocated within a clonal plant from ramets growing in favourable conditions to ramets in less favourable sites (Ong & Marshall, 1979; Newell, 1982 and Hartnett & Bazzaz, 1983). This equitable distribution of resources between inter-connected ramets will affect the way in which the clonal genet experiences a patchy environment. Furthermore, fitness of the genet may be increased when the benefit to ramets growing in locally inferior conditions outweighs the cost to those ramets which are contributing resources (Salzman & Parker, 1985). Physiological integration of ramet growth will also mean that the performance of a particular ramet may be affected not only by its immediate environment but by the environment of neighbouring inter-connected ramets.

An experiment was devised to investigate the influence of small-scale edaphic and biotic environmental variation on the growth of individual ramets within the same genet of *R. repens*.

Method

1. Sequence Pots

A sequence pot technique (Noble, 1976) was used to study the growth of *R. repens* plants as they colonised a patchy environment. In order to subject ramets of a single genet to different conditions, successive nodes along a primary stolon were allowed to root in a sequence of three-inch pots. All pots were filled with John Innes No. 1 compost but the environment in each pot was manipulated experimentally to enable adjacent ramets to experience contrasting edaphic and/or biotic conditions. Individual pots were subjected to one of four treatments:

i)"GF" 10 tillers of *Lolium perenne* and 60 mls of fertiliser/week 18 pots

ii)"G" 10 " " " and NO " 54 pots

iii)"F" NO " " " and 60 mls " 54 pots

iv)"O" NO " " " and NO " 90 pots

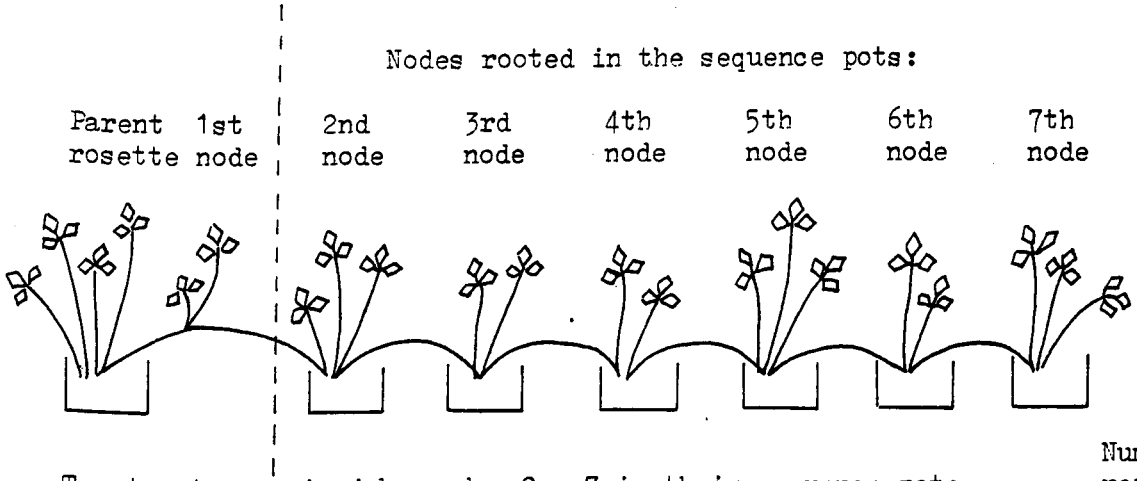
72 pots containing grass were set up in December 1981 using tillers of *Lolium perenne* plants sampled from the main lawn at Treborth Botanic Garden (see Appendix II). Throughout the experiment the grass was clipped every 2-3 weeks to a height of 3-5 cm. Full Long Ashton solution was applied to the pots receiving fertiliser treatment (in three applications of 20 mls per week) after a ramet of *R. repens* had been rooted in the pot.

All 216 pots were arranged in 36 sequences of 6, such that successive nodes of a *R. repens* genet could be rooted in pots receiving the same or alternating treatments (Table 5). The sequences were positioned at random on a bench in the heated glasshouse.

2. Sampling and Cultivation of *R. repens* Phytometers

In November 1981 *R. repens* rosettes were sampled from the main lawn at Treborth Botanic Garden. 36 equivalent rosettes, each with 4 or 5 leaves, were selected from discrete sampling points not less than three metres apart. Soil was shaken from the roots and the rosettes were potted individually in three-inch pots containing John Innes No. 1 compost. These pots were placed in the heated glasshouse for 8-10 weeks. A single primary stolon was allowed to grow from each parent rosette. All primary and secondary stolons that were formed subsequently were "stopped" by cutting off the stolon tips.

Table 5. The order of different treatments employed in the sequence pots. The notation used for the treatments is: G^F - grass and fertiliser; G - grass; F - fertiliser and 0 - control, no grass or fertiliser (for a full description of the treatments see page 54).



Treatments received by nodes 2 - 7 in their sequence pot:							Number of replicates
Grass and fertiliser	G^F	G^F	G^F	G^F	G^F	G^F	3
Grass	G	G	G	G	G	G	3
Fertiliser	F	F	F	F	F	F	3
Control	0	0	0	0	0	0	9
Alternating treatments:	G	0	G	0	G	0	3
	0	G	0	G	0	G	3
	F	0	F	0	F	0	3
	0	F	0	F	0	F	3
	G	F	G	F	G	F	3
	F	G	F	G	F	G	3

3. Experimental Procedure

Typically, the first daughter ramet on a stolon of *R. repens* is formed very close to the parent rosette (Sarukhán & Harper, 1973) and could not be rooted in the first sequence pot. Plants were therefore selected for the experiment after a stolon had developed two nodes. The second node was allowed to root in the first pot in the sequence, the first node remaining unrooted, as shown in Table 5.

8 weeks after sampling, only 12 of the plants bore stolons with two nodes. These were assigned at random, three to the control and one to each of the other sequences. This procedure was repeated in the two following weeks as other plants reached the required growth stage, such that the replicates of each sequence were established over three consecutive weeks. As nodes were produced along a stolon they were encouraged to root in successive sequence pots by placing a loop of wire over the node in each pot to hold it in position on the soil surface.

Stolon extension and the production of daughter ramets were recorded at weekly intervals until a ramet had rooted in the sixth pot. Subsequent stolon growth was prevented by removing the tip of the primary stolon. The birth rate of leaves was calculated by placing, each week, a coloured ring over all newly-formed leaves on the parent and daughter ramets. After 14 weeks all plants were harvested and the total number of leaves and dry weight determined for each ramet. The weekly recordings of leaf birth rate and the lengths of internodes were checked before the plants were dried. Flowering occurred in very few ramets and therefore was not included in the analysis.

Results

Despite the selection of phenotypically uniform plants from the field, internode lengths were very variable, both within and between genets. Long internodes were easily accommodated by moving the pots further apart. However, the occasional production of very short internodes (less than 4 cm) resulted in two adjacent ramets occupying the same pot or in an aerial ramet which could not be rooted. In some cases the growth of the stolon ceased before the sixth sequence pot was reached. This presented problems in analysing the data because "replicates" could sample the environment in different ways (Figure 4). To cope with this problem it was decided to analyse the variation between individual ramets, rather than between genets. All ramets which were unrooted, or shared a pot with a second ramet were excluded from the analysis.

Using the 36 genets as replicates, the differences between individual ramets could be due to:

i) position along the stolon

ii) treatment, grass and/or fertiliser, in the pot

iii) treatment in neighbouring pots

i) The Influence of a Ramet's Position Along the Stolon on its Performance

A one-way analysis of variance was made to investigate the variation between ramets of genets which occupied all six pots in seven of the control sequences. Scheffé's multiple range test was used to compare the performance of ramets from different positions.

There was no significant difference between the performance of the ramets at different positions along the stolon (Table 6); i.e. neither the age of a ramet nor its distance from the original parent affected its

Figure 4. Variations in the stoloniferous growth of R. repens plants which, in (b) and (c), prevented the rooting of consecutive nodes in a sequence of six pots and led to some ramets being discarded from the analysis.

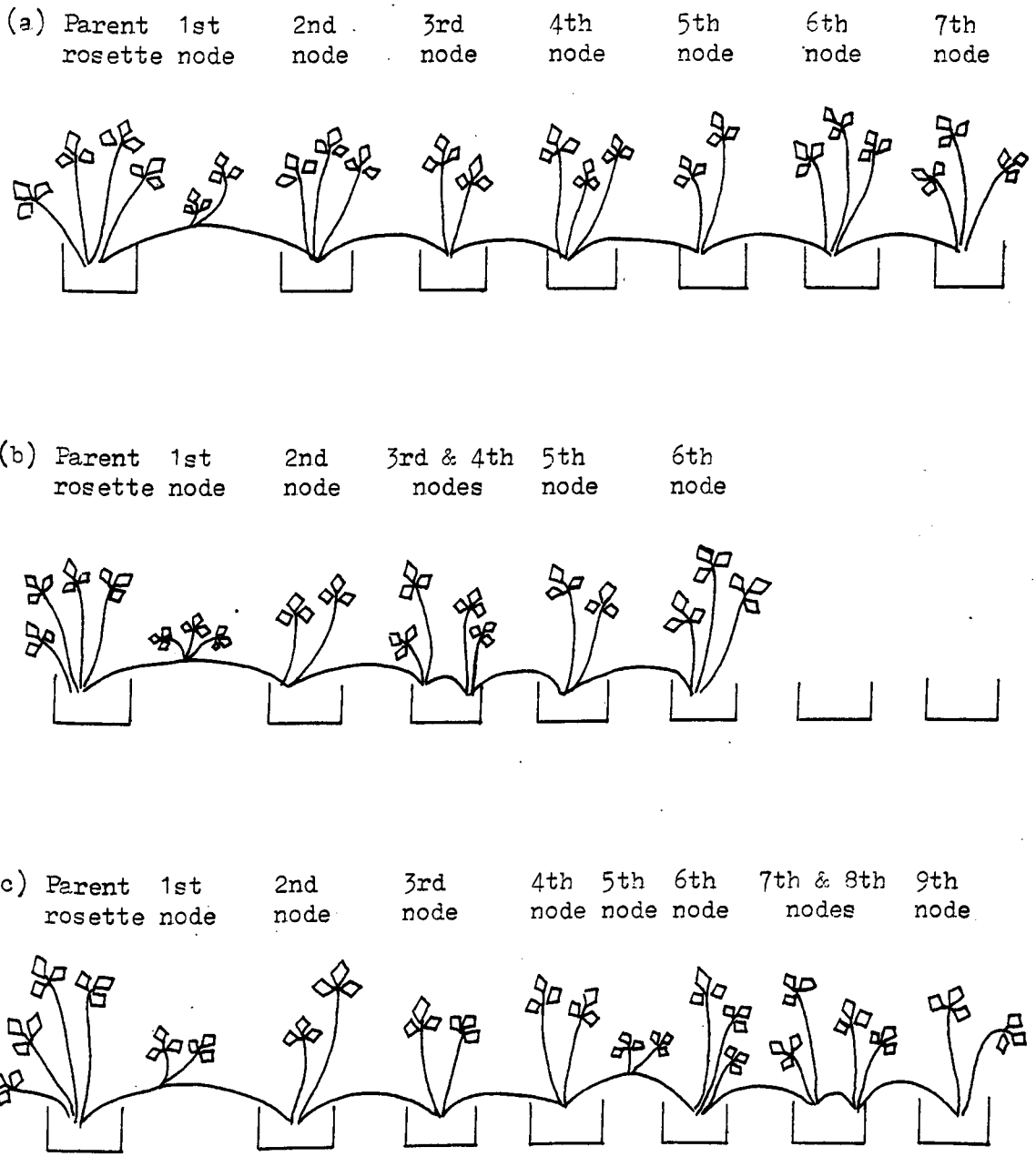


Table 6. The mean values for the performance of rooted ramets of *R. repens* at different positions along a stolon (n=7). Significance of the variance ratio (F) from the ANOVA and the least significant difference, LSD, between means (P<0.05 given by the Scheffé test) are also shown; ns not significant.

	RAMET NUMBER*							
VARIABLE:	2	3	4	5	6	7	F	LSD
Internode length (cm)	7.00	7.81	8.96	7.60	7.63	-	ns	3.13
Dry weight (g)	1.75	1.49	1.78	1.55	1.38	1.33	ns	1.30
Number of leaves	8.29	8.00	8.29	7.43	8.29	7.00	ns	5.63
Leaf birth rate/week	0.74	0.79	0.84	0.84	1.09	1.07	ns	0.54

* ramets are numbered from the parent rosette to the apex of the stolon see Table 5).

Table 7. The significance of the main effects and interactions of fertiliser and grass on the performance of ramets of *R. repens*: ns - not significant; * P<0.05; ** P<0.01; *** P<0.001.

VARIABLE:	EFFECT OF FERTILISER	EFFECT OF GRASS	INTERACTION (FERTILISER X GRASS)
Internode length	ns	ns	ns
Dry weight	***	***	**
Number of leaves	***	***	*
Leaf birth rate/week	***	***	ns

growth over the duration of the experiment. Therefore, in subsequent analyses the performance of individual ramets was considered irrespective of the order in which they were produced on the stolon.

ii) The Influence of Grass and Fertiliser Treatment on Ramet Growth - (in plants experiencing the same regime along their length.)

The ramets rooted in the sequence pots were classified in a two-way system according to: (i) the presence or absence of grass and (ii) the application of fertiliser to their pot. Thus treatments: "G^F"; "G"; "F" and "0" were represented as sub-classes in a 2 x 2 table:

		PRESENCE OF GRASS	
		-	+
APPLICATION OF FERTILISER	-	0	G
	+	F	G^F

We consider first only those ramets experiencing the same treatment as their neighbours (i.e sequences: G^F,G^F,G^F,G^F,G^F,G^F; G,G,G,G,G,G ; F,F,F,F,F,F and 0,0,0,0,0,0) to exclude any differential influence of neighbouring ramets. A two-way analysis of variance with unequal replication (Snedecor & Cochran, 1967 pp. 484-488) was performed to assess the effects of the grass and fertiliser treatments.

The application of fertiliser to a pot increased the birth rate of leaves, the dry matter production and the total number of leaves on a ramet in that pot (Tables 7 and 8). In contrast, the presence of grass caused a significant decrease in each of these parameters. The negative influence of the grass and the positive effect of fertiliser on plant growth were

Table 8. Mean values (number of observations) showing the main effects and significance of grass and fertiliser treatment on ramet growth and the significant comparisons between individual means where the interaction (grass x fertiliser) was significant: ns - not significant; * $P<0.05$; ** $P<0.01$ and *** $P<0.001$

INTERNODE LENGTH (cm)

		Grass −		Grass +	Effect of fertiliser
Fertiliser	−	7·74 (34)		8·04 (8)	7·80
					ns
	+	7·76 (12)		7·38 (12)	7·57
Effect of grass		7·75	ns	7·64	

DRY WEIGHT (g)

		Grass −		Grass +	Effect of fertiliser
Fertiliser	−	1·52 (42)	***	0·34 (8)	1·33
		***	***	**	*
	+	3·80 (14)	***	1·35 (15)	2·53
Effect of grass		2·09	*	1·01	

NUMBER OF LEAVES

		Grass −		Grass +	Effect of fertiliser
Fertiliser	−	7·76 (42)	***	3·38 (8)	7·06
		***	***	**	*
	+	14·36 (14)	***	6·93 (15)	10·52
Effect of grass		9·41	*	5·70	

BIRTH RATE OF LEAVES/WEEK

		Grass −	Grass +	Effect of fertiliser
Fertiliser	−	0·86 (42)	0·46 (8)	0·80
				*
	+	1·33 (14)	0·75 (15)	1·03
Effect of grass		0·98	*	0·65

generally additive. Thus in the presence of both grass and fertiliser (in pots G^F) the growth of ramets did not differ significantly from that in the control treatment (0). This additive effect of grass and fertiliser did not entirely explain the variation in dry weight or in the number of leaves, as indicated by the significant interaction (Table 7). However, a comparison of the individual treatment means, using Student's *t*, revealed that the overall influence of the treatments was consistent with the above observations (Table 8).

Neither the presence of grass nor the application of fertiliser, alone or together, had any significant effect on the length of stolon (internode length) produced by a ramet. This stability in stolon length (and so in exploratory ability of the plant) is remarkable in view of the high responsiveness of other characters to treatment.

iii) The Influence of the Treatment of Neighbouring Ramets on Ramet Growth - (in ramets experiencing alternating treatments along their length)

(a) The effect on a ramet's performance of its own environment and that of ramets located both proximally and distally to it.

Ramets were classified according to (i) the treatment they received and (ii) the treatment of their neighbours. Ramets were only included in the analysis when both their proximal neighbours i.e. older ramets, located along the stolon towards the parent rosette, and distal neighbours, located towards the apex of the stolon, experienced the same treatment, to avoid the problem of a conflicting influence from different neighbouring environments. The number of observations in each class varied (Table 9) and a weighted analysis was necessary to compare the performance of ramets in different classes. Few ramets received both grass and fertiliser (G^F),

Table 9. The number of ramets in each sub-class of a two-way classification of ramets according to: (i) the treatment the ramet experienced and (ii) the treatment of neighbouring ramets located both proximally and distally. The treatments were: G^F - grass and fertiliser; G - grass; F - fertiliser and 0 - control, no grass or fertiliser (for a full description of the treatments see page 54).

		Treatment experienced by the ramet:			
		G^F	G	F	0
Treatment of neighbours:	G^F	9	0	0	0
	G	0	6	7	7
	F	0	10	9	7
	0	0	8	10	27

they were therefore excluded from the analysis.

A two-way regression analysis using GENSTAT revealed a significant interaction between the two factors (i) treatment of a ramet and (ii) treatment of its neighbours (Table 10). However, the two-way analysis assumes an additive relationship between the factors and is therefore, inappropriate for these data.

Three separate one-way analyses were made to investigate the effect of the treatment of neighbouring ramets on the variance of ramets subjected to each treatment (Table 11). The growth of a ramet which was receiving fertiliser was unaffected by the environment in adjacent pots. However, untreated ramets and those growing in association with grass, were significantly affected by the treatment that both their proximal and distal neighbours experienced. Untreated ramets showed an increase in leaf production and dry weight when fertiliser was being applied to adjacent ramets (Figure 5a). The growth of a ramet was generally reduced if its neighbours were growing with grass, particularly in those ramets which were themselves growing with grass (Figure 5b).

Internode length i.e. the length of stolon produced by a ramet, was unaffected by the treatment experienced by neighbouring ramets (Figure 6).

(b) The effect on the performance of a ramet of the environment experienced by ramets distal to it.

The parental rosettes and the first daughter ramets experienced no grass or fertiliser treatment but differed with respect to the environmental conditions in which distal ramets, produced further along the stolon and rooted in the sequence pots, were growing. An analysis of variance was made as described above to determine the effect on the parent and first daughter ramet of the grass and fertiliser treatment experienced

Table 10. The results of a two-way ANOVA showing the significance of : (a) the treatment experienced by a ramet and (b) the treatment of its neighbours on the ramet's performance, ns - not significant; * P<0.05; ** P<0.01; *** P<0.001.

VARIABLE:	Effect of the treatment of: (a) the ramet	(b) its neighbours	Interaction
Internode length	ns	ns	ns
Dry weight	***	**	*
Number of leaves	***	ns	*
Leaf birth rate	***	*	*

Table 11. The significance of the treatment of neighbouring ramets on the performance of ramets themselves subjected to different treatments (summary of ANOVA results). ns- not significant; * P<0.05; ** P<0.01; *** P<0.001

VARIABLE:	Effect of the treatment of neighbours on ramets which experienced: Control (no treatment)	Grass	Fertiliser
Internode length	ns	ns	ns
Dry weight	**	*	ns
Number of leaves	**	**	ns
Leaf birth rate	**	**	ns

Figure 5. Mean values for the growth of R. repens ramets according to: (a) the treatment they received and (b) the treatment of their neighbours. A different letter, a or b, denotes a significant difference ($P<0.05$ calculated using Student's "t") between the performance of ramets in the same treatment but whose neighbours experienced different conditions. Neighbours: ☐ untreated O; ▨ with grass G; ▦ with fertiliser.

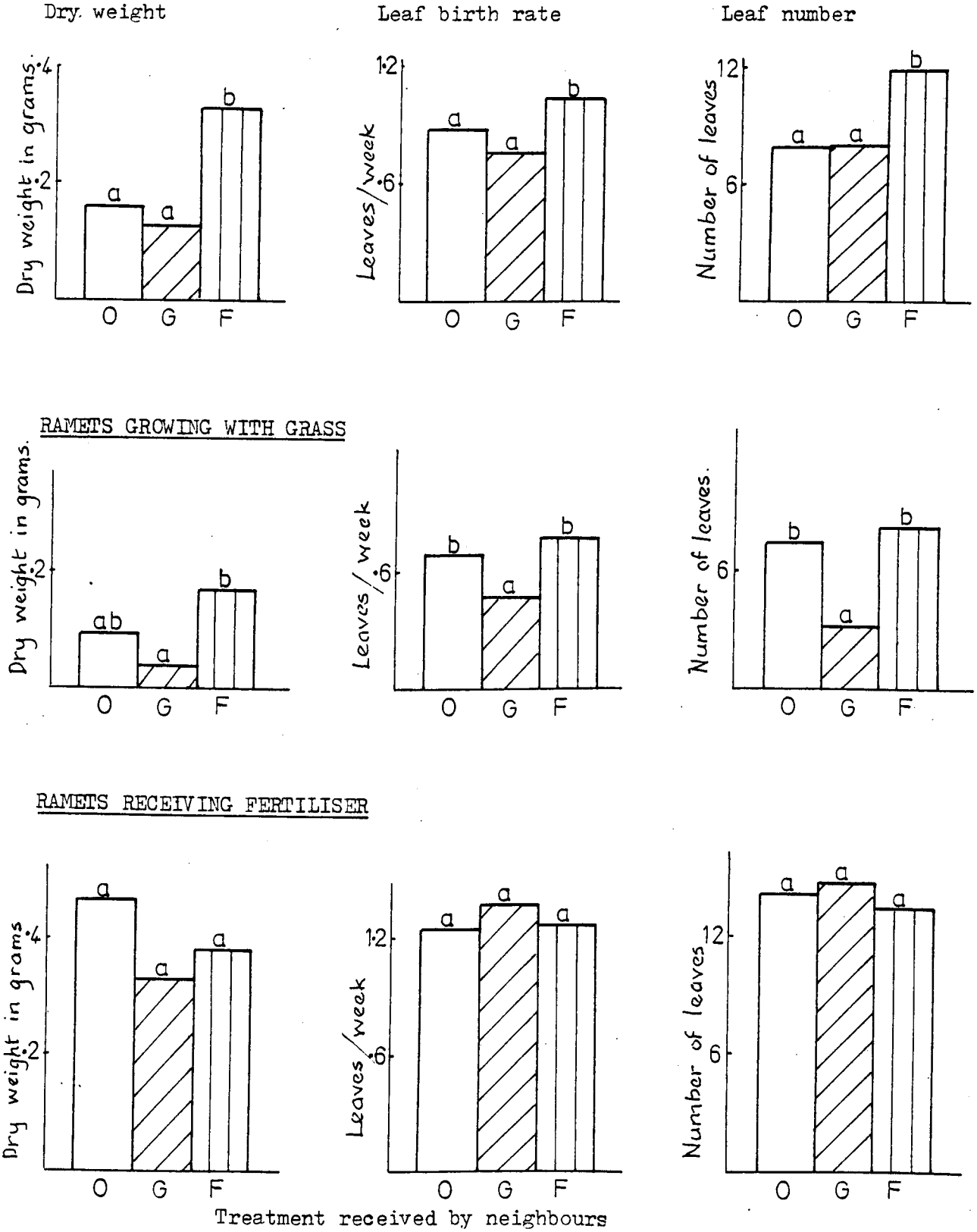
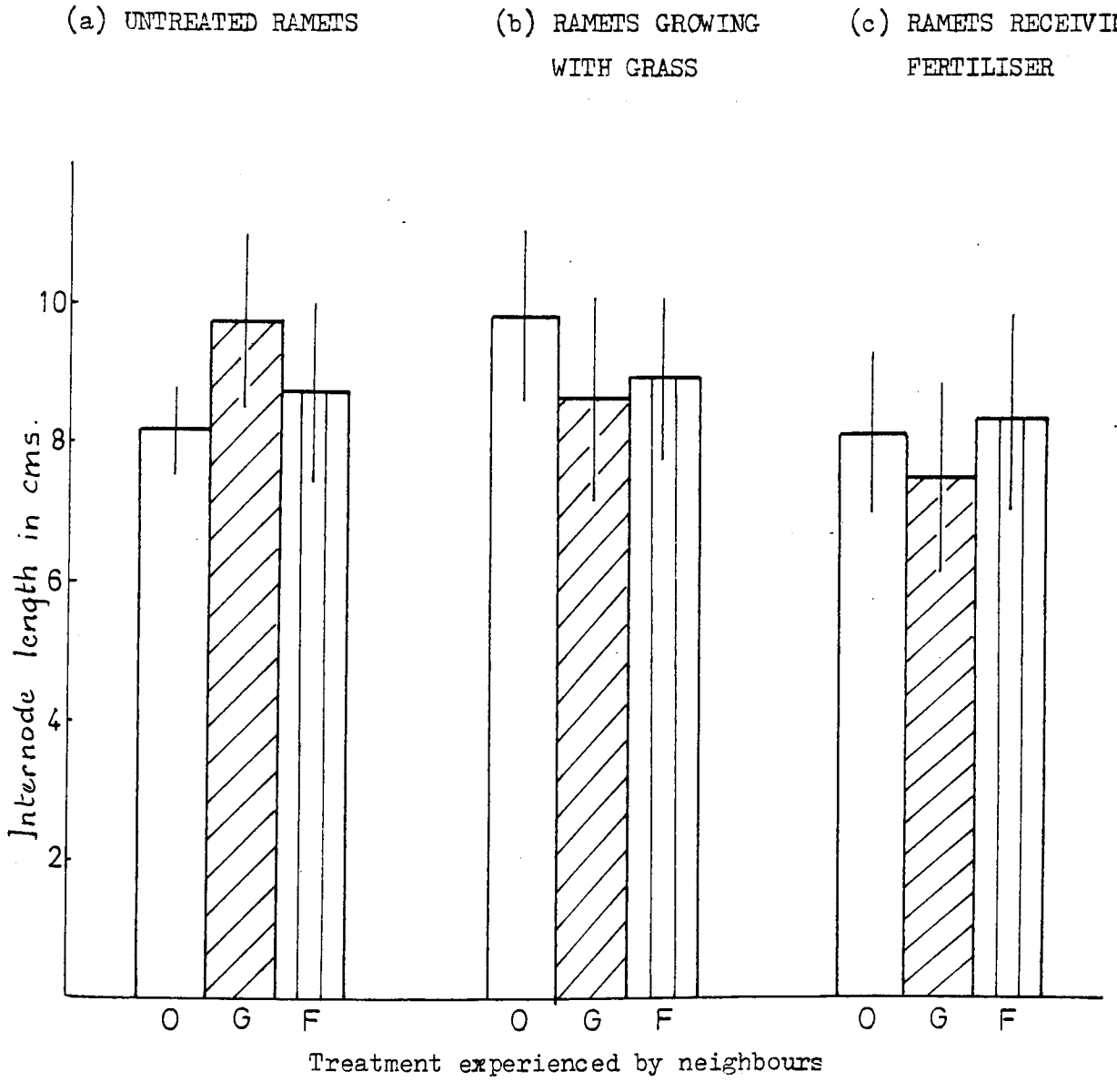


