BREEDING FOR IMPROVED NUTRITIVE VALUE

OF *PHALARIS TUBEROSA* HERBAGE

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GENERAL INTRODUCTION

This thesis examines the potential for increasing the nutritive value of Phalaris tuberosa herbage by breeding. Two important nutritive value criteria for this species are herbage digestibility and crude protein content. One assumption made in advance of the experiments was that, for ruminants grazing P. tuberosa pastures in an environment such as south-eastern Australia, digestibility and protein content of vegetative herbage are unlikely to be major limitations to animal production. For this reason, attention was concentrated on the nutritive value of herbage at various growth stages during the reproductive phase. The heading stage was chosen for many measurements because it is the latest of the easily recognised developmental stages of the reproductive cycle at which a reasonably large plant population may be measured for herbage quality in advance of flowering. In a plant breeding program, each cycle of selection for nutritive value at a later stage would require two years instead of one. However, the quality of more mature herbage was also examined in several experiments.

The response to selection for any character can be predicted provided certain genetic parameters are known; such requirements are briefly outlined and the relevant literature on herbage digestibility and protein content is reviewed with reference to these genetic concepts. The first three experiments, which provide information on the inheritance of the two characters, are then described. In addition, the third experiment provided selection lines differing in crude protein content, and the following two experiments describe the agronomic performance of these lines under field conditions. When it became obvious that crude protein content was negatively correlated with seedling vigour and herbage yield, a final series of four experiments was undertaken to investigate the physiological nature of these adverse relationships.

One of the experiments (experiment 3), conducted while the author was employed by the Division of Plant Industry, CSIRO, Canberra, Australia provides the connecting link between the genetic, agronomic and physiological aspects of this thesis. A brief summary of the results of this experiment which are relevant to the remainder of the work is therefore included.

LITERATURE REVIEW

During the regular dry season in many parts of Australia, the productivity of grazing animals is limited by the low digestibility and protein content of the abundant dead herbage. In such environments improvement of these two characters could substantially increase animal production from dry-season pastures. Phalaris tuberosa is the most important perennial grass sown in areas of southern Australia where drought-resistance is required, but its usefulness is limited by its low nutritive value for sheep during summer and early autumn (Arnold 1962; Biddiscombe 1964; D.B. Jones unpublished). Commencing about the date of ear emergence, herbage digestibility and crude protein content fall rapidly. Accompanying this decline in digestible energy and protein concentrations, and partly resulting from it, is a lowered intake of herbage by grazing sheep (Arnold et al 1964). These factors lead to reduced animal production from P. tuberosa pastures, until summer or autumn rains allow renewed vegetative growth (Arnold et al 1964).

The liveweight maintenance feed requirement for grazing sheep is about 550-680 gm digestible organic matter per day (Coop and Hill 1962; Lambourne and Reardon 1963; Coop and Drew 1963; Grimes 1966). Since intake is closely related to digestibility (eg, Blaxter et al 1961; Arnold et al 1964), grazing sheep cannot obtain their maintenance requirements when the dry matter digestibility of the diet falls below 55-60%. Higher digestibilities are needed for liveweight gain.

At concentrations below 1% nitrogen (6-8% crude protein) in the diet of sheep, the supply of nitrogen limits rumen microbiological activity, which in turn depresses feed intake (Allden 1959; Vercoe et al 1961; Elliott and Topps 1963; Blaxter and Wilson 1963; Milford and Minson 1967). A level of 1.6% nitrogen (10% crude protein) is usually adequate for both animal maintenance and production, although levels of up to 2.5% nitrogen may be needed for maximum growth of early-weaned lambs (Andrews and Ørskov 1970). As is well known, nitrogen wastage can occur if diets have higher levels of crude protein; following protein breakdown and deamination of amino acids by rumen microorganisms, ammonia is absorbed through the rumen wall, converted to urea and excreted. However, it has recently been shown (Reis 1969; Hemsley et al 1970) that if dietary plant protein is

protected from ruminal degradation and digested in the small intestine, substantial increases in wool production occur even at levels of 4%N (25% crude protein) in the diet.

Where the diet contains less than 1% nitrogen, intake responses to nitrogen supplementation can be expected and have frequently been demonstrated (eg, Faichney 1968). The nitrogen concentration of Phalaris-based pastures frequently falls below 1% (Allden 1959) but grazing animals select material with a higher nitrogen concentration than the mean of the available pasture. For this reason, nitrogen supplementation of temperate and Mediterranean grass pastures during the summer will not necessarily improve animal performance, although responses have occurred (Allden 1959; Willoughby and Axelsen 1960; Coombe and Tribe 1962).

It is clear from these considerations that improving the herbage nutritive value of Phalaris-based pastures should result in increased animal production. Breeding for higher quality of the Phalaris component is one possible approach. The important nutritive value criteria are digestibility and crude protein content; a similar conclusion has recently been reached by Hogan et al (1969). The measurement of crude protein content (%N x 6.25) is a standard laboratory procedure, requiring only small amounts of herbage for analysis. Until recently, measurement of digestibility required that relatively large amounts of herbage be fed to each of several animals; such quantities of material were not usually available from plant breeding programs. However, two easy and highly accurate methods are now available for measuring the digestibility of small samples. One, the in vitro technique (Tilley and Terry 1963) uses a simplified artificial rumen; the other method measures in vivo digestibility of samples suspended in the rumen in nylon bags (Quinn et al 1938; Lusk et al 1962). The in vitro technique may be marginally superior to the nylon bag method (Van Dyne 1962; Hopson et al 1963; Van Dyne and Weir 1966; Monson et al 1969).

The overall benefit from breeding for any character depends not only on the improvement of that character, but also on the net result of accompanying changes in other characters. The response to selection for the character is a function of phenotypic variability, heritability, selection intensity, generation interval and the amount of genotype x environment interaction. The associated changes in other characters depend partly on similar factors and partly on the

genetic relationships between characters. Such relationships can be conveniently measured as (additive) "genetic correlations" and the sign and magnitude of these correlations are important. If a significant genetic relationship can be demonstrated, it is helpful if the underlying cause of the correlation (i.e., linkage or pleiotropy) can be defined; associations resulting from linkage are frequently transient and can often be broken, while those due to pleiotropy usually cannot.

There has so far been no systematic attempt by plant breeders to analyse the prospects and problems of breeding pasture plants for improved herbage nutritive value, although a useful start has been made (eg, Smith 1952; Corkill 1965; Oram 1970; Clements 1970). Such an analysis, which must be based on the genetic concepts referred to previously, should aim to evaluate plant breeding gains (or estimated gains) in terms of animal production under realistic conditions. Here, discussion will be restricted to two herbage quality criteria, %N (or crude protein) and digestibility. The potential for plant breeding gains and the problems of assessing the benefits to grazing animals will be discussed in turn.

THE POTENTIAL FOR GENETIC IMPROVEMENT OF HERBAGE DIGESTIBILITY AND PROTEIN CONTENT

(a) Variability

Differences in herbage quality between cultivars, ecotypes, families and individual genotypes within species are well documented. The examples in table 1 are restricted to those describing variation for %N (or crude protein) and/or digestibility. They are chosen for their relevance or recent publication and to include a wide range of species. Some references to other and earlier work are given by the authors; others can be found in reviews by Smith (1952, 1956), Vose (1963) and Cooper (1966). While most experiments can be faulted for one or more reasons (eg, poor definition of growth stages; inadequate replication; failure to test in more than one environment or at more than one growth stage; small numbers of plants or lines) the results as a whole leave no doubt that within-species differences in herbage quality not only exist but are frequently very large. Furthermore, variation for both %N and digestibility has been found within both legume and grass species, tropical and temperate species, species grown as spaced plants or in swards under many different soil fertility regimes. Variation occurs in vegetative herbage, in mature (senescent or dead) herbage, and at all intermediate stages. In experiments with sufficiently large and diverse plant populations, differences of 10-20 digestibility units and twofold differences in %N between entries at comparable growth stages have frequently been obtained. In other experiments with a small number of entries, differences have often been slight, but only relatively rarely has there been no significant variation.

Variability in P. tuberosa is extensive. Among 21 ecotypes grown as undefoliated spaced plants at Canberra in 1966-7, *in vitro* dry matter digestibility at the ripe seeds stage ranged from 36 to 45% (Clements, Oram and Scowcroft 1970). Among 7 entries chosen for more detailed study, the ranges in digestibility of leaf, leaf sheath, stem and head (inflorescence) at the same stage were 6.8, 6.8, 7.8 and 4.6 digestibility units respectively, while ranges of the same fractions at the head emergence stage were 6.7, 11.1, 10.5 and 4.4 units respectively. Nitrogen content was equally variable; for example, at the ripe seeds stage values ranged from 0.67 to 1.11 %N (whole plants)

TABLE 1

EXAMPLES OF PUBLISHED DATA DESCRIBING WITHIN-SPECIES VARIATION IN %N AND/OR DIGESTIBILITY OF HERBAGE

Species	Reference(s)
Medicago sativa, *M. falcata* and derived material	Heinrichs and Troelsen (1965) Lamprecht *et al* (1965) Gil *et al* (1967) Heinrichs *et al* (1969) Troelsen and Campbell (1969) Heinrichs (1970)
Trifolium pratense	Davies *et al* (1966, 1968)
Lolium perenne	Cooper *et al* (1962) Dent and Aldrich (1963, 1966) Rogers and Thomson (1970)
Dactylis glomerata	Cooper *et al* (1962) Dent and Aldrich (1963, 1966) Julén and Lager (1966) Christie and Mowat (1968)
Bromus inermis	Pickett (1950) Christie and Mowat (1968)
Phleum pratense	Dent and Aldrich (1963, 1966) Heaney *et al* (1966)
Festuca arundinacea and *Festuca* x *Lolium* hybrids	Buckner *et al* (1967) Frame *et al* (1970)
Phalaris tuberosa	Clements *et al* (1970) Hoveland (1970)
Phalaris arundinacea	Asay *et al* (1968) Carlson *et al* (1969)
Paspalum species	Milford (1960)
Cynodon dactylon	Burton *et al* (1967)
Lespedeza cuneata	Donnelly and Anthony (1970)
Zea mays	Hoener and DeTurk (1938) Roth *et al* (1970)

among ecotypes. Digestibility and %N levels of commercial varieties were intermediate.

In another experiment (Oram, Clements and McWilliam, unpublished) two-year-old full-sib family swards (described by McWilliam and Latter 1970) which had been grazed by sheep during the spring were sampled on two occasions (December and March) during the summer. The family means for %N and *in vitro* dry matter digestibility ranged from 1.84 to 2.58% and 64 to 73% respectively in December, and 0.50 to 1.60% and 39 to 52% respectively in March. The variation was slightly overestimated because of small differences between families in growth stage at sampling.

(b) Heritability

There is relatively little published information on the heritability of digestibility and protein content. Although it seems certain that much of the observed variation is genetic in origin, few investigators have partitioned the genetic variance into additive and non-additive components, and where this has been done the nature of the plant population usually has not allowed generalisations to be made. In the unpublished work described above (Oram, Clements and McWilliam), heritability estimates for %N and digestibility at the December harvest were 0.22 and 0.23 respectively (the reference unit is a single plot measurement). Non-additive genetic variance was negligible. As already mentioned, these values may be slightly biased. The only other heritability estimates available for *P. tuberosa* are those reported in this thesis. For *P. arundinacea*, Asay *et al* (1968) and Carlson *et al* (1969) reported broad-sense heritabilities of 0.44 to 0.70 and narrow-sense heritabilities of 0.19 to 0.41 for crude protein content; corresponding heritabilities for herbage digestibility were 0.51 to 0.80 and 0.06 to 0.66 in the broad and narrow sense respectively.

Some information is available for other species. Christie and Mowat (1968) estimated broad-sense heritabilities of digestibility of *Bromus inermis* herbage harvested at anthesis to be 0.73, 0.60, 0.62 and 0.73 respectively for leaves, stems, vegetative tillers and whole plants. Cooper (1961) and Cooper *et al* (1962) reported heritabilities of crude protein content and digestibility of vegetative herbage to be 0.20 to 0.75 and -0.29 to 0.17 respectively in *Lolium perenne*, and 0.53 to 0.69 and 0.52 to 0.53 respectively

in Dactylis glomerata. However, their statistical methods for calculating heritability are open to criticism. Later, Rogers and Thomson (1970) obtained narrow-sense estimates in Lolium perenne of 0.14, 0.69 and 0.64 for %N and digestibility of dry matter and organic matter respectively in one year, but in the following year the corresponding estimates fell to 0.14, 0.14 and 0.06. Kneebone (1951) reported significant general combining ability for crude protein content in Bromus inermis, and Knight and Yates (1968) found significant general combining ability for digestibility in Dactylis glomerata. For Medicago sativa, Lamprecht and Stevens (1964), Lamprecht et al (1965) and Gil et al (1967) have reported significant general combining ability for %N (eg, h^2=0.56; Lamprecht et al 1965) and low specific combining ability. General combining ability was also significant and specific combining ability relatively insignificant for digestibility in M.sativa (Gil et al 1967). Roth et al (1970) found significant general and specific combining ability for digestibility and protein content in Zea mays.

It should be remembered that broad-sense heritability, which is the ratio of the total genetic to the total phenotypic variance, cannot be used to predict selection response in cross-pollinating species which are sexually propagated. Such heritability estimates can be misleadingly high; the disparity between broad - and narrow-sense estimates reported from the same experiment for Phalaris arundinacea by Asay et al (1968) and Carlson et al (1969) (see earlier) is a good example of this. However, for species which can be asexually propagated (such as Cynodon dactylon), broad-sense heritability has predictive value.

(c) Generation Interval

(i) Genotype x Growth Stage Interactions

Genetic improvement per unit time depends not only on progress per generation, but also on the generation interval (the time from a given stage in the life cycle in one generation to the same stage in the subsequent generation). Because selection of plants for quality of standing dead (mature) herbage requires a selection cycle of at least 2 years, it would be helpful if such quality could be accurately predicted in advance of flowering, thus enabling a cycle of selection to be completed in 1 year. To compare the relative

efficiencies of such direct and indirect selection requires knowledge of the heritability and standard deviation of each character and the genetic correlation between them.