Figure 6. The mean internode length (± 95% confidence limits) of stolons produced by ramets which experienced different treatments (a,b,c) according to the treatment experienced by their neighbours. Treatment of neighbours: ▭ untreated (O); ▨ with grass (G); ▦ with fertiliser (F).



by the second ramet.

The growth of leaves on a ramet was significantly affected by the environmental experience of a ramet located distally along the same stolon (Table 12). The presence of grass growing with the second ramet resulted in a lower number and birth rate of leaves in the first ramet. Conversely, application of fertiliser to the second ramet was associated with an increased leaf birth rate and higher total number of leaves in ramets proximal to it. No significant differences were recorded in the dry weight or internode lengths of ramets but again, the application of fertiliser tended to be associated with an increase and the presence of grass with a decrease, in the value of these parameters (Table 13).

Summary

Generally, the application of fertiliser to a ramet of *R. repens* increased its production of leaves and overall dry weight whereas, the presence of grass neighbours reduced the growth of the ramet. This positive influence of fertiliser and the negative effect of grass was also reflected in the performance of ramets located proximally to the treated ramet but to a lesser degree (Table 14). In a patchy environment, e.g. where sequential pots were subjected to different treatments, a ramet made more growth when adjacent ramets, located both proximally and distally to it, were occupying relatively more favourable sites. Thus, the growth of an individual ramet was influenced not only by its own environment but by the edaphic and biotic conditions in which neighbouring inter-connected ramets were growing.

Table 12. The significance of the main effects and interactions of grass and fertiliser application to the second ramet on the performance of: (a) the parent rosette and (b) the first daughter ramet.
ns - not significant; * $P<0.05$.

(a)Parent Rosette

VARIABLE:	Effect of grass	Effect of fertiliser	Interaction
Internode length	ns	ns	*
Dry weight	ns	ns	ns
Number of leaves	ns	*	ns
Leaf birth rate	ns	*	ns

(b)First Daughter Ramet

Internode length	ns	ns	ns
Dry weight	ns	ns	ns
Number of leaves	*	ns	*
Leaf birth rate	*	*	ns

Table 13. Mean values (number of observations) showing the main effects and significance of grass and fertiliser treatment of the second ramet on the growth of ramets located proximally along the stolon. Significant comparisons between individual means are shown where the interaction was significant. ns - not significant and *P<0·05.

	PARENT ROSETTE			FIRST DAUGHTER RAMET		
INTERNODE LENGTH (cm)	Grass −	Grass +	Effect of fertiliser	Grass −	Grass +	Effect of fertiliser
Fertiliser −	5·45 (13)	5·74 (7)	5·55 ns	5·90 (12)	6·22 (6)	6·01 ns
Fertiliser +	7·06 (5)	4·07 (3)	5·94	7·52 (2)	6·50 (2)	6·88
Effect of grass	5·89 ns	5·24		6·09 ns	6·29	
DRY WEIGHT (g)	Grass −	Grass +	Effect of fertiliser	Grass −	Grass +	Effect of fertiliser
Fertiliser −	1·73 (13)	1·67 (7)	1·71 ns	0·26 (12)	0·13 (6)	0·21 ns
Fertiliser +	2·56 (5)	2·24 (3)	2·44	0·21 (2)	0·31 (2)	0·26
Effect of grass	1·96 ns	1·86		0·25 ns	0·17	
NUMBER OF LEAVES	Grass −	Grass +	Effect of fertiliser	Grass −	Grass +	Effect of fertiliser
Fertiliser −	5·77 (13)	5·00 (7)	5·50 *	6·83 (12)	3·33 (6)	5·67 ns
Fertiliser +	9·80 (5)	9·67 (3)	9·75	4·00 (2)	6·50 (2)	5·25
Effect of grass	6·89 ns	6·40		6·43 *	4·13	
BIRTH RATE LEAVES/WEEK	Grass −	Grass +	Effect of fertiliser	Grass −	Grass +	Effect of fertiliser
Fertiliser −	0·57 (13)	0·23 (7)	0·45 *	0·56 (12)	0·29 (6)	0·47 *
Fertiliser +	0·76 (5)	0·83 (3)	0·79	0·95 (2)	0·54 (2)	0·74
Effect of grass	0·62 ns	0·41		0·61 *	0·35	

Table 14. A summary of the mean effect (± 95% confidence limits) of: (a) the presence of grass neighbours and (b) fertiliser application to ramets of *R. repens* on their growth and on the growth of untreated ramets located proximally along the same stolon. Significant effects, shown in bold type, were calculated using Student's *t* from the data in Tables 8 & 13.

(a)The Effect of Grass

VARIABLE:	Parent Rosette	First Ramet	Ramets 2 - 7* (treated ramets)
Internode length (cm)	-0.67 ±1.4	0.11 ±1.6	-0.03 ±1.0
Dry weight (g)	-0.14 ±0.7	-0.08 ±0.1	**-1.84 ±0.4**
Number of leaves	-0.58 ±3.3	**-2.30 ±1.6**	**-6.02 ±1.5**
Leaf birth rate	-0.22 ±0.3	**-0.30 ±0.2**	**-0.49 ±0.1**

(b)Effect of Fertiliser

Internode length (cm)	0.40 ±1.5	0.85 ±1.8	0.22 ±0.9
Dry weight (g)	**0.74 ±0.7**	0.06 ±0.1	**1.86 ±0.4**
Number of leaves	**4.27 ±3.5**	0.03 ±2.0	**5.59 ±1.4**
Leaf birth rate	**0.34 ±0.3**	**0.33 ±0.3**	**0.41 ±0.1**

* the ramets are numbered from the parent rosette to the apex of the stolon (see Table 5).

Discussion

Neighbouring plants may interfere with each other's growth by the pre-emption of limited resources, such that less light, nutrients and space are available for growth. A rooted ramet of *Ranunculus repens* may have three different sorts of neighbours:

i) a plant of a different species

ii) a plant of the same species

iii) another rooted ramet on the same plant.

In the experiment reported here the presence of neighbours of different species (*Holcus lanatus* and *Lolium perenne*) and of the same species (i.e. *R. repens*) was associated with a general reduction in the growth of *R. repens* phytometers. Therefore, the presence of such neighbours might be expected to influence the performance of transplants in the field. However, no differential effect of grass neighbours compared with *Ranunculus* plants, or between the two species of grass, could be detected, possibly because of the small number of phytometers used in the experiment. The yield and survival of *Trifolium repens* phytometers has been shown to differ significantly in the presence of different grass species (Turkington & Harper, 1979b). Furthermore, seedling growth of *T. repens* is known to be affected by both the species and density of neighbouring plants (Clatworthy, 1960). *R. repens* has a similar stoloniferous growth habit to that of *T. repens* therefore, we might have expected that interactions with different species of neighbour would also influence growth of *R. repens*.

A single stolon developed on phytometers introduced into a population of *R. repens*, compared with a minimum of three stolons in the absence of neighbours. The concentration of stolon growth on a single axis was also recorded in *R. repens* plants growing at low nitrogen levels (Ginzo &

Lovell, 1973a). Rather than responding to adverse environments by keeping stolon number high and reducing stolon length, the plants reduce stolon number and maintain the growth of the remainder. This ensures lateral spread of a genet currently growing in an unfavourable environment. In the first experiment, stolon internodes were shorter in the presence of other *R. repens* plants. However, in the sequence pot experiment the internodes were remarkably constant; showing no significant variation when growing from ramets in different biotic and edaphic environments This indicates that the physical interference of neighbouring plants in the path of a stolon could be an important factor influencing the distribution of ramets along the stolon. The effect of neighbouring *R. repens* plants on both stolon number and internode lengths of *R. repens* phytometers illustrates the potential influence of intra-specific interactions on the growth form of plants in the field.

The growth of a ramet was affected by the environment of inter-connected ramets of the same plant. Ramets growing in relatively unfavourable sites e.g. in the presence of grass or in unfertilised soil, showed increased leaf production and dry weight when neighbouring ramets were receiving fertiliser. This strongly suggests that resources are translocated between individual ramets. Photosynthates labelled with radio-isotopes have been shown to be translocated in a predominantly acropetal direction, from the parent plant to the newly-formed ramets in *R. repens* (Ginzo & Lovell, 1973b) and in other clonal plants e.g. *Saxifraga sarmentosa* (Quereshi & Spanner, 1971) and *Carex arenaria* (Tietema, 1980). Resources supplied by a parent plant may support daughter ramets during their establishment, enabling colonisation across unfavourable sites and enhancing the ability of the clonal plant to spread laterally.

As the daughter ramets mature, assimilates may be translocated

basipetally, back to the parent plant e.g. in *Viola* species (Newell, 1980) and in spring barley (Anderson-Taylor & Marshall, 1983). This reciprocal transfer of photosynthates is not merely a reflection of the relative ages of the ramets but a complex response to the environmental conditions experienced by different parts of the genet. In *Saxifraga sarmentosa* long distance movement of caesium and photoassimilates in the phloem was reversed (from parent-offspring to offspring-parent) when the parent plant was completely shaded (Quereshi & Spanner, 1971). Similarly, in paired ramets of *Ambrosia psilostachya* both water and photosynthates were transported in the rhizome from a ramet growing in tap-water to its neighbour in a saline environment, irrespective of the ages of the ramets (Salzman & Parker, 1985). In a natural population, basipetal transfer of phosphorous was observed from daughter ramets of *Hieracium pilosella*, which had grown out into an open scree, back to their parent rosette which was shaded by neighbouring plants (Grindey, 1975).

The sequence pot experiment confirmed that individual ramets of *R. repens* are not physiologically independent but act as "integrated physiological units", IPU's, (Watson & Casper, 1984) within the clone. Within an IPU, or ramet of *R. repens*, the development of a meristem is dependent upon its local environment and this in turn determines the role of that meristem as a "source" or "sink" of resources. Actively-growing parts of the *R. repens* clone will act as sinks and draw resources from inter-connected ramets at proximal, and probably also distal, locations along a stolon. An indication of the overall effect of this integration of ramets is that total biomass and shoot growth of *R. repens* rosettes are reduced when stolons are severed (Clegg, 1978). *Solidago canadensis* ramets severed from their parent plant also showed a higher mortality, a decrease in growth rate, dry weight and flowering and produced fewer daughter ramets

than plants which remained connected to the parent (Hartnett & Bazzaz, 1983).

In a patchy environment the movement of resources within a clone will show a complex, changing pattern as new ramets grow into different areas and sample different micro-habitats. The translocation of resources within the clonal genet will buffer the effect of local environmental heterogeneity, as ramets in unfavourable sites are supported by ramets which are already established: conversely, ramets growing out into favourable sites may contribute resources to their neighbours. This physiological integration will reduce the strength of local intra-population selection and may limit the extent to which the clonal genet becomes specialised to its immediate environment (Hartnett & Bazzaz, 1983).

CHAPTER 4

Introduction to Plant Viruses

Introduction

The study of the variation between local populations requires the behaviour of a population to be described from the characteristics of its individuals. This focusses attention on the variability of individual plants both within and between populations. Much of this variation is traditionally attributed to the expression of genetic variation, or to environmentally-induced phenotypic differences between plants growing in different micro-environments. In a standard garden, or within a transplant site, environmental conditions are generally assumed to be uniform. Therefore, the variation between transplants in the same experimental plot is considered to be indicative of the genetic differentiation of plants previously growing in contrasting environments. The genetic diversity within the species is then interpreted in relation to the breeding system and the selective forces acting in different local populations. Differential pathogen infection could account for some of the intra-specific variation hitherto assumed to reflect genetic differences evolved in response to: micro-climatic variation (e.g. Aston & Bradshaw, 1966) ; soil heterogeneity (e.g. Snaydon & Bradshaw, 1961) ; different species of neighbour (e.g. Turkington & Harper, 1979b) and contrasting management regimes (e.g. Warwick & Briggs, 1980b). Yet, ecologists have generally disregarded the possible role of pathogens in influencing the performance of individual plants and, ultimately, as a selective force in the differentiation of plant populations.

Plant Pathogens

The plant represents a series of niches which may be exploited by a variety of predators and parasites which, in occupying a specialised niche cause malfunction or disease of the plant. The infectious nature of disease distinguishes it from other functional disorders of plants induced by climate, toxic agents and nutritional or genetic factors. Transmission of diseases and infection occur whenever the pathogenic agent encounters a vacant, susceptible niche. The spread of the pathogen is generally passive, involving a carrier or vector e.g. wind, water or animals. This contrasts with the "searching" and selective activity of predators.

A pathogen may induce malfunction or disease of the host plant by:

i) sequestration of host nutrients

ii) disruption of the amount and activity of plant growth hormones

iii) secretion of toxins

iv) production of enzymes which degrade host cell walls.

Many of the diseases which infect established plants in natural populations are not lethal. The parasitic pathogen is dependent upon a living host for its own growth but an infected individual will, by definition, be affected to some degree by the pathogen. In a natural ecosystem any detrimental effect will be exaggerated in competition with adjacent healthy individuals. Organisms which can be pathogenic to plants include: fungi; bacteria; algae; viruses and nematodes. Viral pathogens are frequently systemic and once infected a plant will remain infected throughout the life of the genet. Clonal organisms, in which ramet production increases the longevity of the genet, might be expected to accumulate viral infections.

The role of viruses in the population biology of two clonal species, *Ranunculus repens* and *Primula vulgaris*, has been investigated.

Plant Viruses

Definition

Plant viruses were first recognised as distinct from other kinds of infectious disease at the end of the nineteenth century. In 1892, Iwanowski showed that sap from a diseased plant was still infective after it had passed through a filter which would retain bacterial pathogens. The original criterion of a virus was, an infectious entity capable of passing through a filter small enough to hold back known cellular agents of disease. This definition is now known to include infectious agents with cells of less than 1μm which are not viruses; these are the mycoplasma-like organisms (MLO) and rickettsia-like organisms (RLO).

Mycoplasma-like organisms are surrounded by a triple-layered unit membrane approximately 10nm thick. They vary in shape from rounded mycoplasmas, with a diameter of 100-1000 nm, to the elongated helical spiroplasmas, which can be up to 12um in length. Mycoplasma-like organisms, detected by electron microscopy, are frequently found in the phloem sieve-tubes of infected plants. In contrast, rickettsia-like organisms have a definite cell wall 20-25nm thick and can occur in either the xylem or phloem vessels of infected plants (Maramorosch, 1974).

The definition of a virus has been refined as techniques of molecular biology have developed. Gibbs and Harrison (1976) define viruses as "transmissible parasites whose nucleic acid genome is less than 3x10 daltons in weight and that need ribosomes and other components of their host cells for multiplication". This excludes the MLO and RLO which contain all the genetic information and biochemical components to exist as independent cells. Furthermore, the symptoms of disease caused by MLO (yellowing; stunting; proliferation of axillary shoots; virescence i.e. green flowers and phyllody, the conversion of petals to leafy structures)

and the wilting of young shoots associated with RLO, are suppressed by tetracycline and non-tetracycline antibiotics respectively. Viruses are not sensitive to any antibiotics.

Biochemistry

Viruses are often described as "small packages of genetic information enclosed in a protein coat". The nucleic acid of plant viruses is typically single-stranded RNA, though rice-dwarf and related viruses have double-stranded RNA and a few e.g. cauliflower mosaic virus contain DNA. The total genome is usually in one molecule of nucleic acid consisting of from two to ten genes. Plant viruses range in size from approximately 20nm to 300nm. They vary in shape between elongate rigid or flexuous rods, isometric particles and the more complex, bacilliform rhabdoviruses.

Infection

Viruses are inactive outside their host cell and the outer cell wall of plants is impermeable to them. Entry of viruses into the cell, inoculation, normally takes place through wounds or breaks in the cellulose cell wall, often caused by the feeding action of insects or nematodes. On entering the host cell the virus disassembles into its constituent macromolecules. If the cell is susceptible, virus nucleic acids are replicated and translated into viral proteins by the host's ribosome system; infection is then said to have occurred. The replicated viruses move from cell-to-cell within the plant via plasmodesmata and throughout the host's vascular system.

Disease

The normal metabolism of the host cells is disrupted and visible abnormalities or symptoms of infection may develop. Infection can occur

without the production of overt symptoms and is said to be latent. Most of the host's physiological processes can be affected by virus infection. These effects may be deleterious to the host resulting in disease, though not all viruses are pathogenic.

Transmission

Plant viruses are associated with other elements of the environment in addition to their host plant. These include the plant-feeding insects and nematodes which act as vectors and carry viruses from infected to healthy plants. In this way transmission of viruses to new hosts occurs. A consequence of the complex interactions between these components of the ecosystem is that the effect of viruses must be considered, not only at the cellular and individual plant level, but also within the community.

A Review of the Effects of Virus Infection of Plants

The majority of the work on the effects of viruses on their plant hosts has been carried out at agricultural institutions. Agriculturalists are primarily concerned with whole plants and crop communities therefore, this review focusses on changes occurring at this scale.

1. Symptoms of virus infection

The most obvious effects of viruses on plants are the gross pathological changes which alter the appearance of the plant to produce recognisable symptoms of infection. Many of these processes relate to measurable cytological aberrations. 7 major deviations are symptomatic of virus disease in plants (Bos, 1964).

i) The size and number of cells may be increased, or decreased, resulting in malformation of organs.

ii) Gross plant size may be reduced.

iii) The colour of leaves, stems, flowers, fruits and seeds may be altered. Disruption of chlorophyll production frequently causes chlorosis of leaves. This can be localised e.g. in veins or at leaf margins. Light green (chlorophyll deficient) and dark green areas of leaf tissue may form mosaics or vertical streaks, reflecting the pattern of development of dicotyledonous and monocotyledonous hosts respectively. Other pigments may also be affected; increased carotene production causing a yellowing of plant tissue, and anthocyanin, a general reddening.

iv) The uptake and movement of water within the plant may be affected, resulting in water deficiency and wilting.

v) Necrosis of leaves, stems and fruits reflects the local death of infected cells. Necrotic lesions are often localised at the points of entry of viruses into the plant.

vi) Cork formation can be initiated as a response to viral infection.

vi) Individual cells may appear normal but can proliferate abnormally in tissues lacking cellular organisation, leading to the formation of tumorous growths. Alternatively, organisation may be normal but excessive growth of specific cells can occur e.g. in the upper layers of leaves, causing leaf-rolling or curling. The number of cells in the leaf lamina may be reduced, producing fan leaves or "shoe-stringing".

Symptoms vary considerably with different viruses, host species and environmental conditions. The biochemical background of many of the pathological processes underlying symptom expression is complex and not fully understood.

2. Virus effects on plant growth

The most economically important and therefore the best documented effect of virus infection is the reduction of growth of the host plant. Decreased growth results in a decline in yield. This may be reflected as the reduced size and/or number of storage organs. For example, plants infected with shallot virus yellows yielded, on average, 54.5g of bulbs, compared with 91.9g in healthy shallots (Henderson, 1953). Similarly, potato leaf roll virus causes a significant reduction in the number and mean weight of potato tubers (Killick, 1979). A reduction in total organic matter and digestibility of perennial ryegrass is associated with ryegrass mosaic virus infection (Holmes, 1979).

Alternatively, a change in growth form of infected plants may be important. Ryegrass plants infected with cocksfoot streak virus show a decrease in tiller number but increased size of tillers (Catherall, 1966). Infection of ryegrass with ryegrass mosaic virus is most detrimental when conditions for growth are optimal, with necrosis and reduction in yield more evident in high nitrogen plots than in a low nitrogen treatment (Holmes, 1980). It has been suggested that fertiliser should not be applied to diseased grass swards (A'Brook & Heard, 1975).

Growth of roots may also be affected by virus infection: alfalfa mosaic virus has a detrimental effect on root development of alfalfa cuttings (Frosheiser, 1977).

3. Effects of viruses on reproduction

In grain crops and in the flower industry the effect of viruses on flower and seed production is of more direct concern than growth inhibition. In horticulture, viruses such as chrysanthemum aspermy and lily symptomless virus affect flower size and colour, greatly diminishing

the value of the crop (Lawson, 1981). Virus infection may also delay flowering, as in red clover infected with red clover necrotic mosaic virus (Bowen & Plumb, 1979). Conversely, infected mother plants may flower earlier than healthy plants. Glasshouse-grown *Nicotiana* and *Chenopodium* plants infected with arabis mosaic (AMV) or spinach latent virus(SLV) tended to produce flowers before healthy plants. The infected *Nicotiana* bore significantly more mature capsules at an initial harvest but after a second harvest the total number of capsules obtained from healthy and infected plants was not significantly different. Infection with AMV and SLV also caused an increase in seed abortion with a corresponding reduction in the number of normal seed produced. In standard conditions, there was no consistent difference in the rate or percentage germination of seed from infected and healthy plants but in an accelerated ageing test the percentage germination of seed from SLV-infected plants declined more rapidly than that of seed from healthy stock (Walkey *et al.*, 1985).

Many viruses affect both production and germination of seed. Seed set from fertilised ovules of barley was reduced by 10% in plants infected with barley stripe mosaic virus (Slack *et al.*, 1975). The male gametes were also affected, with less pollen produced on infected anthers and a reduction in the proportion of male pollen grains. The percentage germination of seed was consistently lower when gametes came from infected parents. In agricultural systems this detrimental effect of infection is recorded as a decline in crop yield. Barley yellow dwarf virus induced a reduction in grain yield of 44% in wheat, 66% in oats and almost 100% in barley rendering it virtually sterile (Potter, 1982).

The time taken for germination may increase with infection, e.g. in dandelions infected with tomato ringspot virus (Mountain *et al.*, 1983).

4. Effects of viruses on plant survival

Virus-infected plants may have lowered survivorship compared with uninfected plants. Almost all infected clover plants were dead after one year in an experimental plot whereas, few healthy plants had died (Barnett & Gibson, 1977). Similarly, 19% of clover plants infected with red clover necrotic mosaic virus died during an experiment in which no healthy plants died (Bowen & Plumb, 1979).

5. Physiological changes in virus-infected plants

The processes underlying the above-mentioned effects of virus infection are poorly understood. Many studies have focussed on the decline of specific components of yield, rather than on the whole plant. The physiological effects most commonly associated with virus-diseased plants are: decreased rate of photosynthesis; increased respiration rate; change in growth hormone activity; increased activity of polyphenoloxidase and the accumulation of soluble nitrogen compounds (Diener, 1963). We know something of the biochemical changes which accompany the development of virus infection but understand little about the mechanisms of their initiation at the molecular level (Matthews, 1981).

6. The impact of viruses on the role of the plant in the community

(a) Modification of herbivore and vector behaviour

The status of the host as a food plant may be altered. *Kennedya rubicunda* seedlings inoculated with Kennedya yellows mosaic virus survived longer than seedlings inoculated with water. Loss of seedlings was mainly due to grazing therefore, the virus-infected plants may have been less attractive and/or less palatable to the herbivore. In palatability trials,

food containing powdered leaves from infected plants was consumed at a significantly lower rate (11.7% eaten) than food to which virus-free material was added (29.3% eaten), indicating that some decrease in palatability was associated with virus infection (Gibbs, 1980).

The behaviour of virus vectors can be modified when feeding on infected plants. *Trichodorus allius*, the nematode vector of tobacco rattle virus, was shown to feed preferentially and have a higher rate of reproduction on infected tobacco plants compared with healthy plants (Ayala & Allen, 1966). Similarly, peach trees infected with peach mosaic virus were more suitable to their mite vectors than healthy trees (Wilson *et al.*, 1955). However, significantly fewer mite vectors were recorded on ryegrass infected with ryegrass mosaic (Gibson, 1976). The high numbers of cereal aphids observed on plants infected with barley yellow dwarf virus were thought to reflect the increased attractiveness of the chlorotic diseased leaves (Ajayi & Dewar, 1983a). Furthermore, both the overall rate of reproduction and the production of alate aphids were enhanced on infected plants.

(b) Effects on the plant as a host for symbionts and pathogens

Nodulation of soybeans was reduced by 81% when plants were infected with soybean mosaic virus (Tu *et al.*, 1970). Number, size and weight of nodules were all affected. Nodule formation on white clover infected with alfalfa mosaic, peanut stunt or clover yellow vein virus was also significantly lower than that of healthy clover plants (Gibson *et al.*, 1981). Thereby, reducing the plant's capacity for nitrogen fixation and perhaps accounting for some of the growth inhibition associated with virus infection.

Increased incidence of infection with fungal pathogens has frequently been recorded in virus-infected crop plants. The rolled leaves of potato

plants infected with potato leaf roll virus retain moisture longer than healthy leaves and provide a more favourable micro-climate for infection with *Phytophthora infestans* (Richardson & Doling, 1957). Virus-infected potato plants in the field are often heavily infested with this fungus.

(c) Complex interactions within pathogen-host-vector systems.

Plants at known foci of barley yellow dwarf virus in the cereal crop have been observed to be more heavily blackened by mould fungi than plants elsewhere. Aerial photography and laboratory and field investigations have resulted in the proposal of a cycle of events to account for this phenomenon (Ajayi & Dewar, 1983b).

Infection with barley yellow dwarf virus increases the concentration of nutrients, such as carbohydrates and free amino acids, in the host plant and sugary exudates may also be produced on infected leaves. These physiological changes will increase the nutrient value of infected plants as substrates for fungal growth.

The loss of chlorophyll induced in virus-infected plants results in a generalised yellowing of leaves as other photosynthetic pigments, carotene and xanthophyll, are not affected. This increases the attractiveness of the plant to aphids, which alight preferentially on colours near the red-yellow end of the spectrum after long periods of flight.

Infected plants support larger populations of aphids than do healthy plants. Consequently, virus-infected plants are subject to a greater degree of aphid feeding damage which, in turn, increases their vulnerability to attack by saprophytic fungi. The presence of honey-dew on aphid-infested plants may also favour fungal growth.

Thus, the physiological changes induced by virus infection directly enhance fungal growth. In addition, these changes indirectly promote

colonisation by fungi through their effect on increasing aphid infestation. The saprophytic fungus in turn affects its host. Photosynthesis is decreased as a result of the fungus blocking stomata and covering some of the photosynthetic area.

Almost all of the examples quoted in this review concern agricultural plants. However, viruses also infect uncultivated plants, with important consequences both in agriculture and in ecological research.

Occurrence of Viruses in Wild Plants

1. Viral Infection in Agricultural Ecosystems

Interest in the viral infection of weeds has grown since the realisation that they can act as a major source of infection for crop plants. Bos (1981) discusses the role of wild plants as "reservoir hosts", ensuring the maintenance of the virus when the host is absent from the field e.g. during the winter or in crop rotations. Inoculum can build up in the wild hosts and act as a source of infection when the wild crop is re-introduced. Crop viruses may also persist and disperse in the seed and pollen of wild plants. Many viruses of crop plants originated in the wild plants from which the crop species themselves have been developed. Introduction of a new crop to an agricultural region often results in devasting infection by viruses which are subsequently found to be indigenous to the native plants. Finally, plant breeders increasingly look to the wild progenitors of cultivated plants for sources of genetic resistance to virus infection.

2. Incidence of Infection in Natural Populations

Studies of virus infection in wild plants have been biassed towards weed species because of their proximity to crop plants. I have reviewed the literature concerning viruses in wild plants and produced an indexed system of cards recording: the original reference; the host species; and the virus, which is with Dr. Cooper at the N.E.R.C. Institute of Virology in Oxford. Less than half (98) of the 224 studies in my survey report infection in natural habitats. The majority of these papers resulted from chance detection of infection, apparent when symptoms were observed in diseased plants. Many of the remaining references concern studies restricted to a single specified virus, host species or genus. The study of MacClement & Richards (1956) remains the only systematic survey of viruses in a natural population. Thus, it is difficult to draw conclusions about the distribution of virus infection in wild plants. However, the incidence of infection in a natural population can be as high as 10% (MacClement & Richards, 1956) and in one genus, *Plantago*, a 64% infection was recorded in 9 study sites in England (Hammond, 1981). This evidence suggests that virus infection could be prevalent in plants sampled from and/or transplanted into natural vegetation.

3. Problems in the Detection of Infection

Virus infection in natural plant communities has attracted little attention because it is not directly of economic importance. Furthermore, infection in uncultivated ecosystems is often less evident than in agricultural ecosystems. Natural plant populations are variable, containing many species of differing genotypes and at various stages of development. Consequently, an infected individual is unlikely to be in

contact with other susceptible plants and epidemics are less likely to develop. When plants do die in the field it is often impossible to determine the cause of death. The death of a single infected plant may not even be noticed and its space soon occupied by the growth of new or adjacent healthy plants.

Infection of wild plants may often be symptomless. High selection pressure from viruses in natural habitats is likely to lead to the disappearance of susceptible host genotypes and very virulent virus strains will seldom persist for long. This was evident in Australia, where a highly virulent strain of the myxoma virus killed over 99.8% of its rabbit hosts when it was first introduced. After the first winter, when the numbers of both mosquito vectors and susceptible rabbits were much reduced, less virulent isolates of the virus were recovered from the wild. Within 7 years, the original highly virulent strain had disappeared and many strains, of varying virulence, caused the death of only 25% of their rabbit hosts (Fenner & Ratcliffe, 1965). The relationship between a pathogen and its host may be even more complex. An aggressive strain of *Ceratostomella ulmi* caused a severe epidemic of Dutch Elm disease in the Netherlands, Britain and the United States in the 1930's but has subsequently declined in the Netherlands, where the disease is present but at a very low level. In contrast, the aggressive strain of the pathogen has persisted in the United States, causing heavy losses of elm trees whereas, in Britain a decline in the severity of the disease has recently been reversed with the re-appearance of an aggressive fungal strain (Gibbs & Brasier, 1973).

Many of the metabolic changes in virus-infected plants are secondary effects of infection and non-specific to viral disease. Thus, symptoms which do arise often resemble senescence, mineral deficiencies or mechanical injury.

4. Consequences of Viral Infection in Ecological Experiments

If virus infection is common and important in wild plants it is unlikely that it will have been detected for the various reasons given. Yet, such infection would have important consequences in the interpretation of ecological phenomena.

i) Many viruses are transmissible by hand and the experimenter may unwittingly transmit infection from one plant to another in the course of cloning, transplanting and recording experimental data.

ii) Infection is usually systemic therefore, once infected, viruses will persist throughout the life of the plant and all its ramets.

iii) Infection may also be transmitted vertically, through pollen and seed derived from the parent plant, to infect the progeny.

iv) Although often symptomless, infection can affect virtually all aspects of growth and reproduction of the host plant. This in turn may influence the performance of neighbouring plants.

v) Virus infection may modify the status of the plant as a host for other pathogens, symbionts, and plant-feeding animals.

vi) The effects of virus infection may easily be confused with genetic variation. Thus, differential infection of plants, or seed, from different populations may be misinterpreted as genetic differentiation in response to contrasting local conditions. Similarly, variation within a clone, traditionally attributed to, and used as a measure of, different environmental conditions could reflect a differential incidence of virus infection in individual ramets

These factors indicate that viruses should be considered as an experimental variable in ecological experiments.

Methods to Detect Virus Infection in Plant Hosts

Virus infection of wild hosts cannot be reliably identified by visual inspection because infection is frequently symptomless or because the symptoms may not be recognised in a species that has not previously been studied. This latent infection may be detected by:

i) Biological assay i.e. mechanical transmission of the virus to selected indicator plants which are susceptible to many viruses and show symptoms of infection.

ii) Serological assay which is based on the reaction between viruses in the plant sap and specific antibodies from an immunised animal.

iii) Electron microscopy can be used to observe the products of a serological reaction or to directly diagnose the presence of virus particles in the plant sap.

Frequently all of the above techniques will be required to detect and identify plant viruses. Further biochemical procedures may be employed to characterise a new virus species or strain. Noordam (1973) provides a detailed description of the laboratory techniques used in plant virology.

1. Biological Assay

Sap from the plant to be tested is applied to the surface of an indicator plant in such a way that virus, if present, can enter the cells. Indicator plants are species chosen because they are: easy to culture; readily infected by mechanical inoculation; susceptible to many viruses and react promptly to display characteristic symptoms when infected. A number of species are used internationally as indicator plants. These include many species of *Nicotiana*, *Chenopodium album*, *Chenopodium quinoa*, *Cucumis sativa* and *Phaseolus vulgaris*. Four or five indicator plants of different

species may be used to assay viruses in sap from each plant to be tested. This increases the range of viruses and the probability of their detection.

Method of Mechanical Inoculation

The upper surface of two or three leaves of each indicator plant is dusted with fine Carborundum powder. This abrades the leaf epidermis, increasing the points of entry for the virus. 2-3g of leaf from the plant to be tested are ground with a minimum quantity of water in a sterilised mortar. Nicotine is sometimes added as it inactivates inhibitors of virus transmission naturally present in plant sap. However, some species e.g. *Phaseolus vulgaris* are sensitive to nicotine and can suffer leaf damage. A clean finger moistened with the sap is rubbed gently over the leaves previously dusted with Carborundum. Finally, the leaves are rinsed with water to prevent the harmful effects of sap drying on the surface.

The inoculated plants are covered with newspaper overnight and kept in an insect-proof glasshouse, where they are observed for up to 4 weeks for symptoms of virus infection. Plants from which inoculum produces symptoms in one or more indicator plants are recorded as infected. Symptoms shown depend upon: the species of indicator plant; the virus or viruses; host species; environmental conditions and time since inoculation. Typical symptoms which may be seen on the inoculated leaf are necrotic lesions, necrotic ringspots and chlorotic lesions. Systemic symptoms may occur on leaves above the inoculated leaf, these include: necrotic spots; vein necrosis; vein-clearing; mosaic mottling; chlorosis; leaf distortion and stunting.

This method can be used to assay plants for infection with sap-transmissible viruses. However, some plant viruses are not readily transmissible mechanically and insect or nematode vectors, grafting

techniques or dodder may be necessary to facilitate experimental virus transmission.

Inoculation using Aphid and Nematode Vectors

Virus-free vectors may be cultivated in the laboratory and then introduced to the test plant for a period of time. Subsequently, the vectors are transferred to indicator plants to allow transmission of viruses acquired from the test plant. Young, very susceptible indicator plants are necessary to detect infection because only a low dose of virus will be present in the vector.

Inoculation by Grafting

The cut surfaces of different plants are held together until a union is established. To assay a plant for infection it must be grafted onto a healthy but susceptible partner, enabling viruses, if present, to cross the graft union and cause symptoms of infection in the previously healthy partner. Virus may not be transmitted if it is only partially systemic in the host or if the graft union is poor.

Inoculation with Dodder

Cuscuta species are now routinely used to transmit viruses between plants whose tissues are incompatible and fail to unite when grafted. Dodders, e.g. *Cuscuta campestris* are parasitic plants with root-like haustoria which connect with the vascular bundles of their host. In experimental transmission of viruses, dodder is allowed to establish on the plant to be tested and then trained onto a healthy indicator plant. The dodder forms a bridge by which viruses present in the test plant can be introduced into the indicator.

2. Serological Assay Techniques

Plant viruses introduced into vertebrate animals act as antigens, inducing an immune response. This is detectable as the production of antibodies in the lymphatic tissue, which then circulate in the blood of the immunised animal. These antibodies are highly specific and only combine with proteins of a similar structure. Serological assays in plant virology are based on the reaction between virus antigens in the plant sap and known specific antibody from an immunised animal. The detection of infection depends upon the observation of the product of the immunological reaction *in vitro*.

(a) Ouchterlony Gel-diffusion Test

This serological test depends upon the formation of a visible precipitate when an antigen-antibody reaction takes place in an agar gel. Antiserum i.e. serum containing antibodies, is prepared by injecting rabbits with a purified isolate af a specific virus. The occurrence of a reaction between viral antigens in plant sap and antiserum containing specific antibody is used to identify the plant virus.

Method

An agar gel is made by pouring a 1% agarose solution into a petri-dish. The agar is allowed to set. A gel-cutter is then used to make small (4mm diameter) holes, or wells, in the gel. Usually 7 holes are cut, 6 surrounding a central well, with 4mm spacing between wells.

Sap is extracted from the leaves to be tested by grinding them in phosphate buffer in a sterilised mortar. The sap is then squeezed through muslin and a sample placed in the central well of the gel. Alternatively, a purified extract of virus isolated from a test plant may be used.

Antibodies from the antisera and viral antigens from the infected sap diffuse into the agar. 10-20 hours after filling the wells the gels are examined. A cloudy precipitate can be seen in the gel where an antigen meets and combines with its specific antibody. The location of such a precipitin line identifies the presence in the sap of a virus serologically related to the antibody it has reacted with (Figure 1).

This gel-diffusion test is frequently used to identify a virus. More complex tests, with two or more similar viral antigens or mixtures of antibodies, can also be used. The range of precipitin reactions observed e.g. fusion of precipitin lines and spur formation, can indicate the degree of serological similarity between different viruses or virus strains.

Viruses longer than 650nm cannot be detected in the gel test as they are too large to diffuse through the agar.

(b) Enzyme-linked Immunosorbent Assay

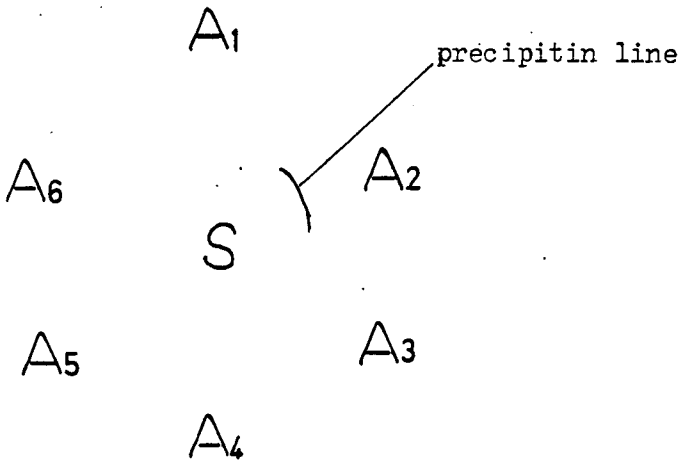
This serological technique is invaluable for the qualitative and quantitative detection of a known pathogen in crude sap or isolates. The product of the immunological reaction is readily observable *in vitro* by the activity of an enzyme to which it is bound.

Comparison of ELISA with classical immuno-precipitation methods:

i) In an immunosorbent assay specific antibodies are adsorbed to a solid phase which then selectively traps the antigen. This enables detection of the pathogen at much lower concentrations.

ii) In an ELISA, the antibody is labelled with an enzyme which, in reaction with its substrate, gives a coloured hydrolysate. The presence of the antigen-antibody complex is detectable visually as the colour change of the substrate. This is more sensitive than immuno-precipitation which

Figure 1. Diagrammatic representation of a precipitin reaction used to identify a virus in plant sap (Ouchterlony gel-diffusion test).



A_1-A_6 wells containing antisera to different viruses, for example: 1 - raspberry ringspot; 2 - strawberry latent ringspot; 3 - broad bean mosaic; 4 - arabis mosaic; 5 - alfalfa mosaic and 6 - cucumber mosaic. S - well containing sap from plant infected with strawberry latent ringspot virus.

Figure 2. Diagram of an ELISA plate used to screen a number of samples of plant sap for arabis mosaic virus (AMV).

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	1	2	3	4	5	6	7	8	9	10	V
B												
C	H	11	12	13	14	15	16	17	18	19	20	B
D												
E	V	21	22	23	24	25	26	27	28	29	30	H
F												
G	H	31	32	33	34	35	36	37	38	39	40	B
H												

B - buffer, H - healthy sap, V - sap from known AMV-infected plant. 1 - 40 are samples of sap from plants to be tested, samples in rows A,C,E and G are duplicated in rows B,D,F and H respectively.

requires a considerable degree of precipitation before the product is visible.

iii) Quantitative assessment of the antigen is possible by measuring the colour intensity of the hydrolysed substrate.

iv) ELISA is able to detect antigens of varied size and morphology.

v) The technique of ELISA is suitable for rapidly processing large numbers of samples.

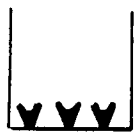
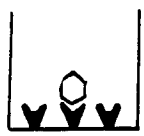
A number of enzyme immunoassay techniques are available to plant virologists (see van Regenmortel, 1982) and many are also used in clinical pathology and immunology. ELISA is widely used in routine indexing of virus infection because of its high sensitivity and suitability for large-scale testing of samples. For plant viruses the double-antibody sandwich form of ELISA has been found to be most suitable.

Double-antibody Sandwich (DAS) ELISA, Method

The reaction is carried out in polystyrene microtitre plates approximately 13x8.5cm, each containing 96 wells in a rectangular grid pattern. A typical scheme for screening forty test samples for a specified virus e.g. arabis mosaic virus (AMV) is shown in Figure 2. The laboratory procedure is presented in schematic form in Figure 3.

A Dynatech Micro-elisa plate reader may be used to determine the colour intensity of the substrate, measured as absorbance at 405nm. The colour in well 1A , containing buffer, (Figure 2) is used as a blank or standard. Infected samples are those with an absorbance of 2-3 times that of healthy samples. Alternatively, the upper negative (healthy) limit may be calculated as the mean of the healthy values plus 2 or 3 standard deviations. In practice, infected samples are frequently assessed by eye.

Figure 3. Schematic representation of DAS ELISA, for formulae and preparation of reagents see Clark & Adams, 1977.

PRINCIPLE		LABORATORY PROCEDURE
1. SPECIFIC ANTIBODY ADSORBED TO PLATE		200µl purified ɣ globulin in coating buffer added to each well. Incubate at 4°C overnight. Wash plates 4 times in PBS tween.
2. ADD TEST SAMPLE CONTAINING VIRUS		200 µl aliquots of test sample (sap) added to duplicate wells. Incubate at 4°C overnight. Wash 4 times in PBS tween.
3. ADD ENZYME-LABELLED SPECIFIC ANTIBODY		200µl aliquots of enzyme-labelled ɣ globulin added to each well. Incubate at 4°C overnight. Wash 4 times in PBS tween.
4. ADD ENZYME SUBSTRATE		250µl aliquots of freshly prepared substrate added to each well. Leave at room temperature for 60-90 minutes or until colour develops. Add 50µl 3M NaOH to each well to stop reaction. Assess results: i) visually ii) measure absorbance at 450nm.

CHAPTER 5

The Incidence and Effects of Plant Viruses in Natural Populations

The Incidence of Virus Infection in Natural Populations of *Primula vulgaris* and *Ranunculus repens*.

Primula vulgaris and *Ranunculus repens* were selected for a study of local specialisation between and within plant populations. Both species have a similar breeding system, being insect pollinated and predominantly cross-fertile with less than 10% selfing. Primroses and buttercups are also capable of clonal growth but this occurs naturally to a far greater extent in the buttercup, in which many ramets are spread laterally over a few square metres, than in the primrose, which typically grows a short branched rhizome with a single rosette of leaves. Genets of these two species will therefore, sample their environment in different ways.

For each species, a reciprocal transplant experiment was carried out in which the relative performance of genets transplanted from different populations was used as an estimate of their genetic differentiation (see Chapter 2). It is important to establish how far the differences between clones are genetic in origin, and those within a clone a response to different environments, and how far this apparent genetic and environmental variation may have been confused with the effects of differential pathogen infection. Therefore, the incidence of virus infection in *Primula vulgaris* and *Ranunculus repens* growing in the study populations was investigated.

Evidence from the Literature

Primula vulgaris

In garden-grown and wild primroses, infection with cucumber mosaic virus has been reported to cause curling and distortion of leaves (Smith, 1955). Primroses and many other herbaceous species were found to be

infected with "hardy primrose virus" but many infected plants showed no symptoms of virus infection (Uschdraweit & Valentin, 1959).

Virus infection has more often been recorded in cultivated *Primulas*. There are many references to mosaic diseases of *P. obconica* (e.g. Severin & Tompkins, 1950), including a mosaic caused by a new strain of alfalfa mosaic virus (Singh & Nagaich, 1976). In 1950, tobacco necrosis virus was so prevalent in cultivated *Primulas* that the U.S. Department of Agriculture prohibited their importation from Australia and the British Isles (U.S.D.A., 1950). Mokra (1964), in a review of virus diseases of *Primula* species, cites many instances of infection with: cucumber mosaic; tobacco necrosis and tomato spotted wilt viruses. Abnormal flower pigmentation and colour-breaking in ornamental primroses has been attributed to an unknown virus isolated from the flowers of diseased *P. obconica* plants (Lisa & Lovisolo, 1976) and to tomato black ring virus (Morand & Poutier, 1978).