Genetic relationships between herbage quality characters measured at different growth stages before and after flowering have not been published for any species, but some phenotypic relationships have been described. Experiments with a representative range of P. tuberosa ecotypes have so far shown no acceptable pre-flowering indicator of quality at maturity (Clements, Oram, and Scowcroft 1970). On the contrary, there were highly significant ecotype x growth stage and genotype x growth stage (Clements 1970) interactions for both %N and digestibility. It is possible to deduce from other work that similar interactions may occur in Bromus in ermis (Pickett 1950), Medicago sativa (Troelsen and Campbell 1969), Dactylis glomerata (Mowat et al 1965b; Julén and Lager 1966) and other species, but the importance of the interactions is not clear.

It must be emphasised that phenotypic correlations cannot be used with confidence to predict changes in herbage quality at a given growth stage resulting from selection at another. Although for characters of moderate to high heritability (Falconer 1960) the phenotypic correlation may be of some predictive value, there will usually be an element of uncertainty in predictions which are not based on genetic correlations. However, genetic correlations are themselves notoriously inaccurate and frequently unstable (for example, in small populations, or populations not in linkage equilibrium, or populations undergoing selection) so that even predictions based on genetic correlations can be misleading.

(ii) Repeatability

Another factor influencing the generation interval is the repeatability of the desired character at a specified growth stage. In the present context, the relevant question is the need for repetition in time rather than in space. Although the requirement for spatial replication is an important aspect of plant breeding, it is more likely to influence selection intensity than generation interval; reviews by Morley (1963) and Latter (1964) contain excellent discussions of this topic. However, the benefits from replication in time (e.g, from season to season or year to year) can be evaluated in a similar manner, provided the repeatability is

known (Falconer 1960). Alternatively, the need for multiple measurements could be examined using genetic correlation analysis or (if seasons or years were markedly different) techniques normally used for investigating genotype x environment interaction effects (eg, Finlay and Wilkinson 1963). So far, no evaluation along these lines for herbage quality characters has been attempted. The only repeatability estimates available are those of Cooper *et al* (1962), for *in vitro* digestibility of vegetative herbage of *Lolium perenne* and *Dactylis glomerata* measured in 2 consecutive months of one year; they were 0.44 and 0.53 respectively.

Other authors who have replicated measurements of %N or digestibility in time have sometimes presented the results either as phenotypic correlations between measurements, or as a genotype x harvest interaction term in an analysis of variance, and these can be taken as an approximate indication of repeatability. The results have been conflicting. Burton *et al* (1967), who measured the digestibility of 24 *Cynodon dactylon* genotypes in swards cut at 2-, 3-, 4- and 6-weekly intervals found little evidence of genotype x cutting frequency interaction. They also showed that in 4 consecutive years the average digestibility of the best genotype exceeded that of a control commercial cultivar by 6.7, 3.6, 6.3 and 9.6 digestibility units respectively. Pickett (1950) measured %N in vegetative herbage of each of 7 first generation inbred clones from each of 25 unrelated inbred *Bromus inermis* families; measurements in consecutive years were highly correlated (r=0.89). However, for the same species Christie and Mowat (1968), who examined 250 clones in each of 2 years at the heading stage, reported low correlations (r=0.00 and r=0.48 respectively) between repeated measurements of leaf and stem digestibility. Among 9 *Phalaris tuberosa* clones grown in a controlled environment there was a high correlation (r=0.70) between measurements of %N at the heading stage repeated in different years (Clements 1970). Roth *et al* (1970) found no evidence of genotype x year interactions for protein content or digestibility of *Zea mays* herbage.

On the other hand, Knight and Yates (1968) reported wide fluctuations in relative ranking of 9 *Dactylis glomerata* parents and their hybrids for digestibility at successive samplings in one year. Digestibility differences between *Trifolium pratense* varieties have been found to vary between years (Davies *et al* 1968). Asay

et al (1968) and Carlson et al (1969) found significant clone x year and/or progeny x year interactions in Phalaris arundinacea for %N and digestibility respectively. Rogers and Thomson (1970) also obtained progeny x year interactions for both %N and digestibility in Lolium perenne; specific combining ability estimates for 5 parents differed significantly for each character in each of 2 years, but general combining ability was significant only for digestibility in the first year. However, the GCA rankings of the 5 parents were more repeatable; for each character, the two parents having superior GCA in year 1 were also superior in year 2.

(d) Genotype x Environment Interaction

Consideration of the repeatability of a character in space and time leads logically to the more general problem of "repeatability" in a range of environments- i.e., genotype x environment interaction. It is usually unwise to assume that genotypes selected in one environment will perform well in another; the more the environments differ, the less likely is the assumption to be true. Should genotype x environment interactions for %N or digestibility be large, breeding programs may need to be modified. In addition to the special case of interaction between genotypes and years, some relevant information is available from experiments which have included a range of environments imposed by the researchers.

Heinrichs et al (1969) found a correlation of only 0.33 between %N in the leaves of approximately 100 Medicago sativa clones grown in a greenhouse and in the field. In Lolium perenne, Lazenby and Rogers (1965) measured %N of 6 clones grown at 5 levels of nitrogen fertilizer (0 to 897 kg N/ha/annum) as spaced plants or simulated swards in each of 2 years. In one year a small but significant genotype x spacing interaction occurred; in neither year were any genotype x N level interactions observed. However, after diallel-crossing 5 of the 6 clones, Rogers and Thomson (1970) later demonstrated highly significant progeny x N level interactions for both %N and digestibility. Within years, the amount of variation in digestibility due to interaction between the general combining ability of the parents and level of applied N was small (20-30%) in comparison with that due to GCA main effects. For %N, the amounts of variation attributable to each source were about equal, indicating quite large interactions for this character. It should be noted

that the range of N levels chosen for these experiments was very high; up to twofold increases in %N and several-fold increases in herbage dry matter yield were obtained.

Dent and Aldrich (1963, 1966, 1968) measured the digestibility of 26 varieties of Lolium perenne, 19 varieties of Phleum pratense, 14 varieties of Dactylis glomerata and 11 varieties of Festuca pratensis grown at each of 2 localities and under 2 management systems (swards cut either 4 or 8 times each year). Location x variety interactions occurred under infrequent cutting in all species except Lolium perenne. With frequent defoliation there was little within-species variation in digestibility averaged over all cuts; however, interactions between varieties and management systems would be confounded with variety x growth stage interactions. Genotype x cutting frequency interactions for %N and digestibility have been found to be of little importance in Phalaris arundinacea (Asay et al 1968; Carlson et al 1969) and Cynodon dactylon (Burton et al 1967). Dent and Aldrich (1968) also found good agreement in the ranking of Dactylis glomerata varieties for digestibility and protein content of herbage from swards grown under 2 levels of nitrogen fertilizer.