Green or foliaceous petals in primroses (e.g. French, 1891 and Bowles, 1918), was formerly thought to be a symptom of aster yellows virus (Mokra, 1964), but are now known to be caused by a mycoplasma, which has been reported in *P. denticulata* and *P. variabilis* growing in nurseries (Stevens & Spurdon, 1972).

Ranunculus repens

Latent infection of *R. repens* with arabis mosaic virus has been reported from West-Europe and with cucumber mosaic virus from South- and East-Europe (Schmidt, 1977). Rhabdovirus-like particles have been observed in *Ranunculus* leaves using the electron microscope but biochemical characterisation and identification of the virus was not carried out (Amici, Faoro & Tornaghi, 1978).

In contrast, there are many reports of infection in ornamental

Ranunculus species. Rod-shaped virus particles have been observed in sap from *R. asiaticus* plants showing mosaic symptoms (Smith, 1955 and Raabe & Gold, 1957). The incidence and insect vectors of "*Ranunculus* mottle virus" have been studied in *R. asiaticus* in California (Laird & Dickson, 1967) and the characteristics of a virus also causing mosaic mottling of *R. asiaticus* have been described in India (Padma, Singh & Verma, 1972). Anemone mosaic (Hollings, 1957) and tobacco necrosis; tobacco rattle; cucumber mosaic and *Ranunculus* breaking viruses (Devergne *et al.*, 1969) have all been isolated from cultivated *Ranunculus asiaticus*.

There are few reports of virus infection in natural populations of either *Primula vulgaris* or *Ranunculus repens* but many virus diseases have been recognised in ornamental species of both genera. It seems likely therefore, that the paucity of data for the wild plants reflects a lack of interest in primroses and buttercups, rather than an inherently low incidence of infection.

A Survey of Virus Infection in Natural Populations of *Primula vulgaris* and *Ranunculus repens*

A preliminary survey of the incidence of virus infection in populations of *Primula vulgaris* and *Ranunculus repens* in North Wales was carried out in June 1982. Plants from five populations of each species were assayed for infection. The populations were the same as those used in the reciprocal transplant experiment (Chapter 2).

Procedure

Native plants i.e. not transplants, were collected from each of the 10 study sites. The plants were sampled systematically over the entire study area in each site, at approximately 2 metre intervals to avoid repeatedly sampling the same genet. Consequently, the number of plants taken from each population varied according to the density of the primroses or buttercups and the area of the site.

The plants were dug up carefully and placed individually in clean, labelled polythene bags to avoid mechanical transmission of viruses during transit. Each plant was then potted in John Innes No.1 compost in a 5-inch pot and placed in a heated glasshouse in Bangor.

After 1 week, 1 or 2 leaves were removed from every plant. Each leaf was handled with, and placed directly into, a clean and appropriately labelled polythene bag. These samples were taken to Oxford and tested for the presence of viruses using the biological assay method. All leaves were stored in a refrigerator at 4°C for up to 6 days prior to testing.

Results

None of the plants of *Ranunculus repens* tested was found to be infected with a sap-transmissible virus (Table 1). 17 of the 88 primroses were shown to be infected. This represents an infection level of 19% over all sites but within sites the incidence of infection varied from 0% at Rhoscefnhir to over 43% at Traeth Bychan.

Discussion and Conclusions

Hollings (1966) in assessing the potential of various species as indicator plants reported that, "most *Ranunculus* species tested were highly immune or highly resistant to the majority of viruses tested." This suggests that *Ranunculus* sap may contain inhibitors of virus transmission.

Table 1. The incidence of virus infection in natural populations of *Primula vulgaris* and *Ranunculus repens* in North Wales, as identified in a biological assay.

SPECIES	STUDY SITE	NUMBER OF PLANTS: INFECTED/TESTED	% INFECTION
Primula vulgaris			
	Penmon	1 / 8	12.5%
	Plas Gwyn	1 / 16	6%
	Rhoscefnhir	0 / 16	0%
	Traeth Bychan	13 / 30	43%
	Vaynol	2 / 18	11%
	Total	17 / 88	19%
Ranunculus repens			
	Treborth main lawn	0 / 19	0%
	Treborth new lawn	0 / 24	0%
	Henfaes	0 / 22	0%
	Cae Llyn	0 / 18	0%
	Cae Groes	0 / 17	0%
	Total	0 /100	0%

Protoanemonin is a volatile unsaturated lactone, which is liberated from the glucoside Ranunculin when *Ranunculus* tissues are bruised (Hill & van Heyningen, 1951). The presence of this chemical could affect transmission of viruses. A biological assay would fail to detect viruses which, though present, were not transmitted to the indicator plants. Serological techniques could not be used as they require prior knowledge or prediction of the viruses likely to occur and this information is not available for *Ranunculus*. A large scale survey using the electron microscope was not within the scope of this project. However, sap from 3 plants was examined using the transmission electron microscope but with negative results. Viruses in *Ranunculus repens* were not investigated further.

Further studies were carried out with *Primula vulgaris* to attempt to answer the following questions:

1. Which virus (or viruses) is responsible for the observed infection?
2. How is the virus transmitted and what are the vectors?
3. What is the distribution and incidence of infection in primroses and other selected species at Traeth Bychan and at other sites?
4. How does infection affect the vegetative growth and reproduction of the plant: (a) in the glasshouse and (b) in its natural environment?

Specific questions relating to the transplant experiment can also be asked:

5. Is infection transmitted to healthy transplants in a site containing infected plants?
6. Do transplants from different sites differ significantly in their susceptibility to infection?

1. Identification of Viruses Infecting *Primula vulgaris.*

Procedure

The Ouchterlony gel-diffusion test was used to determine the identity of the viruses infecting primroses. Viral antigens were obtained from frozen-dried leaves of indicator plants infected in the initial bioassay. Antisera to the following viruses were used:

i) strawberry latent ringspot virus (SLRV)

ii) raspberry ringspot virus (RRV)

iii) alfalfa mosaic virus (LMV)

iv) arabis mosaic virus (AMV)

v) cucumber mosaic virus (CMV)

vi) tomato black ring virus (TBRV)/ arabis mosaic virus (AMV) - a mixture of antisera was used as no antiserum containing antibodies to TBRV alone was available.

Results

None of the plants tested contained antigens to alfalfa mosaic virus, cucumber mosaic virus or raspberry ringspot virus. All but one of the infected primroses tested had transmitted arabis mosaic virus to indicator plants in the bioassay (Table 2).

Table 2. The identity of viruses infecting primrose plants.

POPULATION:	Number of plants Tested	Number of plants infected with: AMV	AMV/TBRV	SLRV
Traeth Bychan	7	7	7	2
Vaynol	2	1	1	0

8 of the 9 primroses tested were apparently infected with arabis mosaic virus. These plants may also have been infected with tomato black ring virus. The antiserum for TBRV contained antibodies to both TBRV and AMV therefore, the presence of TBRV antigens in plants infected with AMV cannot be excluded.

2 primroses were also infected with strawberry latent ringspot virus. Virus infecting one of the primroses from Vaynol showed no precipitin reaction with any of the antisera tested. Subsequent examination of sap from this plant in the electron microscope failed to reveal any distinct virus particles and so it was not possible to identify the infectious agent in this plant.

Conclusion

Arabis mosaic virus was the most prevalent virus infecting primroses at Traeth Bychan. Subsequent investigations concentrated on this virus. No further characterisation of the viruses infecting primroses was carried out.

Characteristics of Arabis Mosaic Virus

Arabis mosaic virus (AMV) occurs naturally throughout Europe, in many species of wild and cultivated monocotyledons and dicotyledons. It has a single-stranded RNA genome and is a nepo-virus i.e. nematode-transmitted polyhedral virus. The virus particles are isometric and approximately 30nm in diameter. *Xiphinema diversicaudatum* is the nematode vector. Arabis mosaic virus is also transmitted in seed, probably through both the pollen and ovule (Lister & Murant, 1967). Transmission occurs when virus particles invade the floral meristem at an early stage of development, before the callose wall has been formed (Carroll, 1981). From 1.2 to 100%

of seedling progeny may be infected (Mandahar, 1981) but transmission is thought to be relatively infrequent in the field (Taylor & Thomas, 1968 and Hanada & Harrison, 1977). Competition from healthy pollen and high seedling death rates may reduce the numbers of infected progeny which survive in field populations.

Using mechanical inoculation the virus is readily sap-transmissible to infect virtually all commonly used indicator species. Antiserum is easily obtained for use in gel-diffusion and other sero-diagnostic tests.

Transmission of AMV by nematode vectors

Xiphinema are large nematodes, 2-12mm long as adults. *Xiphinema diversicaudatum* feeds almost exclusively at the tips of plant roots. The hollow odontostyle, or spear, is used to pierce the root and extract the cell contents. Virus from the sap of infected plants becomes associated with the cuticle of the alimentary tract in the nematode. Subsequently, when the nematode feeds on an uninfected plant the virus is transmitted in secretions during the feeding process. *Xiphinema diversicaudatum* is estimated to move, on average, 0.5 metres per year (Harrison & Winslow, 1961) This slow movement and the patchy distribution of nematode vectors is typically reflected in an uneven distribution of infected host plants within an area. The systemic movement of the virus in plant root systems and dissemination of virus-infected seeds also contribute to the spread and pattern of distribution of nepo-virus infection (McNamara, 1980).

2. Transmission of AMV to *P. vulgaris* at Traeth Bychan.

i) The presence of nematode vectors in the soil

Soil from Traeth Bychan was tested for the presence of nematode vectors. This site slopes steeply, at an angle of about 65°. Soil samples

were taken from equally-spaced locations from the foot to the top of the slope. 3 samples were taken and pooled from each of 3 locations. The soil was stored in polythene bags at 5°C prior to extraction. Nematodes were extracted using the sifting and decanting method.

Procedure

The surface and top 5cm of soil was discarded as most of the roots on which nematodes feed are below this depth. 200g sub-samples of soil were soaked for 30 minutes in 1-2 litres of aerated tap-water. This water was then poured through a coarse sieve leaving much of the silt. The debris retained in the sieve was discarded, removing roots and other plant material. The nematodes were extracted by sifting and decanting the water through a series of progressively finer sieves: 250µm; 140µm and 75µm mesh sizes; the finest sieve catching the smallest larval stages.

Material retained in the sieves was pooled, put onto paper tissue and placed over water in a Baermann funnel. After 15-24 hours at 22°C nematodes collecting in the water in the stem of the funnel were drained off. The water containing the nematodes was examined under a dissecting microscope to enable identification of the species present.

Results

Species of both *Xiphinema* and *Longidorus* were present and were identified by Dr. J.I.Cooper. Many smaller nematodes, less than 1mm in length, were also extracted but these were not identified. *Xiphinema diversicaudatum* was present at mean concentrations of: 4; 12 and 10 nematodes in successive 200g samples of soil from the foot to the top of the site, an overall mean of 8.7 nematodes/200g of soil (see Table 14).

ii) Transmission of AMV in soil from Traeth Bychan

Soil transmission of the virus was investigated by planting "bait plants" in soil taken from Traeth Bychan. The bait plants were healthy seedlings of *Chenopodium quinoa* and *Chenopodium amaranticolor*. Two plants of each species were planted in each of two pots of soil from the site. The nematodes previously extracted from soil from Traeth Bychan were also added. After one month the bait plants showed symptoms of systemic infection. Sap from these plants was assayed using the gel-diffusion test and shown to contain AMV.

Conclusions

Xiphinema diversicaudatum, a nematode known to acquire and transmit AMV, was present in the soil at Traeth Bychan. AMV was transmitted to healthy plants growing in soil from this site. This evidence indicates that *Xiphinema diversicaudatum* is the probable vector transmitting AMV in Traeth Bychan.

3. Arabis Mosaic Virus in Natural Populations

Having established the presence of AMV in natural populations of *Primula vulgaris*, the technique of double-antibody sandwich ELISA can be used to assay many plants for infection with this specific virus. This technique has been used to:

i) determine the incidence of AMV in all primroses at Traeth Bychan

ii) ascertain the distribution of AMV infection in other species at Traeth Bychan

iii) survey *Primula* species from other geographical locations for AMV infection.

(i) The incidence of AMV in *Primula vulgaris* at Traeth Bychan

The site at Traeth Bychan is part of a cliff on the east coast of Anglesey and slopes steeply from the shore, rising 5m in 6m. Primroses, other dicotyledonous species and associated grasses grow mainly on horizontal ledges and less steeply sloping ground between vertical outcrops of limestone rock.

In April 1983, all remaining native primrose plants in Traeth Bychan were labelled by placing a numbered metal peg in the ground beside each plant. 43 plants were located and their positions recorded on a map, together with the location of the transplants (Figure 5). Leaf samples were collected from each of the 43 native plants and placed in clean, labelled polythene bags. The double-antibody sandwich ELISA was used as described in Chapter 4 to assay each sample for the presence of AMV and other viruses.

Results

9 of the 43 plants assayed were infected with AMV, a 21% level of infection (Table 3).

Conclusion

7 of the 30 primroses from this site tested in the initial bioassay were shown, using the gel-diffusion test, to be infected with AMV. The remaining 6 infected plants were subsequently assayed using ELISA and found to contain AMV.

A total of 73 primrose plants from Traeth Bychan has been assayed for viruses. 22 of these 73 plants were infected with AMV, representing approximately 30% incidence of infection in naturally-occurring primroses at this site.

Table 3. Virus infection in native plants at Traeth Bychan.

SPECIES	NUMBER OF PLANTS TESTED	PLANTS TESTED WITH ANTIBODY TO: AMV	AMV/TBRV	SLRV	RRV
DICOTYLEDONS:					
Centaurea nigra	2	-	-	-	-
Cochlearia officinalis	2	-	-	-	-
Filipendula ulmaria	11	-	-	-	-
Fragaria vesca	9	-	-	-	-
Geranium robertianum	3	-	-	-	-
Hedera helix	9	1	1	-	-
Plantago lanceolata	5	-	-	-	-
Primula vulgaris	43	9	9	-	-
Rubus fruticosus	7	-	-	-	-
Sanguisorba minor	1	-	-	-	-
Silene dioica	4	-	-	-	-
Viola riviniana	8	-	-	-	-
MONOCOTYLEDONS:					
Brachypodium sylvaticum	19	-	-	-	-
Dactylis glomerata	16	-	-	-	-
Festuca ovina	7	-	-	-	-
Carex spp.	8	-	-	-	-
FILICOPSIDA:					
Phyllitis scolopendrium	4	-	-	-	-

- denotes a negative reaction in all plants tested with a specific antibody, where a number is given this indicates the number of plants which gave a positive reaction i.e. virus was present in the sap.

ii) The incidence of virus infection in plant species other than primroses at Traeth Bychan.

16 species, representative of the flora at Traeth Bychan were identified and individual plants of each species selected at random throughout the site. The number of plants of each species sampled varied from 1 to 19 reflecting the relative abundance of different species in the site. Leaf samples were taken from each plant and placed in a clean, labelled polythene bag. The samples were assayed for the presence of viruses using ELISA.

Results

AMV infection was detected in three species, *Centaurea nigra, Hedera helix* and *Sanguisorba minor* (Table 3). None of the plants tested was infected with TBRV alone, SLRV or RRV. The antiserum for TBRV also contained AMV antibodies therefore, the possibility that the plants infected with AMV are also infected with TBRV cannot be excluded.

Conclusion

Arabis mosaic virus is present in species other than *Primula vulgaris* at Traeth Bychan. A more comprehensive survey is necessary to enable the incidence of infection to be reliably estimated.

iii) A survey of virus infection in *Primula* species

(a) In Experimental Material

A number of different species of *Primula* in cultivation at Newcastle University were assayed for the presence of viruses using ELISA. The species tested were: *Primula denticulata; P. secundiflora; P. florindae; P. sikkimensis; P. helodoxa; P. verticillata; P. floribunda; P. polyneura; P.*

frondosa; P.chionantha; P. rosea; P. geranifolia; P. anisodora and P. alpicola. None of these species was found to be infected with arabis mosaic virus. However, these results cannot be considered to be indicative of a low incidence (or absence) of AMV in *Primulas* because:

(i) the plants had been cultivated from seed

(ii) only one individual of each species was tested.

(b) In Natural Populations

Primula plants, principally *Primula vulgaris*, from four different geographical regions of England were assayed for infection with AMV using ELISA. 1 or 2 leaves were sampled from a number of different plants, chosen at random within each site. Data from sites in North Wales are also represented for comparison.

Results and Conclusion

AMV occurs frequently in natural populations of *Primulas*; infection was detected in 7 of the 14 sites sampled in this survey (Table 4). The incidence of infection was typically between 5% and 20%. None of the leaf samples tested showed symptoms of viral infection, indicating that many wild plants may harbour such latent infection.

4. The Effect of Viral Infection on Growth and Reproduction of the Host

(a) In the Glasshouse

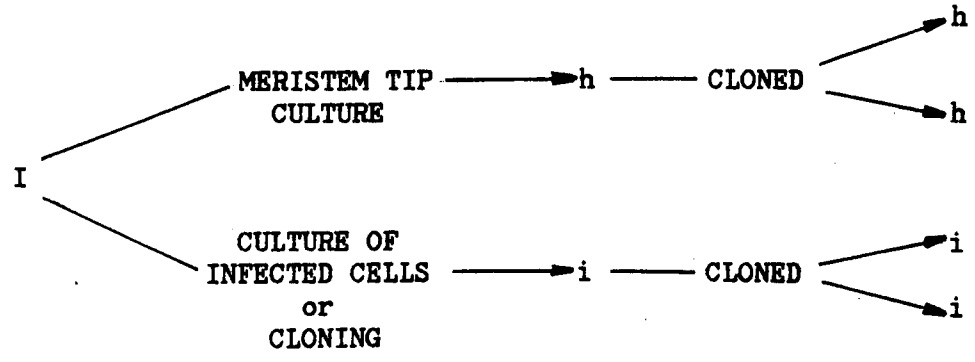
The use of a clonal species enables the comparison between healthy and infected plants to be made within a single genotype. Two different experimental procedures may be followed.

Table 4. AMV infection of *Primula* plants in England and Wales, all plants tested were *Primula vulgaris* except the sample marked with * which were *Primula veris.*

LOCALITY	NUMBER OF PLANTS: TESTED	INFECTED	% INFECTION
Derbyshire			
Lathkill Dale	25	3	8%
Great Rocks Dale	*20	0	0%
Lincolnshire			
East Kirkby "A"	43	0	0%
East Kirkby "B"	23	2	8%
Oxfordshire			
Magdalen College "A"	22	0	0%
Magdalen College "B"	10	0	0%
Sussex			
Site "A"	40	0	0%
Site "B"	40	0	0%
North Wales			
Penmon	20	3	15%
Penrhyn Castle	6	1	17%
Plas Gwyn	22	1	4.5%
Rhoscefnhir	20	0	0
Traeth Bychan	79	26	33%
Vaynol	20	2	10%

Meristem Tip Culture

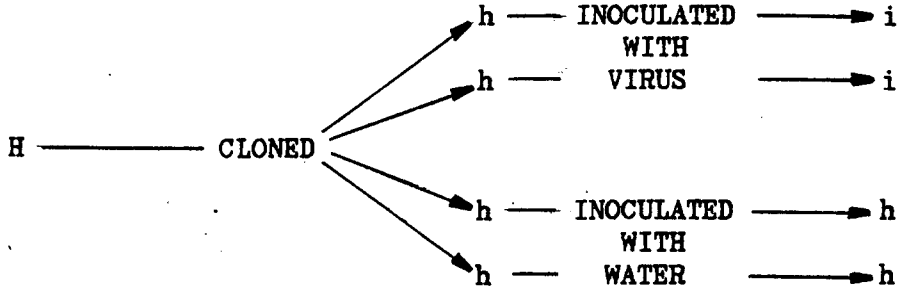
Apical meristems of systemically infected plants may contain little or no virus. Small numbers of cells can be excised from this region and grown in sterile conditions to form virus-free plants. This technique is widely used in horticulture for obtaining virus-free stocks of vegetatively propagated plants and can be used experimentally to produce both healthy (h) and infected (i) ramets from a single infected genet (I).



However, cell cultures may become contaminated or grow very slowly and, particularly with monocotyledons, the growing cells may lack organisation and form callus tissue rather than a whole plant.

Mechanical Inoculation

An alternative procedure is to infect a number of ramets (i) from a healthy genet (H) by mechanical inoculation with a suspension of a known virus and to inoculate an equivalent number of ramets of the same genet with water as a control (h).



This second procedure was adopted in an attempt to infect primrose clones with AMV and assess the effect of this virus on plant growth.

Procedure

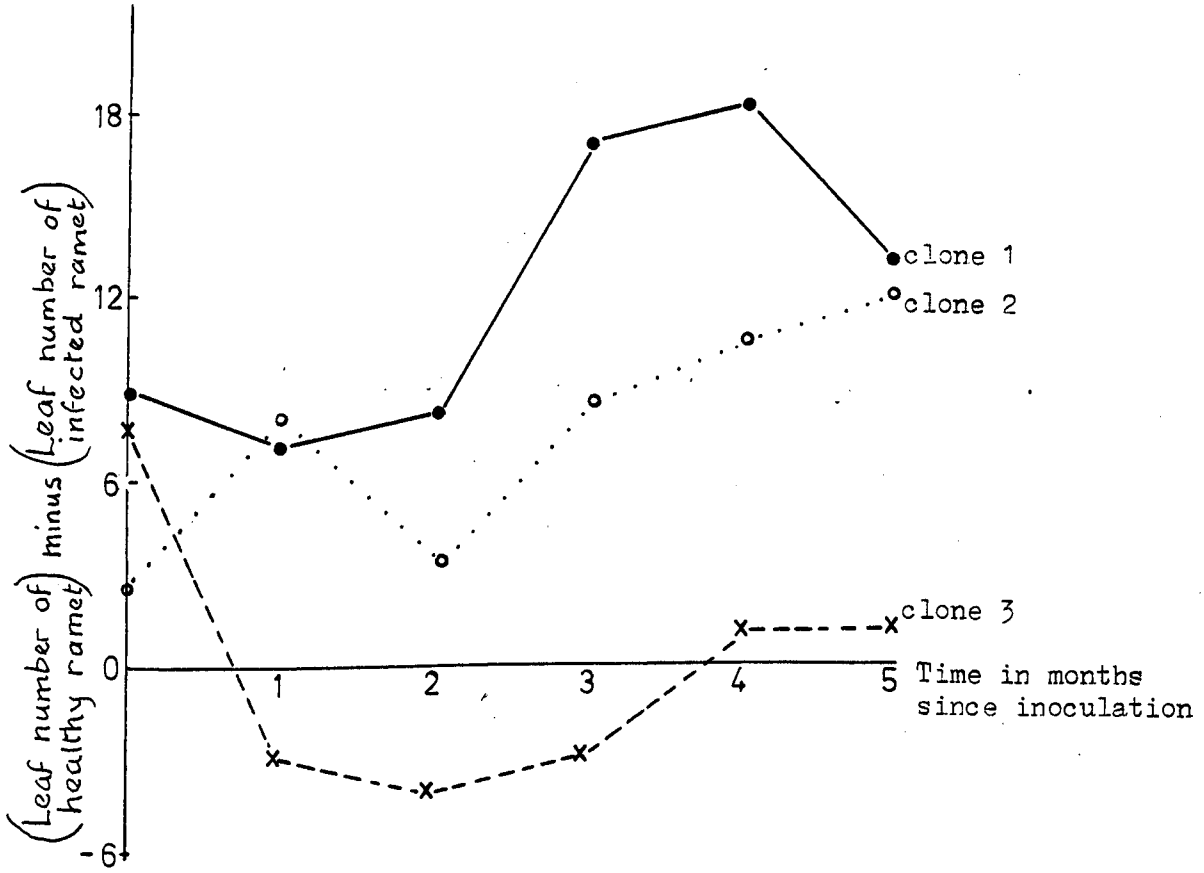
14 primrose plants, in which no virus had been detected in the initial bioassay, were cloned producing 2 ramets from each. The ramets were potted in John Innes No.1 compost in 4-inch pots. These 28 plants were tested for AMV using ELISA and none was shown to be infected.

One plant from each pair was inoculated with AMV using sap from leaves of *Chenopodium album* plants, previously infected with a known preparation of AMV. The remaining plants were inoculated in the same way but with water. All inoculated plants were kept in the glasshouses at Bangor and the numbers of leaves and flowers on each plant recorded at monthly intervals for 5 months. During this period the ELISA technique was again used to assay all plants for infection with AMV.

Results

Only 3 of the 14 clones inoculated became infected, therefore just 3 pairs of infected and uninfected ramets were available for comparison. For each of these three clones, the difference between the number of leaves borne on the healthy ramet and the number on the infected ramet is represented graphically (Figure 1). Statistical analysis was not carried out because of the small number of observations and the high variability of the data. Healthy plants tended to have more leaves than infected plants but there was considerable variation, both between the 3 genets and with time. Thus, infection had little effect on the number of leaves in clone 3, but in clone 2 the healthy ramet consistently bore more leaves than the infected ramet and this difference increased with time. The experiment was terminated during the winter months when a number of plants died.

Figure 1. The difference between the number of leaves on a healthy ramet and the number of leaves on an infected ramet of the same primrose genet for 3 different clones: ●——● clone 1; o····o clone 2; x- - -x clone 3.



Conclusion

Clearly no firm conclusions can be drawn from these data. A larger experiment, using 10 genets with 4 or 5 ramets of each, was planned. However, mechanical inoculation was again unsuccessful; only 1 of 24 inoculated ramets became infected. An experiment using more primroses and of longer duration is necessary to investigate the question of viral influence on host growth. A more successful method of inoculation should be employed. Two modifications which may increase virus transmission are:

(i) the use of a purified virus isolate rather than a crude sap preparation

(ii) the incorporation of a substance such as nicotine in the inoculum to inactivate inhibitors of virus transmission which may be present in primrose sap.

Alternatively, the technique of meristem tip culture may be used to produce virus-free plants from an infected genet.

(b) The Effect of Virus Infection on the Host in a Natural Population

In the initial bioassay, AMV was found to infect native primroses in 4 of the 5 populations used in a reciprocal transplant experiment (Table 1). Therefore, it was decided to inyestigate the incidence of AMV in the plants which had been sampled from, and transplanted back into, these populations. The results of this survey, together with the data collected in the transplant experiment, enabled the effect of virus infection on the performance of plants in a natural site to be assessed.

The incidence of AMV in the primrose transplants

In the reciprocal transplant experiment (Chapter 2) primroses had been sampled from each of 5 sites and cloned repeatedly to obtain a number of

ramets. 60 of these ramets were selected to represent each of the 5 populations, 300 plants in total. These 300 transplants were derived from 100 of the plants originally sampled, with between 1 and 5 ramets representing each genet.The 300 plants were transplanted back into the 5 sites such that every site contained 60 ramets, 12 from each population sampled (Table 5).

Table 5. The distribution of ramets in the transplant sites

	TRANSPLANT SITE:				
POPULATION:	P	PG	R	TB	V
Penmon (P)	12	12	12	12	12
Plas Gwyn (PG)	12	12	12	12	12
Rhoscefnhir (R)	12	12	12	12	12
Traeth Bychan (TB)	12	12	12	12	12
Vaynol (V)	12	12	12	12	12

Procedure

In August 1983 one leaf was sampled from every surviving transplant in all 5 sites. The ELISA technique was used to assay the samples for AMV infection. The infection of the parent genet was deduced from the incidence of infection of its daughter ramets. If all surviving ramets were infected, the plant from which they were cloned was said to be infected. Not all virus-infected plants would give all ramets infected; infection will vary according to the concentration and distribution of the virus in the original host plant. Consequently, genets were also classed as infected if more than half of the surviving ramets were infected. The plant sampled initially was classed as uninfected if at least half of its ramets were uninfected. Infected ramets from uninfected primrose genets

are assumed to have become infected in the site. This interpretation may have led to an over-estimate of infected genets and correspondingly, an under-estimate of the infection of ramets since transplanting.

Results

The incidence of AMV in the original plants sampled, as deduced in this experiment (Tables 6 & 7), is very similar to the results of the initial bioassay, which recorded infection with any sap-transmissible virus (Table 1). This suggests that AMV accounted for most, if not all, of the infection detected in the bioassay. Furthermore, the similarity of the two estimates indicates that they are representative of the true level of infection in this site.

The distribution of infected ramets throughout the sites (Table 8) is mainly due to the random allocation of ramets to sites during transplantation. However, the number of infected ramets from each site reflects the incidence of infection in that site. Thus, 29 ramets from Traeth Bychan were infected, compared with an average of only 8 ramets from each of the other 4 sites. This uneven distribution of infection precluded the use of the full set of data in comparing uninfected and infected plants. Consequently, it was decided to use only the plants from Traeth Bychan to assess the influence of infection on plant performance.

ii) The effect of AMV infection on the performance of transplants from Traeth Bychan

The transplant site and incidence of infection of all transplants from Traeth Bychan is shown in Table 9. It was not possible to determine at what time the two previously uninfected ramets had become infected therefore, they were excluded from the analysis. The performance of 27

Table 6. The distribution of AMV infection in genets with 1 or more infected ramets.

POPULATION	GENET	TRANSPLANT SITE: P	PG	R	TB	V	INFECTION OF GENET
Penmon (P)	2	+		+	+		I
	4	-	-		+	-	U
	7	+	+		+	+	I
	9	+				+	I
	12	+			+	-	I
	20		-		+	-	U
Plas Gwyn (PG)	2		-	-	+	-	U
	3			-	+		U
	6	+	+	+		+	I
	7	-			+	±	U
	11				+	-	U
Traeth Bychan (TB)	1	+	+	+	+		I
	5	+	+	+	+	+	I
	8			-	+	-	U
	10		+	+			I
	13	+			+	+	I
	14					+	I
	16	+					I
	17	-	-		+	-	U
	18		+	+	+	+	I
	19	+		+	+	+	I
	20	+	-		+	+	I
Vaynol (V)	4	-	+		-		U
	6	+			-	-	U
	7	-	-		+		U
	16			+	+	+	I
	18		-			+	U
	21	+	+	+			I

+ denotes a ramet infected with AMV; - a ramet not infected with AMV; where neither sign is used the genet was not represented by a surviving ramet; I denotes a genet estimated to have been infected with AMV in its native site, before transplanting and U a genet not infected with AMV.

Table 7. Summary of the numbers of infected genets sampled from different populations and used in the reciprocal transplant experiment.

POPULATION	NUMBER OF GENETS: TESTED	INFECTED	% INFECTION	INFECTION RECORDED IN INITIAL BIOASSAY
Penmon	20	4	20.0%	12.5%
Plas Gwyn	22	1	4.5%	6.0%
Rhoscefnhir	20	0	0	0
Traeth Bychan	18	9	50.0%	43.0%
Vaynol	20	2	10.0%	11.0%

Table 8. Summary of the numbers and distribution of infected ramets used in the reciprocal transplant experiment, as recorded in August 1983.

	TRANSPLANT SITE:					
POPULATION	P	PG	R	TB	V	TOTAL
Penmon (P)	4	1	1	5	2	13
Plas Gwyn (PG)	1	1	1	4	2	9
Rhoscefnhir (R)	0	0	0	0	0	0
Traeth Bychan (TB)	6	4	5	8	6	29
Vaynol (V)	2	2	2	2	2	10
TOTAL	13	8	9	19	12	61

Table 9. The distribution and incidence of AMV infection in ramets from Traeth Bychan, as deduced from the results of the ELISA (see table 6).

	NUMBER OF RAMETS IN TRANSPLANT SITE:					
	Penmon	Plas Gwyn	Rhoscefn-hir	Traeth Bychan	Vaynol	Total
NOT INFECTED	6	6	3	4	5	24
INFECTED - prior to transplanting	6	4	5	6	6	27
BECAME INFECTED - after transplanting	0	0	0	2	0	2
DEAD - transplant not assayed	0	2	4	0	1	7

infected and 24 uninfected ramets was compared statistically using data from the reciprocal transplant experiment.

The numbers of leaves and flowers (including buds and seed capsules) on each transplant were recorded at intervals from March 1982 to September 1983. The areas of leaves were estimated from their lengths and breadths in July and October 1982. The relationship between the linear dimensions of a primrose leaf and its area was calculated by sampling 100 leaves from native primrose plants in the study populations and recording their length, breadth and area (using an automatic leaf area meter). A regression of the product of leaf length and breadth with area showed a significant correlation, with leaf area equal to:

$$\text{AREA} = 1.323 + 0.492\ (\text{LENGTH} \times \text{BREADTH})$$

(see Chapter 2, Figure 6). The dry weight of leaves was calculated when the transplants were harvested, at the final recording in September 1983.

The data were analysed using a weighted 2-way ANOVA (Snedecor & Cochran, 1967 pp. 484-488) because of the unequal distribution of infected and uninfected ramets between transplant sites. Transformation of the variables was carried out where necessary.

Results

Growth of leaves

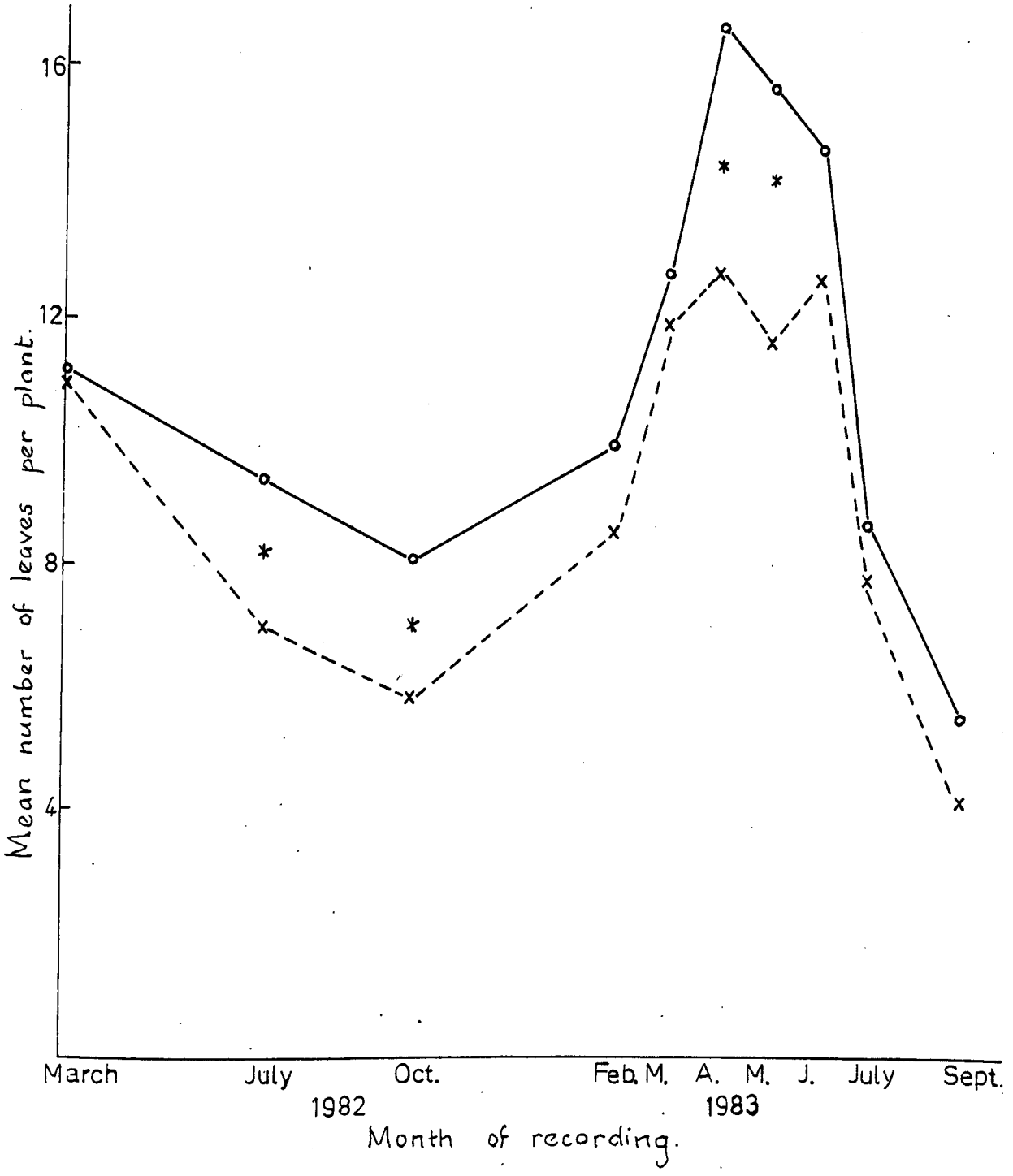
Primroses infected with AMV had consistently fewer leaves than uninfected plants (Table 10 and Figure 2). This difference was significant in July and October, 1982 and April and May, 1983, when infection reduced leaf number by 24 - 27%. There was a tendency for infected leaves to be larger than uninfected leaves, with a significant increase of 25% in mean leaf area in July 1982.

Table 10. The influence of AMV infection on the growth of primrose leaves. D is an estimate of the difference (uninfected - infected) plants, the % difference is the ratio (infected/uninfected) plants and LSD = the least significant difference at the 95% level. The significance of D is given by: ns - not significant and * $P<0{\cdot}05$.

VARIABLE	OVERALL MEAN	D	LSD	% DIFFERENCE
Number of leaves/plant				
1982: March	11·04	0·31 ns	2·42	97%
July	8·12	2·23 *	1·93	76%
October	6·92	2·19 *	1·69	73%
1983: February	9·21	1·40 ns	2·26	86%
March	12·24	0·51 ns	2·95	96%
April	14·69	3·97 *	3·71	76%
May	13·62	4·08 *	3·92	74%
June	13·54	2·20 ns	3·72	85%
July	7·82	1·64 ns	2·66	81%
September	4·77	1·39 ns	1·81	75%
Area of leaves(cm)/plant				
1982: July	105·70	3·83 ns	37·29	96%
October	72·96	16·17 ns	23·86	79%
Area (cm)/leaf				
1982: July	14·09	-4·43 *	4·08	125%
October	11·26	-1·77 ns	4·11	104%
Dry weight (mg) leaves/plant				
1983: September	*180·70	51·53 ns	176·06	133%
Dry weight (mg)/leaf				
1983: September	101·07	-11·76 ns	36·86	112%

* signifies a geometric mean.

Figure 2. Comparison of the number of leaves on infected and uninfected primrose transplants: o——o uninfected plants; x---x infected plants, * difference significant at the 95% level.



Flowering

The data from 1982 was excluded from the preliminary analysis because many plants did not flower. No significant difference in the number of flowers on infected and uninfected primroses was recorded in 1983 (Table 11). There appeared to be a tendency for infected plants to flower earlier in the season (Figure 3). This was also observed in 1982. Therefore, it was decided to calculate the proportion of plants flowering at each date, in both 1982 and 1983 (Figure 4). An arcsine transformation of the proportions was used and infected and uninfected plants compared using a split plot ANOVA.

In March 1982, the proportion of infected primroses in flower was significantly higher (at the 5% level) than the proportion of flowering uninfected plants (Table 12). This same trend was observed in 1983, particularly at the beginning of the season, but was not statistically significant. The data for March 1982 were collected immediately after transplanting and therefore, are likely to reflect the production of flowers initiated in the glasshouse.

Conclusion

The number of leaves borne on primrose plants in a natural site was significantly reduced in plants infected with arabis mosaic virus. From these data it is not possible to assess the influence of the virus on the birth and death rates of individual leaves. The difference between the number of leaves on infected and uninfected primroses during the period of active growth in March and April 1983 suggests a relatively lower leaf birth rate in infected plants. Demographic data recording the fate of individual marked leaves would be necessary to confirm this.

Table 11. The influence of AMV infection on the number of flowers produced by primrose transplants.

VARIABLE:	OVERALL MEAN	"D"	LSD	% DIFFERENCE
Number of flowers/plant				
1983: March	3.97	-0.61 ns	2.14	117%
April	8.38	-1.58 ns	3.94	121%
May	12.47	-0.79 ns	4.92	106%
June	9.31	0.26 ns	4.43	97%
July	7.17	0.20 ns	3.66	97%

"D" is an estimate of the difference, uninfected - infected plants; LSD the least significant difference at the 95% level, ns denotes "D" is not significant; % difference is the ratio, infected/uninfected.

Table 12. The influence of AMV infection on the proportion of plants flowering at different dates.

VARIABLE	OVERALL MEAN	DIFFERENCE BETWEEN THE % UNINFECTED - INFECTED PLANTS IN FLOWER
% of plants in flower		
1982: March	32.1	-36.1 *
1983: February	34.4	-12.9 ns
March	65.7	-1.0 ns
May	85.5	-9.6 ns
July	47.9	-6.9 ns

The least significant difference with 95% confidence limits = 17.1 (calculated according to the method of Cochran & Cox, 1957 p. 298), * denotes a difference which is significant at this level, ns - difference is not significant.

Figure 3. Comparison of the number of flowers on infected and uninfected primrose transplants in 1983: o——o uninfected plants; x- - -x infected plants.

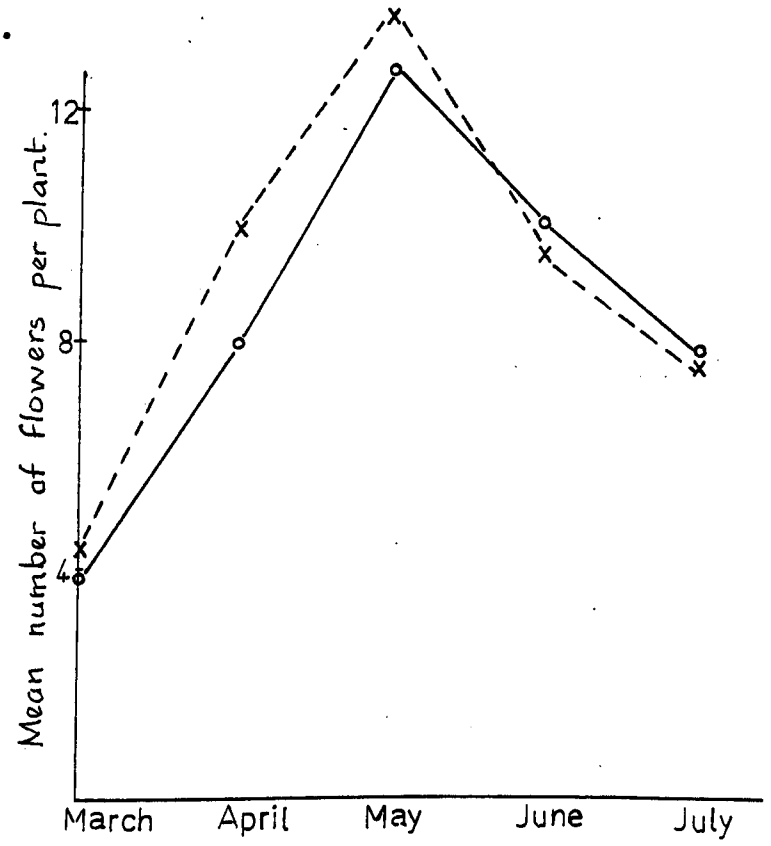
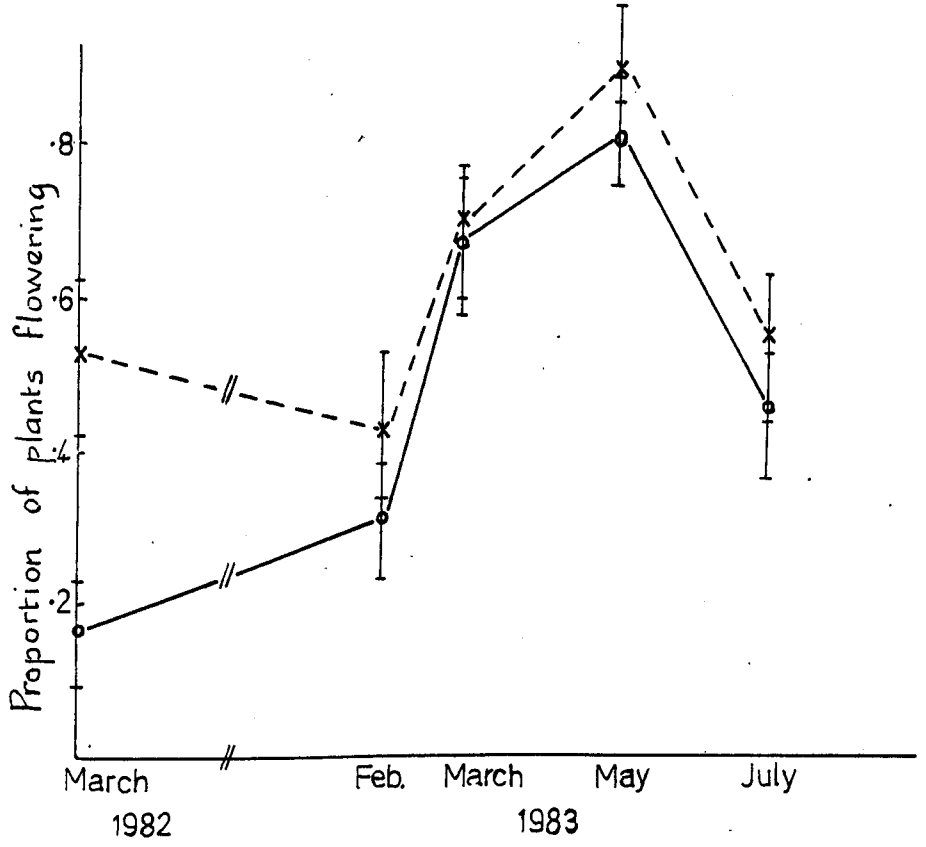


Figure 4. The proportion of infected and uninfected primroses flowering in 1982 and 1983: o——o uninfected plants; x- - -x infected plants ± 1S.E.



In July and October 1982, infected plants had significantly fewer leaves but showed no significant decrease in total leaf area compared with that of uninfected plants, possibly because their individual leaves were larger. This may reflect a compensatory growth of individual leaves in plants with a reduced leaf number.

No conclusive evidence of an effect of viral infection on flowering emerged from this data. This may be due, in part, to the extreme variability of flower production and therefore, the high associated variance. There was a trend towards early flowering in infected plants but this trend was less evident in 1983 and may have been primarily a response to infection expressed in the glasshouse environment prior to transplanting. A third season's data would be necessary to establish whether early flowering is a response to viral infection which is also exhibited in a natural environment.

5. The Transmission of Infection to Healthy Transplants

A more detailed analysis of the data presented in Table 8 was possible because the identity and incidence of infection of the parental genet of each ramet was known.

13 out of a total of 204 uninfected ramets became infected in the transplant sites, an infection rate of over 6% (Table 13). Transmission of infection was greatest in Traeth Bychan where 18% of the transplants became infected, reflecting the high incidence of AMV in this site. A plan of the location of infected and uninfected plants in this site was constructed (Figure 5). This shows the patchy distribution of infection typical of a nematode-borne virus. Transmission of the virus occurred exclusively to ramets in the lower 2/3rds of the sloping site and this can be clearly seen

Table 13. The incidence of infection with AMV which occurred during the transplant experiment.