Van Dyne (1965) analysed herbage from 7 annual grasses and 4 annual legumes grown as pure swards at one site. He compared the results with analyses of the same species growing together (i.e. in competition) at 20 sites on a nearby annual range and found species x source interactions for both %N and digestibility. Newell (1968) measured %N of 10 strains each of Panicum virgatum and the Andropogon geradi/A. hallii complex, grown as swards at each of 3 locations under 2 nitrogen fertilizer levels and 2 defoliation treatments. Effects of strains, nitrogen levels and locations were highly significant for %N, while interactions were usually small and nonsignificant.

THE BENEFITS OF INCREASED DIGESTIBILITY AND PROTEIN CONTENT

Assuming that increases in %N and digestibility of Phalaris tuberosa herbage could be obtained by plant breeding, could increased animal productivity be expected? From earlier considerations it might be expected that the benefits would be substantial. However, evaluating the effects of even small changes in grazing ecosystems is not easy. The difficulties of predicting the benefits of improved herbage quality

of grazed pastures in a Mediterranean climate with low and erratic rainfall can be appreciated from recent computer simulation studies (Freer et al 1970). In a model constructed to simulate the grazing of summer pasture by sheep, the digestibility of dry feed was only 1 of 21 variables included for the purpose of predicting animal liveweight changes. Many important variables (eg rainfall and stocking rate) are beyond the control of the plant breeder but a number of complexities that should be considered by plant breeders will be discussed.

(a) Animal selectivity

Grazing animals are selective in what they eat, so that in a pasture mixture some species may contribute more or less to the diet than their relative abundance would suggest. Further, animals select certain parts of a given species in preference to other parts. For example, in mature stands of both Medicago sativa and Phalaris tuberosa Arnold (1960a) showed that sheep select leaf in preference to stem, and green material in preference to dry material. Thus, there may be no point in improving the digestibility of a given species - or even a part of it - if the environment and stocking rate do not force animals to eat that species or part. It is possible that breeding for a character such as stem digestibility in Phalaris tuberosa might raise animal productivity in some years but not in others.

Three aspects of animal selectivity deserve comment. First, a reasonable approach to improving the nutritive value of a sward might be to improve the resistance of preferred species to selective grazing that could eliminate them from the pasture. This approach is unlikely to be helpful for Phalaris/subterranean clover pastures under Australian conditions, since both species are highly persistent under grazing. Second, it could be argued that selection for late flowering or summer green leaf yield would be beneficial. Breeding non-flowering strains of perennial plants might achieve the same effect. All of these objectives could be achieved in Phalaris tuberosa (McWilliam 1962; McWilliam et al 1965; Latter 1965; McWilliam 1968; McWilliam and Latter 1970; Clements, Oram and Scowcroft 1970). Although all these characters could prejudice summer survival in drier areas (Hoen 1968; Oram unpublished), Axelsen and Morley (1968) found that a Phalaris tuberosa x arundinacea summer-growing hybrid persisted under grazing at Canberra. This tableland environment usually has a relatively mild, though dry, summer climate but during this grazing experiment a severe drought occurred which killed dryland cultivars of Dactylis glomerata and Festuca

arundinacea.

Third, animal selectivity within species might be unimportant if genotypes with high leaf nutritive value also have high stem quality; that is, if genetic correlations among nutritive value characters of different plant parts are high. No such genetic correlations have been published but some relevant information is available. Clements, Oram and Scowcroft (1970) found high phenotypic correlations between nutritive value (%N and digestibility) of leaf blade, leaf sheath, stem and head in a range of Phalaris tuberosa ecotypes at heading, but at senescence the digestibility relationships had deteriorated to an unacceptably low level. There are reports both of low correlations (less than 0.30: Heinrichs and Troelsen 1965; Heinrichs et al 1969; Heinrichs 1970) and high correlations (0.77: Ellington and Davies 1966) between nutritive values of leaves and stems of Medicago species, and low to moderate correlations (0.29 to 0.60) between digestibility of plant parts in Bromus inermis (Christie and Mowat 1968). Julén and Lager (1966), comparing digestibility of leaves and "straw" of 10 Dactylis glomerata clones found good agreement between parts; a similar result for this species was obtained by Mowat et al (1965b) at heading but not at later growth stages.

In a very practical sense, differences in nutritive value between plant parts, between vegetative and reproductive tillers, and between plants differing in heading date (see later) should be considered by the plant breeder during the development of herbage sampling techniques. For example, in many species, stem digestibility is initially higher than leaf digestibility, but decreases more rapidly with advancing maturity (Pritchard et al 1963; Terry and Tilley 1964; later work reviewed by Raymond 1969); thus, regular differences between genotypes in proportions of leaf and stem could account for some of the observed genotype x growth stage interactions. Similarly, at a given plant growth stage (eg heading), within-plant variation in growth stage of individual tillers can differ markedly from plant to plant and cause consequent differences in nutritive value between plants (Clements, Oram and Scowcroft 1970). The plant breeder must take these factors into account when deciding at what growth stage(s) selection is to be applied and what herbage is to be sampled for analysis.

(b) Correlations with other Agronomic Traits

Yet another aspect of the sampling problem concerns the relationship

between nutritive value (especially digestibility) and maturity time. It is well known that pasture species and varieties differ in heading date and flowering date in any given environment, and it is equally well known that growth stage influences herbage quality. Thus, if a population of plants is sampled at some point in time during the reproductive stage, variation in nutritive value will partly result from variation in growth stage. On the other hand, if each plant is sampled as it reaches a defined stage, there can be considerable variation in harvest date, and therefore in climatic conditions at different developmental stages. A good deal of evidence suggests that early-maturing strains or genotypes within a species have higher digestibility at comparable growth stages than late-maturing strains (Dent and Aldrich 1963; Mowat et al 1965b; Breese and Thomas 1966; Walters et al 1967; Christie and Mowat 1968; Davies et al 1968). Variation in temperature and light conditions is known to affect crude protein content in plants (see, for example, Jones 1961; Alberda 1965; Deinum 1966; Marten 1970) and recently has been shown to influence digestibility (Garza et al 1965; Smith and Jewiss 1966; Hidiroglou et al 1966; Deinum et al 1968 and personal communication; Smith 1970; Marten 1970; Minson and McLeod 1970). Whether there is some additional, underlying biological relationship between nutritive value and maturity time is a matter for speculation. However it is a matter of practical importance that selection for herbage quality either at a point in time or at a fixed growth stage might actually reduce the value of the product; in the first case, a late maturing variety might result, with consequent low quality at a given stage, while in the second case selection could produce an early variety and consequently prolong the period of nutritional stress for grazing animals. Clearly, genetic correlations between nutritive value and maturity time are important; so far, no estimates have been published.

One of the major requirements of any pasture species is a high herbage yield. The relationship between yield and herbage quality among an array of genotypes measured at the same growth stage in a given environment is therefore of special interest. Negative relationships between %N and yield have been described by Pickett (1950), Kneebone (1951), Van Hee et al (1963), Vose and Breese (1964), Lackamp (1965), Lamprecht et al (1965), Burton et al (1967), Gil et al (1967), Pacucci and Patruno (1968), Newell (1968), Asay et al (1968), Roth et al (1970) and Clements et al (1970). Earlier evidence (mainly unpublished)

has been reviewed by Smith (1952). The correlations vary considerably in magnitude but appear to be consistently negative.