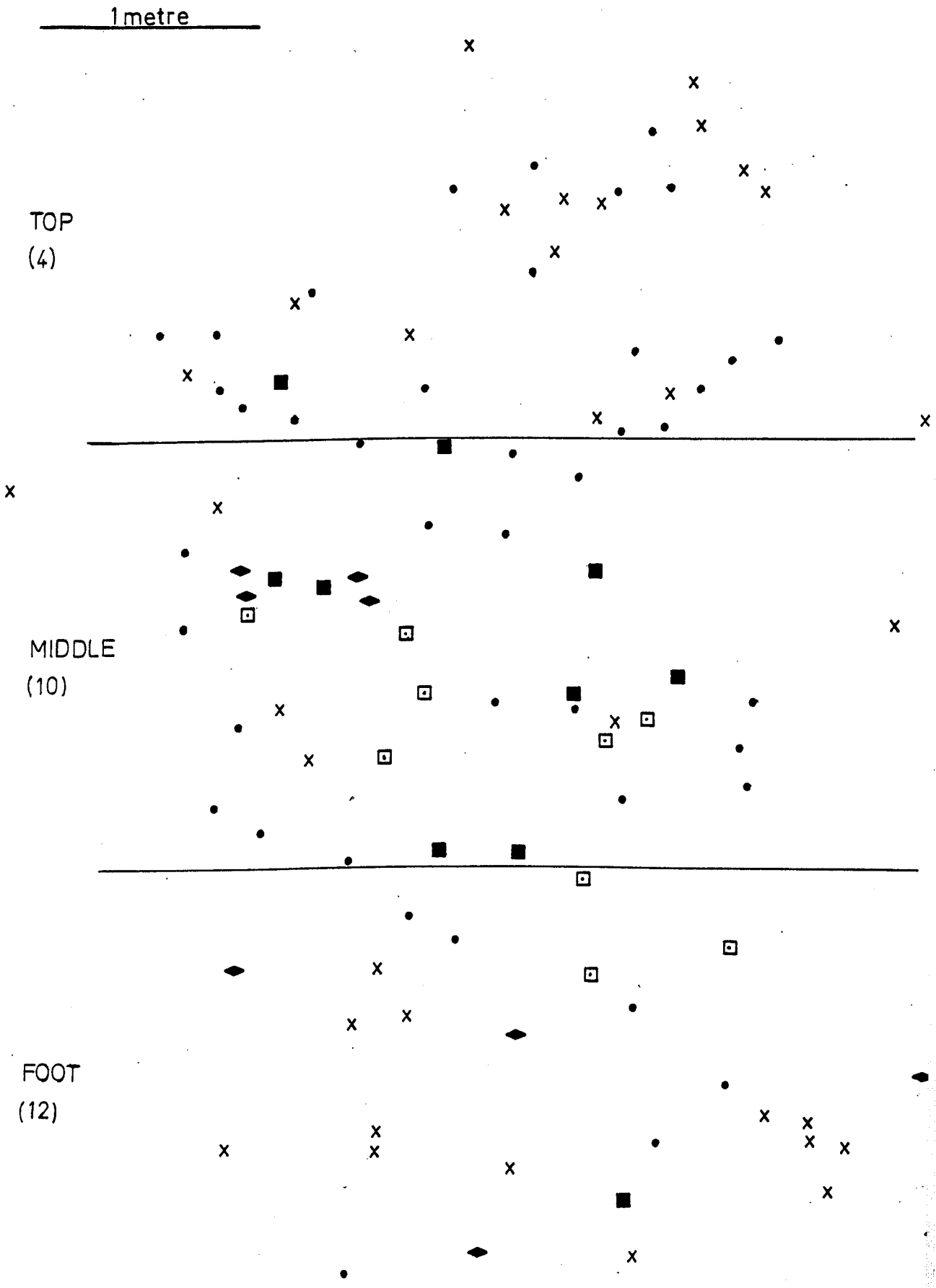
	TRANSPLANT SITE:					
POPULATION	P	PG	R	TB	V	Total
Penmon	0/ 8	0/ 8	0/ 1	2/ 9	0/ 9	2/ 35
Plas Gwyn	0/10	0/ 8	0/ 9	4/12	1/ 8	5/ 47
Rhoscefnhir	0/10	0/ 9	0/ 8	0/12	0/ 8	0/ 47
Traeth Bychan	0/ 6	0/ 6	0/ 3	2/ 6	0/ 5	2/ 26
Vaynol	1/11	1/ 9	0/ 7	1/11	1/11	4/ 49
Total	1/45	1/40	0/28	9/50	2/41	13/204

The denominator gives the number of ramets uninfected in March 1982 and the numerator, the number of these ramets which had become infected by August 1983. Of the remaining 96 transplants, 48 were already infected before transplanting and 48 ramets died before August 1983 and were not assayed.

Table 14. The distribution of *Xiphinema diversicaudatum* and AMV-infected plants in relation to the slope at Traeth Bychan.

REGION OF THE SLOPE	NUMBER OF nematodes per 200g soil	NUMBER (%) OF TRANSPLANTS: initially/total infected	becoming/uninfected infected	NATIVE PLANTS infected/ total
Top	4	11/20	0/19	0/15
		(5%)	(0%)	(0%)
Middle	10	8/30	6/22	4/10
		(27%)	(27%)	(40%)
Foot	12	1/10	3/9	5/18
		(10%)	(33%)	(28%)

Figure 5. The distribution of AMV infection in primrose transplants and native primroses at Traeth Bychan. Transplant: • uninfected; ■ infected; ⊡ became infected in the site and native plant: x uninfected; ◆ infected. ( ) number of nematodes/200g soil at different regions of the slope.



to be related to the distribution of *Xiphinema diversicaudatum* nematodes and infected native plants in the site (Table 14).

Conclusion

Transmission of viruses to healthy transplants can occur within a site containing infected plants. 6.4% of all infectible transplants became infected within 18 months of transplantation. In each site, the frequency of transmission reflected the incidence of infection in that site.

The pattern of distribution of infection in one site, Traeth Bychan, was consistent with transmission of the virus by a nematode vector; infection of both native plants and transplants being concentrated towards the foot of the slope, in the region where the highest concentrations of nematode vectors were found. The number of nematodes tends to increase in soil near the base of a slope as a result of their passive movement in water draining downwards.

6. Do Transplants from Different Sites Differ in their Susceptibility to Infection?

Infection of transplants was a rare event, 6.4% in 18 months over all sites. Furthermore, the infectible ramets from the different sites were distributed unequally amongst the transplant sites. These 2 factors prevented statistical analysis of the data in Table 13. However, it is interesting to note that none of the ramets from Rhoscefnhir became infected whereas, 2 - 5 ramets from each of the other sites were infected. In addition, this site was unique in that none of the ramets transplanted here contracted infection. These observations suggest that:

(i) primroses from Rhoscefnhir are, relatively, less susceptible to AMV than primroses from the other 4 sites or

(ii) AMV is absent from, or present only at a very low level in, plants naturally occurring in this site.

These hypotheses are in accordance with the results of the earlier surveys, in which a total of 36 primrose plants from this site were assayed for AMV with negative results. A formal test of the first hypothesis would require exposing uninfected primroses from Rhoscefnhir and other sites to AMV. Either mechanical inoculation with a known concentration of virus, or the addition of viruliferous nematodes to sterile soil in which the plants were growing, could be used. The latter method is less precise but more representative of the natural situation. The resultant infection of primroses from Rhoscefnhir and elsewhere could then be compared.

To investigate the second hypothesis, the absence of AMV in Rhoscefnhir, 3 approaches could be adopted:

i) the assay of large numbers of plants for AMV

ii) uninfected bait plants could be placed in the site and subsequently tested for infection

iii) establishment of the presence or absence of nematodes in the soil and their potential as vectors.

Any one, or all three, of these experimental procedures could be used in Rhoscefnhir and in the other sites to obtain a comparative estimate of the incidence of AMV.

Discussion

Plant pathogens are, by definition, infectious biotic agents which cause malfunctions in plant performance recognised as disease. The role of the pathogen in the plant community is determined by this detrimental effect. The influence of pathogens in the ecology of plants can be divided into two main categories. Firstly, the pathogen has an immediate effect on the phenotype of the infected individual. All aspects of vegetative growth and reproduction may be affected. Continued exposure to a pathogen will ultimately result in the decreased fitness of genotypes which are susceptible to infection. Secondly, and as a consequence, the pathogen also functions as a selective factor in the environment, influencing the genetic and spatial variability of the host species.

1. The Influence of Pathogen Infection on Host Phenotype

Detection of Infection in Wild Plants

Ecological experiments frequently involve the comparison of plants subject to different experimental treatments and/or from contrasting environments. The incidence of pathogens in these plants is largely ignored. Infection with viral pathogens, particularly in natural populations, is often latent. Consequently, the presence of viruses cannot be reliably identified by eye and infection may be undetected. In my study of *Primula vulgaris* biological and serological techniques revealed infection in 13% of plants assayed yet, no symptoms were apparent in any of the 458 plants tested. Similarly, Hammond (1981) recorded a 64% incidence of infection in *Plantago* plants, the majority of which were symptomless.

Infection with fungal and bacterial pathogens generally produces more

reliable and characteristic symptoms than virus infection. In addition, the hyphae and fruiting bodies of fungal pathogens may be visible on the leaf surface of the host plant. These more overt signs of disease make it less likely that fungal and bacterial infection will be overlooked. One exception is the root pathogenic fungi such as *Fusarium* species which cause rotting of the host's root cortex (Kommedahl & Windels, 1979). Above-ground symptoms of disease may be slight with only a small degree of stunting.

Effects of Infection in Wild Plants

The extent and complexity of the influence of infection on the host plant, even where infection is detected, is rarely recognised. The review of the effects of virus infection (in Chapter 4) illustrates the variety of ways in which a viral pathogen can affect its host in an agricultural ecosystem but few workers have attempted to quantify the effects of pathogens on wild hosts in a natural environment. I have demonstrated that *Primula vulgaris* plants naturally infected with arabis mosaic virus and growing in field sites had significantly fewer leaves than uninfected plants. In April and May 1983, infected primroses had an average of only 12 leaves, compared with 16 leaves in healthy plants, a reduction of 25%. Infection significantly increased leaf area by 4cm^2 (25%) in July 1982. A tendency for infected plants to flower earlier was noted but was not statistically significant.

In a field trial, infection of *Lotus corniculatus* with tobacco ringspot virus was associated with a significant decrease in many parameters of growth and reproduction (Ostazeski *et al.*, 1970). However, this experiment may have under-estimated the role of the pathogen in the natural environment, where the average inter-plant distance would be considerably

less than the 60cm used in the field trial. *Capsella bursa-pastoris* plants infected with *Albugo candida* and *Peronospora parasitica* were surveyed in their natural population to assess the impact of these fungal pathogens on the growth and reproduction of the host (Alexander & Burdon, seen in manuscript). Significantly fewer infected plants survived to maturity; those which did fruit produced significantly fewer seeds per fruit and their total stem length was less than that of healthy plants. Thus, the pathogen affected both the current size of its host population and the potential numbers of plants in the succeeding generation.

In a natural population, neighbouring uninfected plants are often able to take advantage of the incomplete utilisation of resources by an infected individual. The increased growth of the adjacent plants accentuates the reduction in vigour induced by the pathogen. Infection with cocksfoot streak virus caused a 30-40% decrease in tiller number of *Dactylis glomerata* plants. The total dry matter yield of infected plants in a sward was significantly lower than that of healthy plants but infected plants grown individually in pots showed no overall decrease in dry weight because of an increase in tiller size (Catherall & Griffiths, 1961). Conversely, tiller production of ryegrass was greater in plants infected with barley yellow dwarf virus and this resulted in an increased relative growth of the infected plants in a sward which also contained healthy plants (Catherall, 1966).

Many pathogens affect the reproduction of their host. The fungus, *Epichloe typhina*, prevents the emergence of the inflorescence in *Agrostis tenuis* and renders its host virtually sterile (Bradshaw, 1959b). Sterile infected plants showed increased vegetative growth, possibly reflecting the utilisation of resources otherwise employed in flower and seed production. Persistence of the infected genets was high but all of the ramets they

produced were infected with the systemic pathogen, therefore their potential for reproduction and dispersal of progeny was lost and fitness became zero. *Ustilago violaceae* is also a systemic fungal pathogen which infects all shoots and completely supresses seed production in its host. In natural populations of *Silene dioica*, the fungus differentially affects the male and female plants (Lee, 1981). Vegetative growth of male plants decreased significantly whereas flowering was unaffected. In contrast, the growth of female plants was not adversely affected by the pathogen, indeed a fourfold increase in flower number was recorded.

Consequences of Infection in Wild Plants

Ecologists traditionally explain the variation between plants in different experimental treatments in terms of the pre-selected treatment variables. Yet, a pathogen can, directly or indirectly, affect all the parameters of plant performance which ecologists measure. Furthermore, the effect of a pathogen varies between hosts in different environments and even between male and female plants within a population. Therefore, the variation apparently reflecting chosen experimental variables may have been brought about by a differential in the incidence and effect of pathogen infection between individual plants. When studying local specialisation, the dangers inherent in ignoring pathogen infection of experimental material lie in the assumption that all of the observed differences in performance between individual plants reflect genotypic variability. Clearly differential pathogen infection may account for some of the differences between plants hitherto attributed solely to genetic variation.

In electrophoretic studies, the genotype of a plant is characterised in terms of its isoenzymes. Yet, virus-induced cell death and the differential distribution of viruses in various organs and tissues can

directly affect the pattern of isoenzymes in the host plant (Matthews, 1981). Pathogen infection may also alter the developmental sequence of isoenzymes, causing the activation of new enzymes or of those enzyme systems normally associated with senescence (Gáborjányi, *et al.* 1973). Thus, the effects of differential pathogen infection of plants subjected to electrophoretic analysis may be wrongly interpreted as evidence of genetically-determined enzyme polymorphism.

Ecologists frequently study plants which are sampled from and/or transplanted into natural populations. Pathogen infection in wild plants often affects individuals or regions of tissue in which there are no overt symptoms of disease. Therefore, it is likely that some of the plants used in an ecological study will harbour undetected pathogen infection. Consequently, experimental results based on the performance and enzyme activity of these plants may be misinterpreted.

2. The Pathogen as a Selective Factor in the Environment

The occurrence of disease has, by definition, a detrimental effect on the growth and reproduction of affected plants. However, within a population individual plants vary in their susceptibility to pathogens. An individual may escape infection by spatial or temporal avoidance of a pathogen. Alternatively, or additionally, resistance to disease may result from the action of genetic resistance factors incorporated into the host genotype as an active evolutionary response to the pathogen. The possession of disease resistance confers a selective advantage on the resistant individual. At the population level, the evolution of resistance mechanisms affects the genetic composition and spatial distribution of the host species.

The Impact of Pathogens on Host Distribution

Avoidance of disease in space may exclude a host species from areas in which the growth of a pathogen is particularly favoured. This may affect the geographical distribution of the host. Thus, *Eucalyptus* species in South East Australia are restricted to the dry ridge top sites which are unfavourable to the pathogen *Phytophthora cinnamomi* (Burdon & Shattock, 1980).

The enhancement of pathogen activity at high host densities (Burdon & Chilvers, 1975) may influence the number and spatial distribution of host plants within a population. Wide dispersal of hosts and progeny will be favoured, as it increases the chance that some individuals will escape pathogen infection.

The Effect of Pathogens on Species Diversity, Inter- and Intra-specific Interactions

The limitations imposed by a pathogen on host growth and distribution lead to incomplete utilisation of resources, enabling colonisation by other species. There is little direct evidence for the role of pathogens in determining the diversity of plant communities. However, species diversity in North American forests has increased following the virtual elimination of the dominant, *Castanea sativa* by the fungal pathogen *Endothia parasitica* In the Appalachians, chestnuts have been replaced by the co-dominants: chestnut oak; red oak and red maple and secondarily by subordinate species such as tulip poplar (*Liriodendron tulipifera*) and hickories. Most of the rhododendron and mountain laurel plants are believed to date back to the time of the death of the chestnut trees (Day & Monk, 1974). Natural populations, in which many species are intermingled, typically have a considerably lower incidence of disease than the less diverse agricultural

systems. This comparison is further evidence of the interaction between the species diversity of a plant community and the incidence of pathogen infection.

Pathogen infection affects the growth of the host species relative to that of non-susceptible species in the population and thus influences the outcome of inter-specific competition. In a replacement series experiment, the decreased growth of *Lolium perenne* could not be entirely accounted for by the increase in growth of *Anthoxanthum odoratum*. Van den Bergh and Elberse (1962) hypothesised that a higher incidence of viral infection reduced the growth of *Lolium perenne* and contributed to its elimination in competition with *Anthoxanthum odoratum*, which was less susceptible to the pathogen.

Intra-specific interactions may also be affected. Within the apomictic species *Chondrilla juncea*, two forms (B and C), both with a restricted distribution in Australia, increased markedly in frequency when a fungal pathogen, *Puccinia chondrillina*, was introduced to control the widespread and susceptible form A of this weed. The decline in the susceptible form of *C. juncea* was paralleled by the expansion of the resistant plants, which occurred to such a degree that a new strain of the fungus is currently being introduced to attack form B and limit its expansion. This interaction was studied in an experimental system, in which *Puccinia chondrillina* reduced the yield of *Chondrilla juncea* form A in pure stands by 30% but in a mixed stand, where the susceptible host grew together with the resistant forms of *C. juncea*, the presence of the pathogen reduced the yield of form A by over 40% (Burdon *et al.*, 1981).

Pathogen Influence on Host Phenology

Temporal avoidance of pathogen infection requires the plant to complete its important stages of growth at a time when conditions are unfavourable to the pathogen. In practice, this is generally brought about by the early flowering of host plants, before conditions favour an increase in the pathogen population. Thus, pathogen pressure may effect a genetic change in the plant population, selecting for precocious host development. This occurs despite the fact that the resulting phenology is often less ideal for the host. However, if all the host individuals in the population develop precociously, selective pressure on the pathogen will ultimately result in synchronous early growth of the pathogen. Burdon (1982) cites the early flowering of *Avena barbata* as a mechanism of avoiding infection with the pathogen *Puccinia coronata*. Sympatric populations of *Avena fatua* have a greater ability to withstand the pathogen and consequently are able to flower 2-3 weeks later.

Co-evolution of Host and Pathogen

A host species may respond actively to pathogen infection by the evolution of genetically-determined disease resistance factors. The chance mutation which produces a novel resistant allele in a host individual is, initially, at a selective advantage. However, as the frequency of the new genotype increases, selective pressure on the pathogen will also increase until a new virulence gene emerges. The relative frequency of the new, now susceptible host genotype declines in the population. Eventually, another mutation will result in a host genotype which is resistant to the main virulent strain of the pathogen and the cycle continues.

This co-evolution of host and pathogen is a continuing process, reflecting the genetic feedback between two closely interacting components

of the ecosystem. The interaction between the continually diversifying pathogen and its host promotes the establishment and maintenance of a complementary level of genetic diversity in the plant population and prevents the congealing of the host genome (Levin, 1975). Co-evolution in natural plant populations results in an incidence of infection such that the host suffers relatively little from the pathogen. The occurrence of a range of different mechanisms and levels of disease resistance within host plants leads to a long term stability in natural host-pathogen systems.

Conclusion

In this study I have been concerned with the effect of pathogens on the performance of individual plants within natural populations. I have demonstrated that latent viral infection can account for apparent genetic differences between plants. Therefore, pathogen infection should not be overlooked as a variable in ecological experiments.

Plant pathogens also operate as selective factors in the environment, bringing about real genetic differences as a result of evolutionary processes. A pathogen may influence the distribution of host plants within and between plant communities. The species diversity of plant communities may be increased, as the incidence of disease on a dominant affects competitive interactions between and within species. Variability of host phenology is promoted, with selection favouring temporal separation of host and pathogen growth. Genetic diversification of the host population is a consequence of the co-evolutionary interaction between the host and pathogen populations. Howevever, these observations are based largely upon the effects of pathogens in agricultural system and on the predictions of population genetics theory. Obtaining practical evidence relating to the

more complex natural communities will require experimental disturbance of the existing community balance and observation of the mechanisms operating to restore it.

Pathogens function as part of the ecosystem therefore, it is important that their role is acknowledged:

i) in the proximal observation of an organisms' behaviour and distribution

ii) in evolutionary speculation about the ultimate explanation of a species' characteristics.

An understanding of the ecological role of pathogens in plant communities requires a full appreciation of the mechanism of plant-pathogen interactions at the level of the individual and the population. To obtain a realistic assessment of the impact of pathogens it is essential that they are studied in natural plant communities, rather than just in agricultural or experimental systems. The use of transplants is a valuable technique in achieving this.

CHAPTER 6

The Carryover of Phenotypic Differences Between Transplants

Introduction

Individual plants of the same species growing in different habitats often differ strikingly in form. Many investigators have been interested in asking whether these differences are genetic or represent local plastic responses to different environmental conditions. The classical approach to this question has been to compare the growth of transplants or plants raised from seed from contrasting habitats when grown in the same environment (Turesson, 1922a,b; Clausen & Hiesey, 1958 and Antonovics & Primack, 1982). Those individual differences which persist under common conditions are assumed to be genetic in origin whereas, the differences which are lost in cultivation are considered to represent phenotypic modification. Yet, plants and seeds, even from the same population will be genetically heterogeneous and this may cause difficulties in interpretation. Clonal plants offer, at least in theory, a more powerful means to distinguish between genetic and phenotypic differentiation. Parts of a clone should be genetically identical (with the exception of somatic mutation); thus, a single clonal genet can be replicated in one, or a number, of environments. If plants from the same clone are grown in different environments and express different phenotypes this is taken as evidence of phenotypic plasticity (e.g. Bonnier 1890, 1920; Clements & Hall, 1918 and Evans, 1939). Correspondingly, if ramets from different clones are grown together in the same environment and behave differently this is taken as evidence of genetic difference between them (Turkington & Harper, 1979b and Warwick & Briggs, 1980a).

In both these types of experiment it is assumed that the phenotypic effects from a plant's previous history are rapidly lost in a new environment and that carry-over effects do not obscure the issue. To

minimise such carry-over, plants from diverse origins are often cloned and cultivated for a period of time in a common glasshouse or garden environment. Ramets of apparently uniform size and form are then selected. In such an experiment *Festuca rubra* clones from different locations showed no significant differences after twelve months' cultivation in a uniform environment (Harberd, 1961). However, in contrast, phenotypic differences persisted within clones of *Bellis perennis* which had been growing for six months in a glasshouse (Warwick & Briggs, 1980a). Furthermore, during a prolonged period of cloning individual ramets may respond to different micro-environmental conditions in the glasshouse (Libby, 1962) and these differences may be carried over into the new environment. Thus, "even ramets have a certain individuality" (Marsden-Jones & Turrill, 1938). A period of cultivation in a common environment and selection of equivalent ramets may not guarantee phenotypically uniform behaviour, or ensure that subsequently it is only genetic differences that are expressed by plants of different clones.