The relationship between digestibility and yield is not so clear. Weak to strong negative genetic associations have been reported by Allinson et al (1966), Gil et al (1967), Burton et al (1967), Carlson et al (1969) and Roth et al (1970) for the species Medicago sativa, Cynodon dactylon, Phalaris arundinacea and Zea mays. Clements, Oram and Scowcroft (1970) found negative phenotypic correlations for Phalaris tuberosa at the heading stage but not at maturity. Dent and Aldrich (1963), who examined digestibility and yield of a total of 70 varieties of 4 different species (Lolium perenne, Phleum pratense, Dactylis glomerata and Festuca pratensis) in swards at 2 locations and under different managements, found that relationships between yield and digestibility were usually negligible but occasionally negative. Knight and Yates (1968) also found no relationship in Dactylis glomerata but quite close positive correlations have been reported for this species by Breese and Thomas (1966) and for Lolium perenne by Rogers and Thomson (1970).

(c) Genetic Relationships between Quality Criteria

Finally, while digestibility and crude protein content are important herbage quality criteria, they cannot be considered in isolation from the many other components of nutritive value. The nature and measurement of forage quality has been extensively studied and frequently reviewed (eg Raymond 1969) and need not be described here. However, since many of the components are interrelated, it is reasonable to expect that selection for %N or digestibility will result in correlated responses in other indicators. Cooper (1961) has shown strong negative genetic relationships between %N and water-soluble carbohydrate content in Lolium perenne and Dactylis glomerata; similar relationships for Phalaris tuberosa are described elsewhere in this thesis. Roth et al (1970) reported close negative genetic correlations (-0.81 to -0.99) between digestibility and acid detergent fibre, acid detergent lignin and cell wall constituents in silage maize (Zea mays), but protein content was not as closely related genetically to any of these characters (-0.16 to -0.43). Protein content and digestibility were not correlated genetically (0.07). As far as is known, no other genetic correlations relating either %N or digestibility to other nutritive value indicators have been published for these or any other species. It is apparent from

several experiments that %N and digestibility are not closely related genetically in Medicago sativa (Gil et al 1967), Cynodon dactylon (Burton et al 1967) or Lolium perenne (Rogers and Thomson 1970), although a positive genetic relationship between these characters has been observed in Phalaris tuberosa (Oram, Clements and McWilliam, unpublished). It also appears that digestibility in Medicago sativa may be only moderately related to acid detergent fibre and cell wall constituents (Gil et al 1967).

One interesting possibility is that high digestibility among Phalaris tuberosa genotypes may be in some way associated with low levels of the tryptamine alkaloids thought to cause Phalaris staggers and sudden death. Although the rankings of ecotypes for digestibility (Clements, Oram and Scowcroft 1970) and total tryptamine alkaloid concentration (Oram and Williams 1967; Oram 1970) at Canberra showed no obvious relationship, Arnold (1970- personal communication) recently observed a striking depression of digestibility of Dactylis glomerata herbage to which these alkaloids had been added. Another alkaloid (perloline), extracted from Festuca arundinacea herbage has recently been shown to inhibit cellulose digestion in vitro (Bush et al 1970)

CONCLUSIONS

Digestibility and protein content of herbage, especially mature herbage, are useful indicators of herbage quality in environments with regular dry seasons. There is genetic variation for these characters in many grass species, including Phalaris tuberosa. This variation and the heritability estimates so far reported suggest that, for a given environment and plant growth stage, plant breeders could produce varieties with superior herbage quality. Whether this superiority would be maintained at different growth stages or in different environments is not clear. Most workers have not differentiated between these two sources of interaction, and many of the results which are claimed to show genotype x environment interaction for herbage quality are in fact uninterpretable.

The problem of assessing the likely benefits of increased herbage quality to animals is complicated not only by the possibility of interactions such as those above, but also by the complexity of the grazed pasture ecosystem in Mediterranean climates with unreliable rainfall. Even using computer simulation techniques it will not be possible to

predict animal production changes until the effects of improved herbage quality on the other important parameters in the models are known. In particular, more information is needed on the importance of genotype x environment and genotype x growth stage interactions for both characters, and the nature and magnitude of the relationships between herbage quality and agronomic characters.

EXPERIMENT I

VARIATION IN HERBAGE DIGESTIBILITY AND NITROGEN CONTENT AMONG SPECIES AND STRAINS OF PHALARIS

INTRODUCTION

Alternative plant breeding methods for cross-pollinated pasture grasses such as Phalaris tuberosa include selection within an existing variety and selection within a more broadly based population produced by crossing a number of diverse ecotypes among themselves and/or with a suitable tester (McWilliam and Latter 1970). However, in some genera, populations resulting from interspecific hybridisation can provide an opportunity to combine the useful features of different species (McWilliam 1964). The potential value of such hybrid populations may be limited by reduced adaptation to the local environment, but selection or backcrossing to the adapted parent may overcome this limitation. Although interspecific hybridisation has not often been used intentionally to improve herbage nutritive value, there are some relevant examples. Corkill (1945), combined some of the superior nutritive value of Lolium multiflorum with a degree of the persistence of L. perenne in the hybrid H1 (short-rotation) ryegrass, now renamed "Grasslands Manawa" ryegrass. More recently, Pritchard (1965) has successfully crossed Sorghum almum with perennial sweet sudan grass (S.vulgare x halepense) to produce hybrids with 20% higher stem sugar content and markedly greater stem digestibility than local S.almum cultivars. In many countries, Lolium x Festuca intergeneric hybrids are being produced to combine the nutritive value and seedling vigour of the ryegrasses with the desirable features of the fescues.

Many interspecific hybrids in Phalaris have been produced, although not all have been described in the literature. Of those involving P.tuberosa, the hybrids P.tuberosa x minor (Trumble 1935; Hutton 1953, 1954, 1955) and P.tuberosa x arundinacea (e.g. Jenkins and Sethi 1932; McWilliam 1962; Allison and Starling 1963; McWilliam et al 1965; and many others) have been most intensively studied. Other known hybrids are P.tuberosa x canariensis (Oram 1961, 1970 and unpublished; K. Hoen, unpublished), P.tuberosa x brachystachys (Hoen, unpublished) and P.tuberosa x coerulescens (Trumble 1935; McWilliam unpublished; Oram unpublished). These hybrids have been produced for several reasons, but probably in no case has the intention

been to transfer herbage quality characteristics from one species to another. In fact, there is little information on the comparative nutritive value of Phalaris species. Vegetative herbage of P.canariensis has been reported to contain higher concentrations of nitrogen (Scurfield 1963) and lower levels of tryptamine alkaloids (Oram 1970) than that of P.tuberosa, but no species is entirely free of tryptamine alkaloids (Culvenor et al 1964; Oram 1970). P.arundinacea is less palatable than P.tuberosa (Robards 1965; McWilliam et al 1965; Andrews and Hoveland 1965), and the hexaploid races of P.arundinacea are particularly unpalatable (Roe and Mottershead 1962). P.tuberosa x arundinacea hybrids have usually been found to be similar to P.tuberosa in palatability (Robards 1965; McWilliam 1962; McWilliam et al 1965) but there are some reports of unpalatability (e.g. George and Croft 1968). Hutton (1953) has stated that P.minor is more palatable than the perennial species. Protein contents of P.tuberosa, P. arundinacea and their hybrids are similar and the hybrids have been found to resemble P.tuberosa in digestibility (McWilliam et al 1965; Clements, Oram and Scowcroft 1970). Digestibility of mature herbage of P.arundinacea may be lower than that of P.tuberosa (Clements, Oram and Scowcroft 1970).