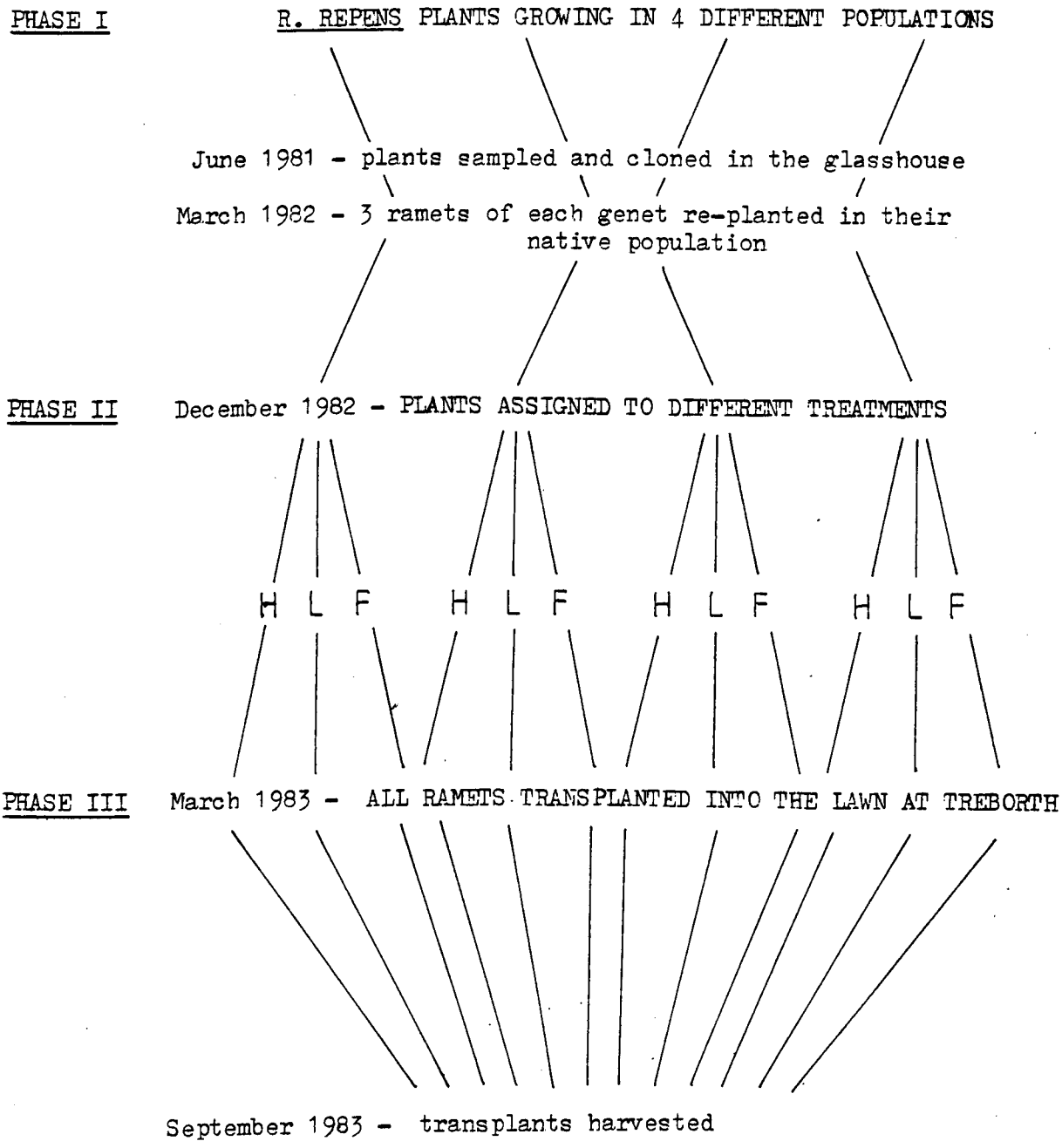
An experiment was designed to examine the "carry-over" of phenotypic characteristics and the extent to which it might confuse the results of transplant experiments.

Method

The plant material and site used in this experiment previously formed part of the *Ranunculus repens* reciprocal transplant experiment (for detail see Chapter 2). A schematic representation of the experimental procedure is shown in Figure 1.

PHASE I : 3 plants of *R. repens* were sampled from each of 4 different populations. Care was taken to sample plants at not less than two to three

Figure 1. Schematic representation of the carry-over experiment; ramets were growing in different populations in Phase I, subjected to contrasting treatments in Phase II and transplanted to a common environment in Phase III. The experimental treatments during Phase III were: H ramets grown in high nutrient conditions; L ramets grown in low nutrient conditions and F ramets remained in the field.



metre intervals and it was assumed that the plants represented 12 different genets. The plants were cloned in the glasshouse and 3 ramets of each genet were planted back into the $3m^2$ subsite in the population from which the genet originated. These plants remained in their native population for a further 9 months.

PHASE II : In December 1982 the 3 plants from each clone were re-located and allocated at random to one of three different treatment regimes:

i) transplants were grown in a high nutrient regime in the glasshouse (H)

ii) transplants were grown in a low nutrient regime in the glasshouse (L)

iii) transplants remained in the field (F).

For the nutrient treatments (H and L) one daughter ramet with 5-8 leaves was taken from each plant, labelled and potted in in a 5-inch pot. In the high nutrient regime the ramets were grown in John Innes No. 1 compost and 30mls of "Vitafeed" solution with a high nitrogen content (3.0.1) applied per pot each week. The low nutrient treatment involved no application of fertiliser and a John Innes No. 1 compost with reduced concentration of nitrogen was used. The ramets were watered regularly and clipped every 2-3 weeks to remove any stolon growth. Plants allocated to the field treatment remained in their field site. A single daughter ramet with 5-8 leaves was chosen to represent each plant. Stolon connections with neighbouring ramets were severed and a labelled metal peg sunk into the ground to identify the selected ramet.

PHASE III : In March 1983, the ramets in the field treatment were dug up with some of their surrounding soil and potted in 5-inch pots. These plants, together with those subjected to the two nutrient regimes, were placed out of doors at Treborth Botanic Garden for one week prior to transplanting in the main lawn at that site. All ramets were then

allocated at random to positions in a 5-metre square grid pattern, with 1 metre separating adjacent rows of transplants. At each transplant position a soil-corer was used to remove a core of soil approximately 10cm in diameter. Compost was shaken from the roots of the ramets and each plant placed into the centre of the soil core, which was then replaced in the ground. A numbered metal peg identified each transplant and a separate record related this to the ramet's population of origin (Phase I) and the treatment it experienced in Phase II. Growth of transplants was then recorded at 3-4 week intervals for 26 weeks by mapping the extent of stolon growth on a co-ordinate grid and recording the location of ramets, together with the numbers of leaves, buds and flowers produced. After 26 weeks all surviving transplants were harvested and their dry weights determined.

Results

One transplant, originally from Henfaes, died two weeks after transplantation . The remaining transplants from this site were excluded from the analysis and a balanced 2-way analysis of variance conducted to estimate: (i) the effect of the three original populations in Phase I (Treborth main lawn; Treborth new lawn and Cae Groes) and (ii) the effect of the treatment experienced in Phase II (high nutrient; low nutrient and field conditions) on the growth that the transplants made in the Treborth lawn during Phase III. Logarithmic and square -root transformations of the data were made where appropriate.

Number of Leaves

Transplants from different populations (Phase I) differed significantly in the number of leaves borne during Phase III of the experiment (Table 1). The differences were maintained over the 26 weeks of this Phase and, over

Table 1. The significance of main effects and interactions in an analysis of the variance of leaf number of *R. repens* transplants originating from 3 different populations (Phase I) and subjected to different experimental treatments prior to transplanting (Phase II). The significance of the variance ratio is given by: ns - not significant; * $P<0.05$; ** $P<0.01$ and *** $P<0.001$.

EFFECT	d.f.	Variance Ratio
Genotype within Population (Error 1)	6	
Population (Phase I)	2	2.71 *
Treatment (Phase II)	2	29.21 ***
Population x Treatment	4	2.11 *
Time x Genotype within Population (Error 2)	42	
Time x Population	14	1.42 ns
Time x Treatment	14	3.35 **
Time x Population x Treatment	28	1.28 ns

all recording dates, plants from Cae Groes had an average of 10.49 leaves compared with only 8.33 and 8.42 leaves on plants from the two lawn populations (Figure 2).

The treatment received in Phase II affected the growth in Phase III and this effect varied with time (Table 1). A high nutrient regime experienced in Phase II (H) was associated with a higher number of leaves developed in Phase III and a significant differential was maintained for the duration of the experiment, 27 weeks after the last application of fertiliser (Figure 3). Transplants which had remained in the field (F) bore consistently fewer leaves than those subjected to low nutrient conditions in the glasshouse (L) and this difference was significant at five recording dates. There was no indication that the variation induced by the different treatment regimes during Phase II diminished with time in the common environment of the transplant site in Phase III (Figure 3).

In the high nutrient (H) and field treatments (F), transplants from Cae Groes bore the highest number of leaves, but after a low nutrient regime (L) it was the plants originating from the new lawn that produced most leaves (Figure 4a), accounting for the significant population x treatment interaction (Table 1).

Flowering

Only one third of all transplants flowered during the course of the experiment, therefore it was not possible to analyse flowering characteristics.

Stolon Growth

Stolon growth in Phase III reached a peak in July and August (after 16 and 21 weeks) when the majority of transplants (23/27) had produced stolons. Analysis of stolon characteristics at this time indicated that

Figure 2. The mean number of leaves, over all treatments and recording dates, of 9 ramets originating from 3 different populations, n = 72. Means which differ significantly ($P < 0{\cdot}05$, according to the Scheffé multiple range test) are designated by a different letter. Transplants from: ▭ Cae Groes; ▨ Treborth main lawn and ▥ Treborth new lawn.

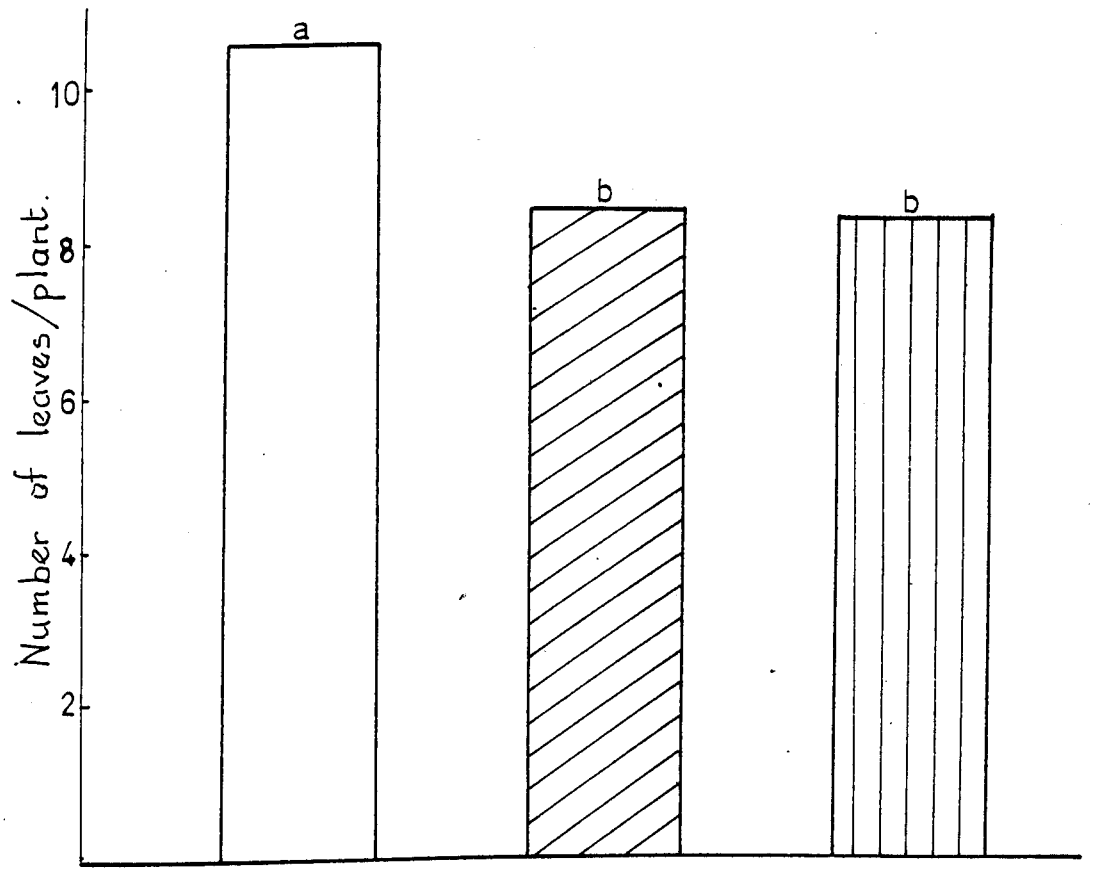


Figure 3. The change in the mean number of leaves of 9 transplants subjected to different treatments before transplantation (Phase II), over time in Phase III. Means which differ significantly ($P < 0{\cdot}05$, Scheffé test) are designated by a different letter. ●——● plants grown in high nutrient regime (H); o- - -o plants grown in low nutrient regime (L) and x——x plants grown in the field (F).

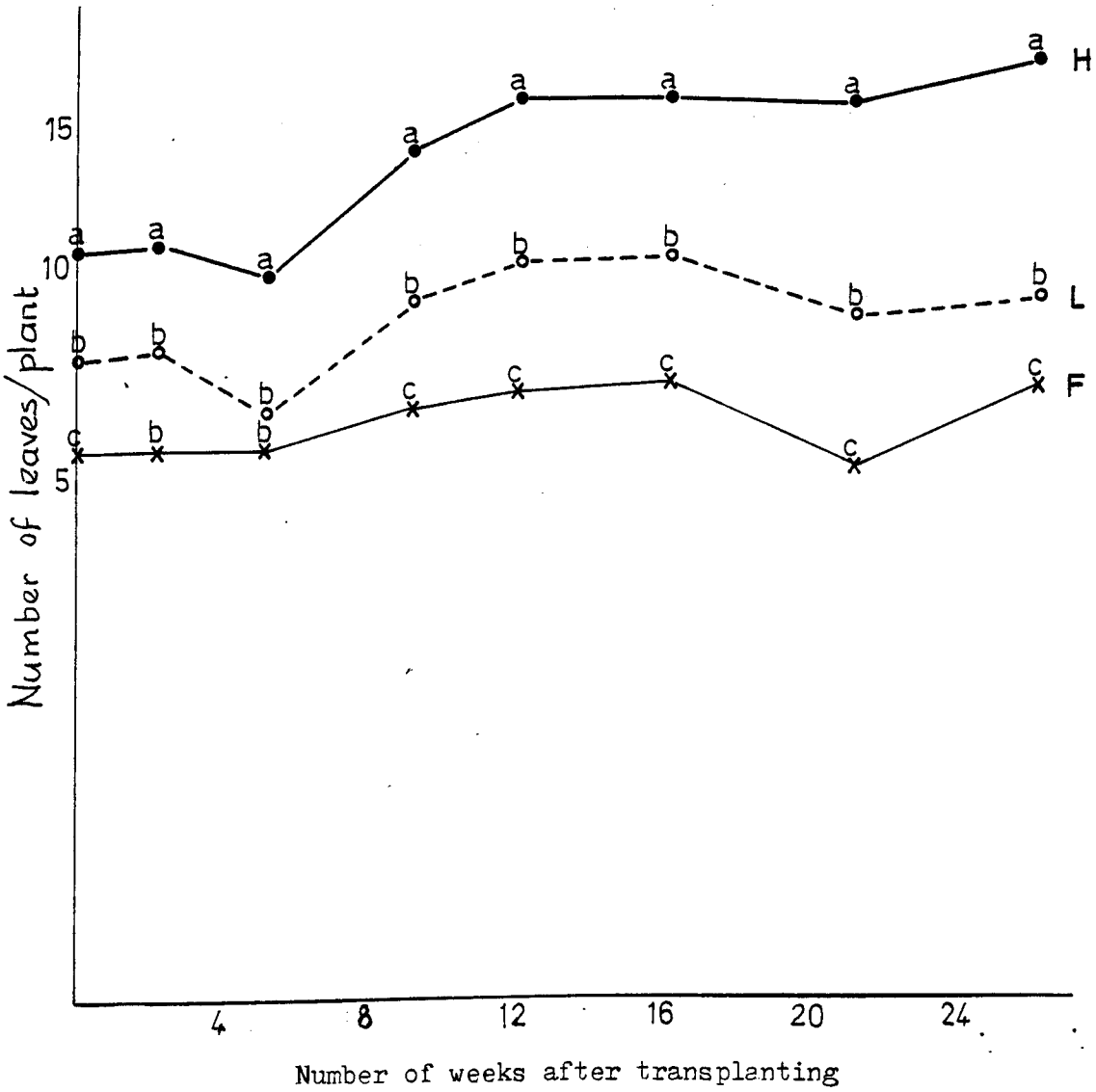
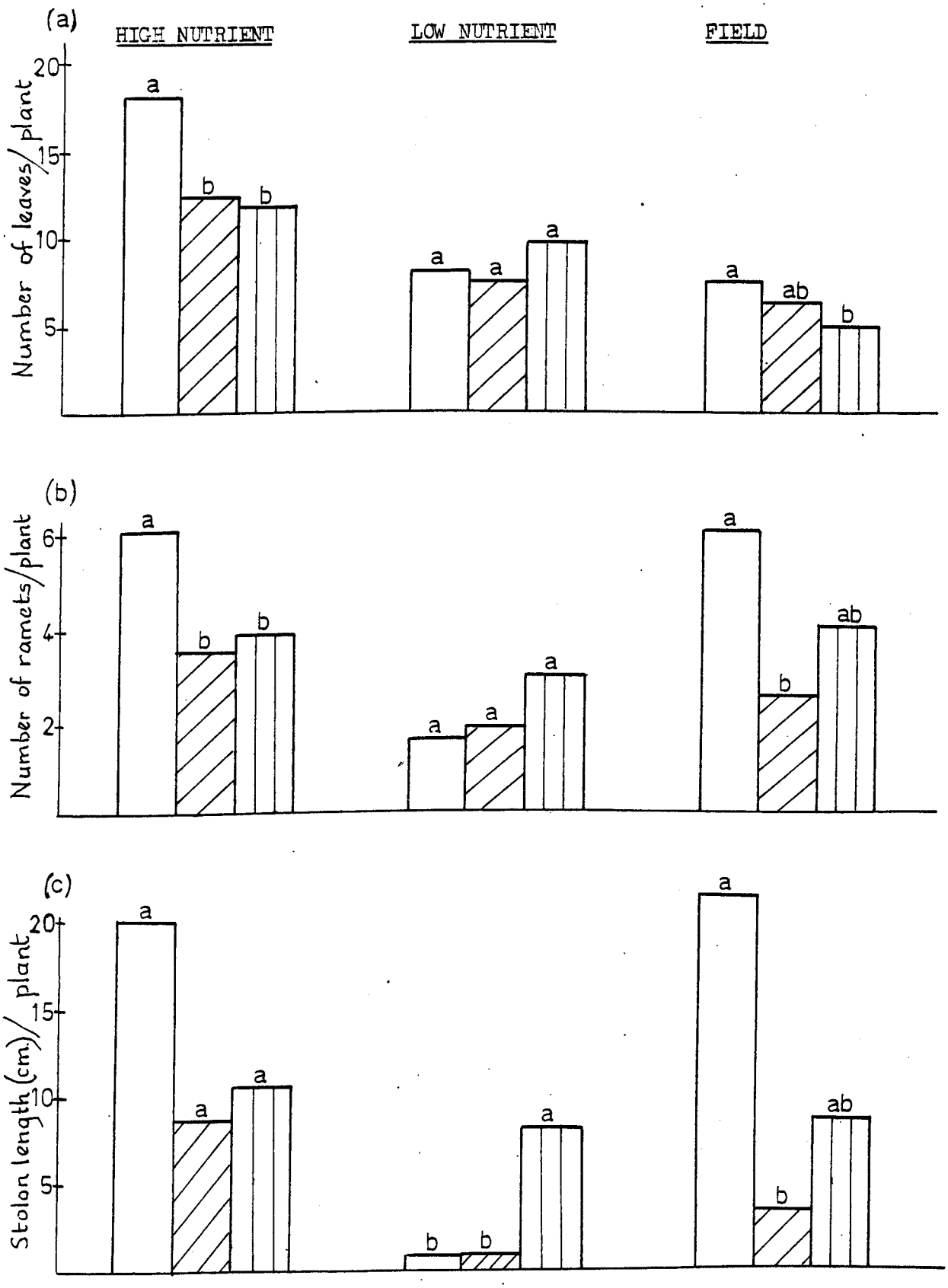


Figure 4. The mean number of: (a) leaves over 26 weeks; (b) ramets after 16 weeks and (c) the length of stolons after 16 weeks on transplants from 3 different populations according to their treatment regime before transplanting, n=3. Means which differ significantly within a treatment ($P<0.05$, Scheffé test) are designated by a different letter. Transplants from: Cae Groes ▭ ; Treborth main lawn ▨ and Treborth new lawn ▥.



both the population (Phase I) and treatment of a ramet before transplantation (Phase II) influenced its stolon production in July (Phase III) and there was a significant interaction at this time (Table 2). Growth at a high nutrient regime before transplanting was generally associated with a high number and length of stolons and a large number of ramets (Table 3ii). The plants which had remained in the field during the winter months also showed extensive growth of stolons but this declined in mid-August.

The treatment experienced in Phase II accounted for much of the variation between transplants but there were also significant differences between genets sampled from different populations in Phase I. Overall, the plants from Cae Groes, the disturbed arable site, tended to produce more stolons of greater total length than plants from the lawn populations (Table 3i). In July, plants sampled from the new lawn in Phase I also showed much stolon growth and this effect was most marked in plants that had spent Phase II in a low nutrient regime; these bore a significantly greater length of stolons than plants from Cae Groes (Figure 4b & 4c).

Dry Matter Production

The treatment applied to a ramet in Phase II significantly affected the dry matter produced over the 26 weeks of Phase III (Table 4). Transplants originating from different populations in Phase I did not differ significantly, neither did they respond differently to the treatments of Phase II. Plants that had spent Phase II in a high nutrient regime produced a significantly greater weight of shoots, roots and total dry matter during Phase III (Table 5). Transplants that had remained in the field during the winter months of Phase II (F) made less growth than those kept in the glasshouse (H & L), yielding a lower root and total dry weight

Table 2. The significance of main effects and interactions on the stolon growth of *R. repens* transplants during Phase III. ns - not significant; * $P<0.05$ and ** $P<0.01$.

VARIABLE	POPULATION (Phase I)	TREATMENT (Phase II)	INTERACTION
<u>July</u>			
Number of stolons	*	**	ns
Stolon length	**	***	**
Number of ramets	*	***	*
<u>August</u>			
Number of stolons	ns	ns	ns
Stolon length	ns	ns	ns
Number of ramets	ns	ns	ns

Table 3. The stolon growth of *R. repens* transplants: (i) from different populations (Phase I) and (ii) previously subjected to contrasting treatments (Phase II). Values = mean of 9 ramets and those which differ significantly within (i) and (ii), $P<0.05$ Scheffé multiple range test, are designated by a different letter.

(i) PLANTS FROM DIFFERENT POPULATIONS

VARIABLE	Cae Groes	Treborth main lawn	Treborth new lawn
July			
Number of stolons	1.75 a	0.78 b	1.75 ab
Length of stolons, cm	8.58 a	3.32 b	9.02 a
Number of ramets	3.86 a	2.59 b	3.60 a
August			
Number of stolons	1.77 a	0.69 a	0.91 a
Length of stolons, cm	10.93 a	4.56 a	6.94 a
Number of ramets	4.61 a	3.65 a	3.64 a

(ii) PLANTS IN CONTRASTING TREATMENTS

VARIABLE	High nutrient	Low nutrient	Field
July			
Number of stolons	2.12 a	0.65 b	1.31 ab
Length of stolons, cm	12.26 a	2.20 b	8.79 a
Number of ramets	4.45 a	2.09 b	3.65 a
August			
Number of stolons	1.94 a	0.65 a	0.82 a
Length of stolons, cm	17.34 a	4.50 a	4.21 a
Number of ramets	5.89 a	3.52 a	2.73 a

Table 4. The significance of main effects and interactions on the performance of *R. repens* ramets from 3 populations (Phase I), previously grown in contrasting conditions (Phase II), 26 weeks after transplantation into a common environment (Phase III). ns - not significant; * P<0.05; ** P<0.01 and *** P<0.001.

VARIABLE	POPULATION (Phase I)	TREATMENT (Phase II)	INTERACTION
Dry weight in mgs of:			
Shoots	ns	**	ns
Roots	ns	*	ns
Total	ns	**	ns
Mean per leaf	ns	ns	ns

Table 5. The mean yield of *R. repens* ramets according to the treatment experienced before transplanting (Phase II), over all populations. Values = mean of 9 ramets, those which differ significantly, P<0.05 Scheffé test, are designated by different letter.