In the following experiment, the variation in herbage digestibility and % N at the heading stage among a range of Phalaris species and hybrids is described.

MATERIALS AND METHODS

A total of 39 cultivars, ecotypes and experimental lines of 6 annual and 5 perennial Phalaris species and 4 categories of species hybrids, was chosen for study (table 2). Further details of individual entries are given in appendix 4. In autumn 1968 (May 26), 2-month-old seedlings were transplanted from seed boxes and established at 60 x 60cm spacings in the field at Palmerston North. The soil was a fertile Ohakea silt loam, i.e. a strongly gleyed yellow-grey earth (J.A. Pollok, personal communication), previously drained and limed (to pH 6.7). Each entry was represented by an 8-plant row in each of 3 replicates in a randomised block design, and each previously undefoliated row was harvested individually in the spring when 6 of the 8 plants had reached the heading stage. A plant was considered to have reached this stage when inflorescences had emerged clear of the flag leaf on at least 4 individual tillers. Only tillers at the defined growth stage were harvested, and an equal number was taken from each plant in the row and bulked to give a single row sample (except that occasionally, because of variability in maturity time between plants within entries, extremely early or late plants were not sampled). This procedure was adopted because previous studies (Clements, Oram and Scowcroft 1970) had shown that, at a defined plant growth stage, within-plant variation in the proportions of vegetative and reproductive tillers could significantly affect whole-plant nutritive value.

Harvested material was dried in a forced-draught oven at 80°C, ground in an Apex cutter mill to pass through a 1mm screen and analysed for Kjeldahl nitrogen concentration (%N, dry matter basis) and in vitro digestibility of organic matter (IVDOM). The analytical methods are described in detail in appendices 1 and 2.

RESULTS

Analyses of variance of observed % N and IVDOM values showed that there were highly significant differences between the species and hybrids (tables 3, 4). The range of values of each character was large, and several species were superior to P.tuberosa in one or both characters. There was also variation between individual entries within species; although the overall analysis indicated little within - species variation for IVDOM, a separate analysis showed that the variation

among P.tuberosa entries was significant. For IVDOM, the P.tuberosa x arundinacea hybrid resembled P.arundinacea, while other P.tuberosa hybrids did not differ significantly from the midparent values. However, for % N, the P.tuberosa x minor and P.tuberosa x canariensis hybrids resembled P.tuberosa and the P.tuberosa x arundinacea hybrid was intermediate. The nitrogen concentration of the P.brachystachys x minor hybrid was lower and its IVDOM higher than the midparent values.

The variation among strains and hybrids of P.tuberosa and P.minor is shown in table 5. The outstanding features were the large range of %N values in P.minor (1.07-2.54%N), the differences in %N and IVDOM between the 2 hybrids and the significant variation in IVDOM of P.tuberosa entries. Correlations between % N and IVDOM among strains of these 2 species were nonsignificant (0.66 and -0.08 respectively for P.tuberosa and P.minor strains). Correlations between IVDOM and heading date were also nonsignificant (-0.27 and -0.11 respectively). Nitrogen content was closely related to heading date among P.minor strains (-0.96) but not among P.tuberosa strains (-0.40).

Because of the variation in maturity time among the species and strains, it was decided to examine the % N and IVDOM of the species after adjusting to a common heading date. This proved to be more difficult than expected. Preliminary analyses of regression of IVDOM and % N on heading date showed that neither individual nor average within-species regressions were significant in the case of IVDOM, whereas both were highly significant for %N (table 6). Inspection of table 3 suggested that much of the species variation in IVDOM could be accounted for by a simple grouping of species as annuals or perennials, although this was not so apparent for %N. Further analyses after this grouping was made (tables 6, 7, 8) showed that (a) the average within-group regression was significant for each character; (b) the annual and perennial group regressions of % N on heading date differed significantly, but those for IVDOM did not; (c) curvilinearity was significant in the relationship between %N and heading date in the perennial group; (d) in an overall analysis, ignoring groupings, curvilinearity was indicated in the regressions of both IVDOM and %N on heading date. Accordingly, both linear and curvilinear regressions were fitted to the data, and the adjusted group means are given in table 9. The interpretation of these analyses is straightforward for IVDOM: annual species of Phalaris were significantly higher in IVDOM than perennials. Before adjustment, the difference in IVDOM between groups was 8.0 digestibility units, and after adjustment for linear regression it was

4.6 units. The inclusion of a quadratic term in the regression had a negligible effect.

The interpretation of the % N results is not so simple, because the regression slopes differed between the groups. However, a comparison of curvilinear regressions showed that, while the group regressions differed significantly, the additional reduction in sums of squares of deviations from regression which resulted when individual group regressions were fitted was very small (6% of the reduction due to the average group regression; table 8). Therefore the difference between adjusted group mean %N values (table 9) is taken as real. The relationship between %N and heading date is given in detail in figure 1, where both species regressions and group regressions are shown. Taken as a whole the data leave little doubt that, as a group, the perennial Phalaris species have higher %N levels than the annuals - a conclusion which was not immediately obvious from the results in table 4.

Because of the complexity of the relationship between %N and maturity time, adjusted species mean %N values are not given. However, the adjusted mean IVDOM values are presented in table 4. It can be seen that the adjustment had little overall effect on species ranking but reduced the species variation between and within the annual and perennial groups.

TABLE 2

CHARACTERISTICS OF THE PHALARIS SPECIES AND SPECIES HYBRIDS EXAMINED FOR NUTRITIVE VALUE

(Details of individual entries are given in appendix 4).

Species or hybrid*	Number of strains examined §	Somatic chromosome number	Breeding system (self- or cross-pollinated)	Habit (annual or perennial) at Palmerston North
P.brachystachys	2	12	self	annual
P.minor	6	28	self	annual
P.amethystina	1	14	self	annual
P.caroliniana	1	14	self	annual
P.californica	1	28	self	perennial
P.canariensis	2	12	self	annual
P.paradoxa	2	14	self	annual
P.truncata	2	12	cross	perennial
P.coerulescens	5	14	cross	perennial
P.tuberosa	8	28	cross	perennial
P.arundinacea	2	42	cross	perennial
P.brachystachys x minor †	1	40	self	annual
P.tuberosa x minor	2	56	mainly self	annual/weak perennial
P.tuberosa x canariensis	3	40 (c.28)	mainly self	perennial
P.tuberosa x arundinacea	1	70	cross	perennial

* 6 of the hybrids are fertile allopolyploids. The exception, P.(tuberosa x canariensis)x tuberosa is a fertile backcross hybrid with a somatic chromosome number of about 28.

§ "Strains" include cultivars, ecotypes and experimental lines (see appendix 4)

† This previously unreported hybrid was developed by Dr. R.N. Oram, CSIRO, Canberra, Australia.

TABLE 3

ANALYSES OF VARIANCE OF HEADING DATE, OF IVDOM AND % N (WHOLE TILLERS) AT HEADING AMONG 15 PHALARIS SPECIES OR SPECIES HYBRIDS

Source of variation	d.f	Sums of squares attributable to source		
		IVDOM	%N	Heading date
Blocks	2	34.70	0.0174	0.07
Between species groups	14	2721.02***	12.7966**	40133.79***
Between strains in species	24	265.08(NS)	6.8694***	6081.95***
Error	76	502.89	2.0635	210.51
Total	116	3523.69	21.7469	46426.32

** P <0.01 *** P <0.001 NS = not significant

TABLE 4

VARIATION BETWEEN SIX ANNUAL AND FIVE PERENNIAL PHALARIS SPECIES, AND FOUR CATEGORIES OF SPECIES HYBRIDS, FOR IVDOM AND %N OF TILLERS WITH HEADS EMERGED

Species or hybrid	Species mean values (S.E.)			
	IVDOM		% N, observed values †	Heading date (days after 1-10-68)
	Observed values	values adjusted to common heading date §		
(1) P.brachystachys	75.1 (0.3)	72.5	1.34 (0.08)	32.6 (0.2)
(2) P.minor	74.4 (0.6)	69.5	1.81 (0.03)	14.7 (0.5)
(3) P.amethystina	74.1 (1.7)	70.0	1.24 (0.05)	20.9 (0.1)
(4) P.caroliniana	72.6 (1.7)	69.9	1.54 (0.06)	32.4 (0.1)
(5) P.californica	68.9 (1.7)	66.5	2.08 (0.17)	34.6 (1.0)
(6) P.canariensis	68.7 (1.7)	69.6	0.91 (0.05)	59.8 (0.5)
(7) P.paradoxa	67.1 (1.3)	67.1	0.76 (0.04)	53.1 (0.7)
(8) P.truncata	66.4 (1.8)	67.4	1.35 (0.15)	61.0 (1.7)
(9) P.coerulescens	65.8 (1.2)	65.2	1.60 (0.02)	48.5 (0.3)
(10) P.tuberosa	64.4 (0.4)	65.6	1.38 (0.02)	61.9 (0.2)
(11) P.arundinacea	58.6 (0.5)	61.7	1.58 (0.03)	77.1 (0.7)
(12) P.brachystachys x minor	76.7 (0.5)	75.1	0.91 (0.07)	40.5 (0.1)
(13) P.tuberosa x minor	70.2 (1.8)	69.2	1.38 (0.02)	45.6 (0.4)
(14) P.tuberosa x canariensis	66.1 (0.4)	67.3	1.32 (0.01)	62.0 (0.1)
(15) P.tuberosa x arundinacea	58.5 (2.7)	60.9	1.45 (0.07)	71.3 (0.8)

§ Analyses of covariance and preliminary regression analyses showed that the error regressions and within-species regressions were nonsignificant, and that adjustment for such regressions was negligible. A more suitable adjustment, which is used here, was the average within-group linear regression of IVDOM on heading date obtained after sorting entries into annual and perennial groups.

† Problems of adjusting these data are discussed in the text.

TABLE 5

VARIATION AMONG STRAINS OF P.TUBEROSA AND P. MINOR AND TWO SPECIES HYBRIDS FOR IVDOM AND %N OF TILLERS WITH HEADS EMERGED

Species and strain §	IVDOM (S.E)	%N (S.E.)	Heading date (days after Oct.1) (S.E.)
P. minor			
19215	76.9 (1.0)	1.28 (0.14)	22.8 (1.42)
19197	75.8 (0.5)	2.54 (0.25)	-2.8 (0.8)
19195	74.4 (0.9)	2.11 (0.07)	5.5 (0.7)
19224	73.8 (1.5)	1.07 (0.07)	38.8 (0.4)
32270	73.7 (1.7)	1.90 (0.14)	10.1 (0.7)
32269	71.9 (1.4)	1.98 (0.08)	13.9 (0.8)
Significance of strain differences	(N.S)	***	***
P.tuberosa			
19280	67.7 (0.4)	1.55 (0.06)	52.7 (0.8)
Seedmaster	66.4 (2.3)	1.43 (0.01)	68.8 (0.3)
General Select	65.4 (1.7)	1.43 (0.01)	63.1 (0.5)
19315	65.1 (1.7)	1.42 (0.07)	61.2 (1.1)
High N	64.8 (0.2)	1.55 (0.07)	59.6 (1.5)
Low N	63.7 (1.5)	1.15 (0.00)	56.2 (0.2)
19351	61.4 (1.9)	1.18 (0.02)	80.0 (0.5)
19305	61.0 (1.7)	1.37 (0.10)	53.7 (1.4)
Significance of strain differences	*	*	***
P.tuberosa x minor cv. Australian X			
19203	68.3 (2.3)	1.12 (0.07)	50.6 (1.0)
"P.daviesii"	72.0 (1.4)	1.64 (0.08)	40.5 (0.5)

§ Numbers used are CSIRO Commonwealth Plant Introduction (CPI) accession numbers.
See appendix 4 for further details of the strains.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ NS = not significant.

TABLE 6

PRELIMINARY ANALYSES OF LINEAR REGRESSION OF IVDOM AND %N ON HEADING DATE

Source of variation	d.f.	Sums of squares attributable to source	
		IVDOM	%N
(a) Strains grouped in species:			
Total sums of squares within species	102	802.6230	8.9503
SS due to average within-species regression	1	0.7223 (NS)	3.5067 (***)
Deviations from average regression	101	801.9007	5.4436
SS due to individual within-species regressions	14	141.1363 (NS)	1.9379 (***)
Deviations from individual regressions	87	660.7644	3.5057
(b) Strains grouped as annuals or perennials:			
Total sums of squares within groups	115	1716.0800	21.7229
SS due to average within-group regression	1	448.3613 (***)	11.5357 (***)
Deviations from average regression	114	1267.7188	10.1872
SS due to individual group regressions	1	10.0758 (NS)	1.5125 (***)
Deviations from individual regressions	113	1257.6430	8.6746

*** $P < 0.001$ NS = not significant

TABLE 7

PRELIMINARY ANALYSES OF CURVILINEAR REGRESSION OF IVDOM AND %N ON HEADING DATE, STRAINS POOLED INTO ANNUAL AND PERENNIAL GROUPS

Source of variation	d.f	Sums of squares attributable to source	
		IVDOM	%N
(a) Within perennial group:			
Deviations from linear regression	64	725.989	3.7494
Deviations from curvilinear regression	63	687.671	2.7707
Curvilinearity of regression	1	38.318 (NS)	0.9787 (***)
(b) Within annual group:			
Deviations from linear regression	49	531.654	4.9252
Deviations from curvilinear regression	48	500.424	4.7320
Curvilinearity of regression	1	31.230 (NS)	0.1932 (NS)
(c) Groups ignored:			
Deviations from linear regression	115	1608.826	15.6141
Deviations from curvilinear regression	114	1541.619	13.0055
Curvilinearity of regression	1	67.207 (*)	2.6086 (***)