VARIABLE	Treatment experienced before transplanting: High nutrient	Low nutrient	Field
Dry weight in mgs of:			
Shoots	314.36 a	121.91 b	60.70 c
Roots	260.10 a	114.69 ab	50.72 b
Total*	583.66 a	265.87 a	112.61 b
Mean per leaf	18.00 a	17.33 a	12.22 a

* Significance testing was carried out on the means of the transformed data, therefore these values are geometric means and are not additive.

and having smaller individual leaves (12.22mg/leaf compared with 18.0 and 17.33 mgs in plants from high and low nutrient treatments respectively) at the end of Phase III.

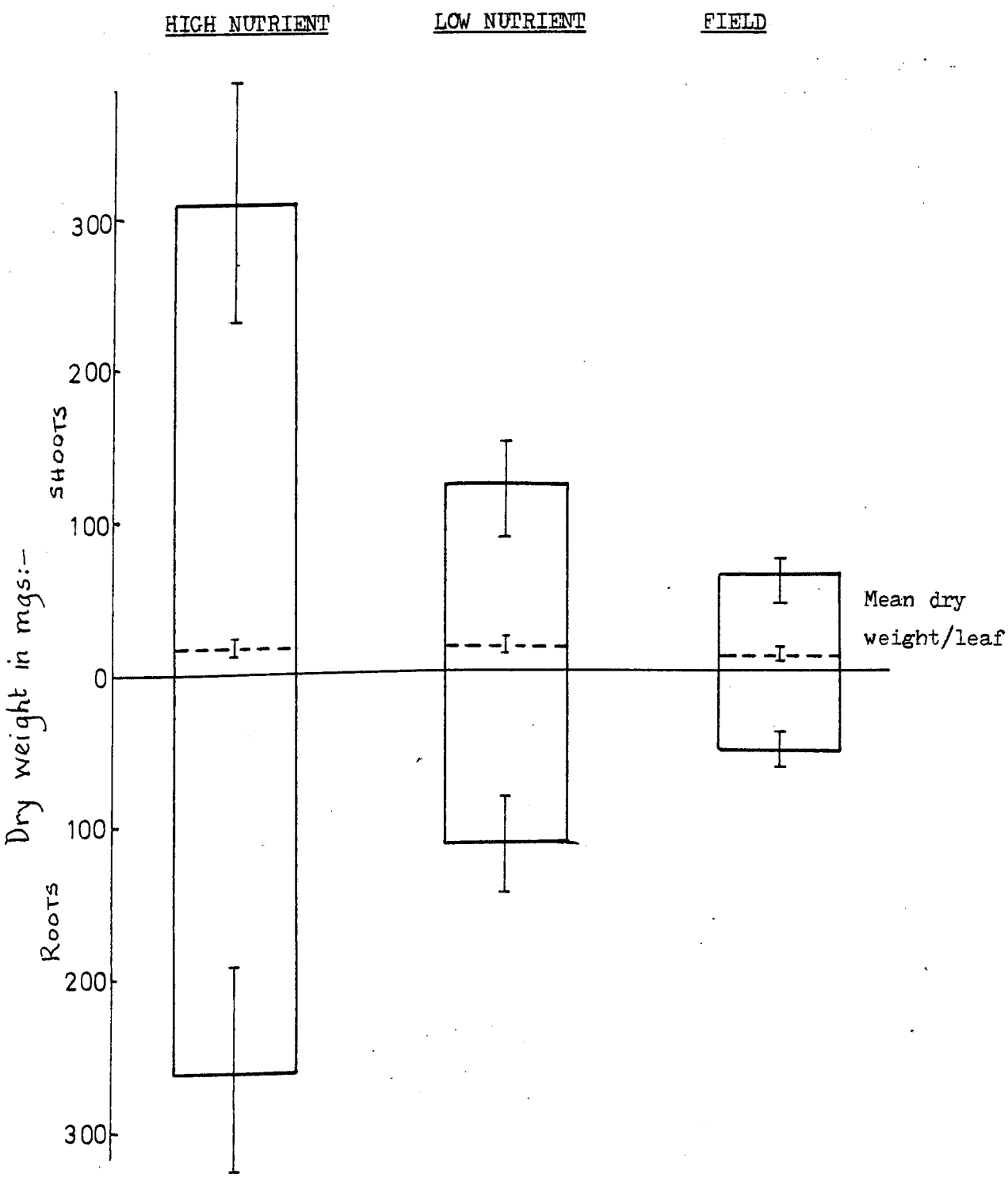
The strong differences between transplants of the same genet, induced by differential nutrient and environmetal conditions in Phase II, were still evident even after 26 weeks' growth in a common environment during Phase III (Figure 5).

Discussion

It is generally assumed that phenotypic differences between plants diminish during cultivation in a common environment and that variation due directly to the environmental origin of transplants is out-grown by the time experimental results are recorded (Bradshaw, 1959a and Harberd, 1961). However, in *R. repens*, phenotypic variation between ramets of the same genet persisted after 26 weeks' growth in an experimental plot. Initial differences in the number of leaves borne on individual transplants increased with time. Thus, variation in ramet size apparently had a cumulative effect and the phenotypic differences induced by contrasting experimental treatments were accentuated with further growth of the plants, even in a common environment. Furthermore, the different populations from which the plants originated also accounted for a significant component of the variation in the number of leaves borne by transplants during the experiment.

The assumptions that: (i) the phenotypic characteristics of transplants entirely reflect their present environment and (ii) the variation between clones is always indicative of genetic differences are clearly invalid.

Figure 5. The distribution and total dry matter of roots and shoots of R. repens transplants grown during Phase II in: high nutrient; low nutrient and field conditions, determined after 26 weeks in a common environment (Phase III). Values = mean of 9 ramets, bars indicate ± 1 S.E.



1. Standardisation of Transplant Size

Plants from diverse origins

The size of a ramet determines its initial food reserves and the way in which it experiences conditions and utilises the resources available when it is transplanted. For this reason, plants of a uniform size are often selected for planting out (e.g. Hiesey, 1953; Bradshaw, 1959a and Mark, 1965). Visual assessment of leaf and shoot characteristics generally forms the basis of this selection (Leach & Watson, 1968) but fresh weight (Walton & Smith, 1976) may provide more indication of the amount of leaf tissue, particularly if there is marked variation between individual leaves (as shown in Figure 5).

Seed and Seedling Transplants

Seed and seedling transplants often show less evidence of phenotypic differences induced by their previous environment. Yet, seeds from different plants within a habitat and even from different positions on the same plant have been shown to vary in weight and germination behaviour (Cavers & Harper, 1966). Seeds may vary because of the carry-over of different maternal effects i.e. nutrients or hormones transmitted from parent to offspring in the cytoplasm (Schaal, 1984). Differences between parents growing in contrasting environments can also apparently be sometimes transmitted through the seed of successive generations of offspring; indicating that changes have been induced in the nucleus or hereditary components of the parent plant. (Durrant, 1962 and Hill, 1965). DNA in the chloroplasts or mitochondria of plant cells could be involved in the inheritance and expression of such environmentally-induced variation.

Seed of a cultivated species is likely to show greater genetic

uniformity than that of wild plants. However, oat seedlings which were cultivated in contrasting conditions reached different developmental stages and consequently, varied considerably when grown as transplants in a common environment (Walton & Smith, 1976). Standardised cultivation procedures and selection of seedlings of uniform size reduced the differences between transplants (Leach & Watson, 1968) but damage to seedlings before and during transplanting still contributed to transplant variability (Evans, 1972).

A selection of plants of a standard size will necessarily be an unrepresentative sample of the species or population. Clausen, Keck & Hiesey (1948) selected transplants which represented the entire range of sizes expressed in seedling progeny from different environments. To ensure that this full spectrum of variability is represented equally in all of the transplant sites the plants must be classified into groups of individuals of similar size; equal numbers of plants from each group are then allocated, at random, to each transplant site. This procedure has been used in assigning pairs of similar plants to contrasting treatments (Bolas & Melville, 1933) and to two transplant sites (Lewis Smith, 1971) and in distributing transplants to many sites in my own reciprocal transplant experiments (Chapter 2).

If standardised transplants from different populations show significant variation when growing in the same environment this may then be associated with their diverse genetic origins. However, even after standardisation of transplants by weight or size, differences may remain in, for example, nutrient status or viruses (see Chapters 5 & 6) and could affect future growth and produce carry-over effects.

2. Genotypic Standardisation of Transplants

On average, transplanted ramets of the same clone are more similar than transplants of different genets. They share the same genotype and can be selected to show the same morphological characteristics at the time of cloning and are frequently employed as "standard" transplants for use as replicates in transplant experiments (e.g. Turkington & Harper, 1979b). Within a clone, transplants are presumed to be genetically uniform but genetic and cytoplasmic abnormalities can result in differences between individual ramets.

Genetic Differences

Mutations of chromosomal DNA are rare and random events yet, under certain environmental conditions, both germinal and somatic mutations have been shown to occur frequently in *Antirrhinum majus* (Harrison & Fincham, 1964). Changes affected the activity of genes at specific alleles; they were expressed as somatic variation in all, or part of individual plants and transmitted to further generations . Gene expression is also affected by "controlling elements" which occur sporadically at certain chromosome loci and block the activity of specific genes in maize and other species (Fincham & Sastry, 1975). In barley, the abnormal gene activity of plants previously infected with barley stripe mosaic virus was thought to reflect the integration of part of the viral genome into the host's chromosomes (Sprague & McKinney, 1971). A fuller appreciation of the factors controlling gene activity is necessary before the genetic stability of clonal lines can be assured.

Cytoplasmic Differences

Cytoplasmic variation between daughter ramets has been linked with

differences in the age of the clonal parent. Successive daughters, produced asexually from the same frond of *Lemna minor*, varied in size; each having a smaller area, fewer cells and a shorter life-expectancy than the preceding daughter ramet (Ashby & Wangermann, 1951). This diminution in area was offset by an increase in the size of fronds subsequently produced from these daughters. Phenotypic variation within clones of perennial ryegrass has been induced by experimentally selecting and cultivating ramets with different rates of tillering (Breese *et al.*, 1965). The differences between ramets were more evident in seedling clones than in those derived from vegetative growth at the start of the experiment and were therefore not merely a reflection of degenerative cytoplasmic changes. Although phenotypic differences were transmitted to subsequent tiller generations, seedling progeny were not obtained and so the somatic variation was not proved to be heritable. Indeed the variation could have reflected differential virus infection acquired during the experiment and subsequently transmitted systemically to all daughter ramets.

3. Phenotypic Standardisation of Clone-transplants

Differences between ramets at the time of cloning may reflect inequalities in genetic, hormonal, viral or nutritional materials received from the parent. Furthermore, after cloning, individual ramets develop in response to their micro-environment and may vary phenotypically when they are transplanted. These differences may persist, as in the above experiment. To minimise the variation associated with environmental differences during cultivation two stages of cloning may be used, with the ramets randomly allocated to positions in the glasshouse (Libby & Jund, 1962). Environmentally-induced variability within clones of *Lolium perenne*

persisted for six weeks in a uniform environment but seven weeks after a second stage of cloning the differences were no longer significant (Hayward & Koerper, 1973).

Conclusions

The transplant technique is a powerful tool for estimating the extent and genetic basis of population differentiation and the influence of different environmental conditions on plant behaviour. However, if the phenotypic characteristics of transplants reflect their previous habitats it will be difficult to correlate observed variation between transplants with their genetic characteristics, or with environmental differences between transplant sites: the results of transplant experiments may easily be misinterpreted. Phenotypic uniformity of transplants is difficult to ensure. In a long term experiment, transplants may out-grow the variation resulting from pre-transplant experiences but if initial phenotypic differences are large (as in my experiment) or are expressed in woody plants (Turesson, 1961) this variation may persist. Consequently, transplant studies may need to be large and long term experiments, with careful selection of plants to represent the entire range of variation from each population and transplanted into each of the sites of origin if the effects of genotype and environment are to be clearly distinguished.

CHAPTER 7

General Discussion

DISCUSSION

Variation between plants of the same species can be of many kinds, involving differences in the morphology, physiology and biochemistry of genetically or phenotypically differentiated individuals. Much of the genetic intra-specific variation is the result of natural selection and, on a local scale, it may be possible to correlate individual differences with the selective effect of specific habitat factors. The initial stage in studying this micro-evolution is to identify the actual variation exhibited between plants in different localities. In doing this it is necessary to assess plant characteristics in the same conditions in which selection has acted. The morphological and physiological variations shown by transplants in a common garden or culture solution and the polymorphisms of specific enzyme systems may not be those that are relevant to the performance of plants in their native population. In a reciprocal transplant experiment we observe the ecologically-relevant differences expressed between plants growing in their natural habitats. The phenotypic variability of transplants within a site is assumed to reflect their genetic differentiation and this is then related to different environmental factors experienced in the original populations. This view may be an over-simplification because phenotypic differences, even between plants growing in the same environment, are not exclusively the product of different genotypes.

In *R. repens*, experimentally-induced variation between ramets of the same clone was maintained, and even increased, during 26 weeks in a common environment. Such variation could be wrongly interpreted as indicating genetic differences between these ramets. Cultivation in uniform conditions and careful selection of transplants of a standard or comparable

range of phenotypes is essential to reduce the carry-over of phenotypic differences from previous environments. These measures will however still be insufficient to ensure that only genetic differences are expressed between transplants if pathogen infection is present.

Sixteen out of a hundred primrose plants, sampled from their natural populations for use in a reciprocal transplant experiment, were estimated to have been infected with arabis mosaic virus before transplanting (see Chapter 5 Table 7). After cloning in the glasshouse, the virus was present in 61 of the 300 ramets which were transplanted. None of these transplants showed visual symptoms of infection, yet the number of their leaves was significantly decreased (by as much as 27%) and individual leaves were up to 25% larger in area than those on uninfected plants. Virus infection of experimental material causes many problems in the interpretation of transplant experiments. Proximally, the virus may influence the phenotype of its host and be mis-interpreted as the effect of the present environment on plant performance. Differences between infected and uninfected transplants in the same site may be wrongly assumed to indicate genetic differentiation which has occurred in response to the obvious habitat differences e.g. in climate and soils, between the plants' original sites. Ultimately, the virus itself must be acknowledged as a selective factor in the environment. In addition, the presence of viruses may modify the effect of other environmental forces, as, for example, when infection affects the behaviour of grazing animals (see pp. 73-74 and Gibbs, 1980) or the performance of the plant at low temperatures (Pratt, 1967).

Ecologists all too frequently ignore viruses both as factors affecting the current behaviour of a plant and as potent selective forces alone and interacting with other components of the ecosystem. Yet viruses can affect all aspects of plant demography, phenology and inter- and intra-specific

interactions and potentially influence evolutionary processes. Therefore, it is important to establish whether viruses are present or absent, not only in transplants but in all plants used in ecological studies, to avoid wrongly attributing the effects of differential infection to environmental and genotypic variation and to attempt to understand their role in natural populations.

The locally differentiated population is the product of ecological processes which involve not only the physical factors of the environment, the climate and soils, but also the biotic interactions with neighbouring plants, herbivores, viruses and other pathogens. By observing the continuing effect of these ecological forces on plants transplanted into a natural environment and in controlled environments (e.g. the sequence pots or experimental swards of the present study) we can speculate about their role in evolution.

The individual plant responds to fluctuations in its environment by phenotypic changes. These changes are generally considered to be "good" for the plant i.e. to maximise its fitness in the present conditions and may indicate the direction of long-term evolutionary change in allele frequency. The low-growing phenotype of plants from mown or grazed populations increases plant fitness and is a genetically fixed characteristic in *Plantago major* and *Poa annua* from these habitats (Warwick & Briggs, 1980b). Alternatively, individual plants may express a range of phenotypes in different environments but within limits set by their genotype. Plants of *Achillea millefolium, Bellis perennis, Plantago lanceolata* and *Prunella vulgaris* were all phenotypically dwarf or prostrate in a grazed site but exhibited a variety of growth forms under cultivation (Warwick & Briggs, 1979).

Phenotypic plasticity is itself under genetic control. It is therefore subject to selection and may be selected for in clonal genets which, by virtue of their growth form and long life, may experience a variety of biotic and physical conditions. A clonal plant such as *Ranunculus repens* can respond to this heterogeneous environment by the differential growth of a number of short-lived ramets, each affected by its immediate conditions but with the possibility of integrated growth through interconnections (see Chapter 3). The variability achieved through this plasticity confers a degree of differentiation which can be carried over to subsequent clonal generations as a differential in nutrient content (see Chapter 6), or the accummulation and transmission of different virus infections (see Chapter 5). Population differentiation of persistent and widespread clonal genets may be achieved by such phenotypic plasticity, particularly if seedling recruitment is rare, and may reduce the intensity of selection for genetic specialisation e.g. in my *R. repens* and in clones of *Achillea millefolium* and *Bellis perennis* in old lawns (Warwick & Briggs, 1979). In contrast, plants of *Primula vulgaris*, which produce few ramets and have a relatively compact growth form, were found to be genetically differentiated in response to conditions in local populations.

Different species show different degrees of plasticity and, within species, plasticity may also vary between different populations (Bradshaw, 1965). In changing or unpredictable conditions, a genotype which shows a high degree of plasticity may be favoured, rather than a highly differentiated one. Individual plants of *Ranunculus flammula* from environments with fluctuating water levels, showed a marked degree of heterophylly when transplanted into different habitats, whereas plants from permanently aquatic or permanently terrestrial sites had specialised genotypes which showed little or no heterophylly and were unable to survive

in extreme conditions (Cook & Johnson, 1968). Furthermore, the plasticity of individual characters varies. This may, in part, reflect the selective influence of different environmental factors, but is probably also subject to evolutionary constraints. In R. repens, the number and size of ramets was very variable in different biotic and edaphic environments. However, the lengths of stolon internodes remained remarkably constant (except when the physical presence of neighbours reduced internode lengths), presumably reflecting the importance of its exploratory ability to the clonal genet.

Phenotypic plasticity is considered inconvenient by the taxonomist who only recognises characters which are environmentally stable. Yet the ecologist must acknowledge this plasticity and understand its genetic control, as it represents the response of the plant to its environment and will determine the level of genetic specialisation in the species.

APPENDICES

APPENDIX I: The Study Sites used for *Primula vulgaris*

Site 1 Penmon

The site is at the top of a limestone quarry on the eastern-most tip of Anglesey (National grid reference SH 634808). On three sides the area is bounded by hawthorn bushes (*Crataegus monogyna*) and brambles (*Rubus spp.*). Vegetation in the study area is mainly *Dactylis glomerata*, associated species include: *Endymion non-scriptus; Fragaria vesca; Lotus corniculatus; Plantago lanceolata* and *Pteridium aquilinum*. The density of primroses throughout the site is low but many large plants, with 30-40 leaves, grow under the hawthorn. The area of the site is approximately 7m x 6m.

Site 2 Plas Gwyn

This site is on the Plas Gwyn estate, approximately 2 miles south-west of Red Wharfe Bay on the east coast of Anglesey (National grid reference SH 5257820). *Quercus petraea, Fagus sylvatica* and *Acer pseudoplanatus* partially shade the site. The ground cover consists primarily of *Anthoxanthum odoratum, Conopodium majus, Endymion non-scriptus, Fragaria vesca* and *Viola riviniana*. There is a low density of primroses and individual plants are small, with only 5-10 leaves and few flowers. The site is frequently grazed by a few sheep and occasionally by cattle which stray from an adjacent pasture. It is located on a west-facing slope of approximately 50° and covers an area 9m x 12m.

Site 3 Rhoscefnhir

This is a roadside verge on a minor road between Pentraeth and Llangefni on Anglesey (National grid reference SH 490752). *Fraxinus excelsior* trees shade the verge, which rises almost vertically from the road and is topped by a hawthorn hedge (*Crataegus monogyna*). There is a

high diversity of dicotyledonous species, including: *Arum maculata; Galium aparine; Geranium robertianum; Silene dioica* and *Viola riviniana.* The main grasses are: *Brachypodium sylvaticum; Dactylis glomerata* and *Holcus lanatus.* Primroses occur at a moderate density of at least 2-3 plants per metre, with the majority of plants bearing 10-20 leaves. This vegetation is cut back from the road two or three times during the summer. The study area extended approximately 30 metres, with a depth of 1.5m, on both sides of the road.

Site 4 Traeth Bychan

This site is part of a cliff on the east coast of Anglesey, approximately 1.5 miles from Benllech (National grid reference SH 518846). Ground cover is not continuous, with limestone outcropping between tufts of *Brachypodium sylvaticum, Dactylis glomerata* and *Festuca ovina.* Other species present include: *Cochlearia officinalis; Fragaria vesca; Plantago lanceolata; Rubus spp.* and *Viola riviniana.* The density of primroses is high but individual plants are small, usually with 10-15 leaves. The site slopes steeply from the shore (at over 60°) facing north-east and covers an area approximately 5m x 6m.

Site 5 Vaynol

This site is in parkland on Vaynol Estate, 3 miles south-west of Bangor, in Gwynedd (National grid reference SH 539695). The study area encircles the base of a large *Castanea sativa* tree. It supports a high density of primroses with: *Anthoxanthum odoratum; Conopodium majus; Fragaria vesca; Hedera helix* and *Narcissus spp.* A lawn surrounding the site is mown frequently but close to the tree mowing is infrequent. The circumference of the tree is approximately 12.5m, around which the site forms a band 1.25m wide, with an area of approximately $23m^2$.

APPENDIX II: The Study Sites used for *Ranunculus repens*

Site 1 Treborth main lawn

This site comprises part of the Botanic Garden of the University College of North Wales, Bangor, on an estate beside the Menai Strait (National grid reference SH 553706). Much of the estate is mixed woodland of *Betula pubescens*, *Fagus sylvatica*, *Pinus sylvestris* and *Quercus robur*. In 1965, an area of woodland was felled, ploughed and sown with commercial grass seed, mostly *Dactylis glomerata*, *Festuca rubra* and *Lolium perenne*. For 2 years the grassland was grazed by sheep but has subsequently been managed by mowing. *Ranunculus repens* is common throughout the site and in the open areas of the surrounding woodland.

Site 2 Treborth new lawn

This is an area of about 5m x 35m, approximately 100m from the main lawn. Originally wasteland with many weeds, the site was ploughed in 1977 and sown with a commercial grass seed containing a high proportion of *Lolium perenne*. The lawn has been mown regularly since then. It is bordered by a cotoneaster hedge (*Cotoneaster lacteas*) and gravel and tarmac paths. A Japanese cherry (*Prunus serrulata purpurascens*) and a bush of *Viburnum bodnantense* have been planted in the lawn. *Ranunculus repens* is a common species in the site.

Site 3 Henfaes

This site is a field of permanent pasture on the University College Farm at Aber (National grid reference SH 653733). It has received no ploughing or chemical treatment for at least 60 years. A small number of sheep are present throughout the year, with larger numbers of sheep and a few cattle occasionally introduced for short periods. The field, of

approximately 1 hectare, is bounded on two sides by hedges, by a row of ash trees (*Fraxinus excelsior*) and, on the fourth side, by a wire fence. *Agrostis tenuis, Holcus lanatus* and *Lolium perenne* are the most abundant grasses. *Trifolium repens* is the dominant dicotyledonous species, with *R. repens* also present in the sward.

Site 4 Cae Llyn

This field is used as a temporary holding site for livestock and is located close to the University Farm, approximately 1km south of Henfaes (National grid reference SH 650725). It is grazed by cattle and sheep and cut for hay in the summer. The sward is composed mainly of *Cynosurus cristatus* and *Lolium perenne*. *Ranunculus repens* occurs throughout the site but particularly along the course of a spring which runs over the surface of the field in wet weather.

Site 5 Cae Groes

This site (National grid reference SH 739773) is a small area, approximately 6m x 4m, formerly cultivated for vegetables with infrequent applications of fertiliser. The site supports a high density of weed seedlings, principally *R. repens*, in the early spring but these are removed annually prior to planting the vegetables.

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