* $P < 0.05$ *** $P < 0.001$ NS = not significant

TABLE 8

ANALYSES OF CURVILINEAR REGRESSION OF IVDOM AND %N ON HEADING DATE, STRAINS POOLED INTO ANNUAL AND PERENNIAL GROUPS

Source of variation	d.f	Sums of squares attributable to source	
		IVDOM	%N
Perennials, deviations from perennials regression	63	687.671	2.7707
Annuals, deviations from annuals regression	48	500.424	4.7320
Total within-group deviations	111	1188.095	7.5027
Pooled within-group regressions	112	1215.566	7.9930
Difference between group regressions	1	27.471 (NS)	0.4904 (**)
Between-plus pooled within-group regressions	113	1539.18	12.9827
Between adjusted group means §	1	323.61 (***)	4.9897 (***)

** $P < 0.01$ *** $P < 0.001$ NS = not significant

§ Although the group regressions of %N on heading date differed significantly, the additional reduction in deviations sum of squares due to fitting separate regressions for each group is only 0.4904/7.9930, or approximately 6%. For this reason, and taking into account fig. 1, the test for significance between adjusted group means for %N is taken to be valid (i.e., over the range of heading dates encountered the groups do differ significantly).

TABLE 9

ANNUAL AND PERENNIAL GROUP MEAN VALUES FOR IVDOM AND %N OF WHOLE TILLERS AT HEADING, BEFORE AND AFTER ADJUSTING TO COMMON HEADING DATE

Character, and adjustment §	Group mean values and differences		
	Annuals	Perennials	Difference
IVDOM, unadjusted values	72.5	64.5	8.0***
IVDOM, adjusted for linear regression	70.6	66.0	4.6***
IVDOM, adjusted for curvilinear regression	70.5	66.1	4.4***
%N, unadjusted values	1.44	1.47	0.03 (NS)
%N, adjusted for linear regression	1.14	1.71	0.57***
%N, adjusted for curvilinear regression	1.15	1.70	0.55***

*** $P < 0.001$ NS = not significant

§ adjustments were made using the average within-group coefficients. These were

(a) <u>linear regression coefficients</u>: for IVDOM on heading date, $b = -0.1295$; and for %N on heading date, $b = -0.0208$

(b) <u>curvilinear regression coefficients</u>: for IVDOM on heading date and heading date squared squared respectively, $b_{1.2} = -0.0032$ and $b_{2.1} = -0.0014$; corresponding coefficients for %N were $b_{1.2} = -0.0467$ and $b_{2.1} = 0.0003$.

Heading date was measured in days after 26.9.1968.

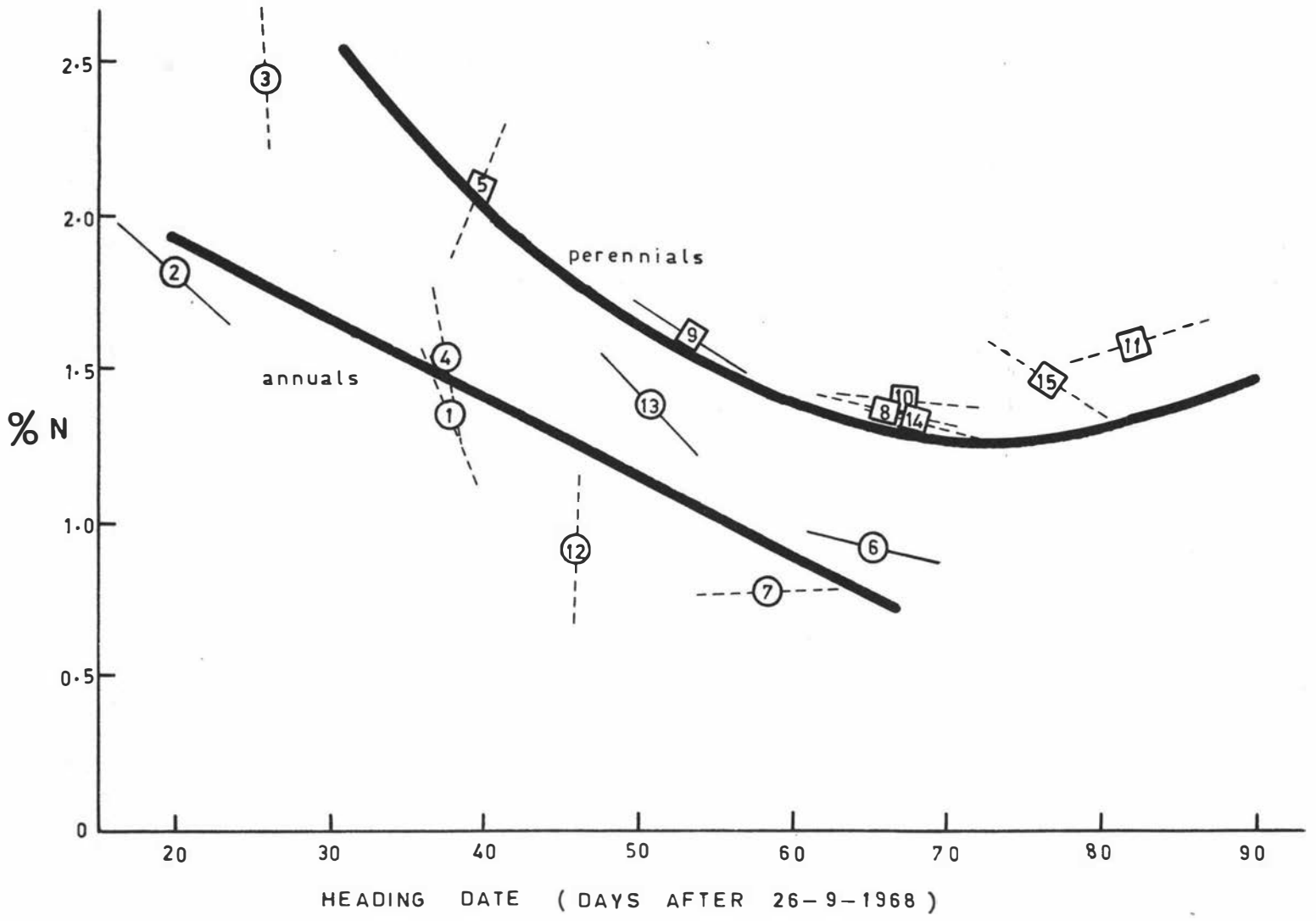

Fig. 1.- Regression of % N on heading date. The overall regression lines for perennials and annuals are indicated. Means and regressions of individual species (◯ annuals ; □ perennials) are superimposed. Numbers refer to species listed in table 4 , while broken and unbroken lines indicate nonsignificant and significant regressions respectively.

DISCUSSION

The outstanding results in this experiment were the differences between annual and perennial species or hybrids for IVDOM and %N. Although P.tuberosa was, on average, inferior in IVDOM to most other species, only the annual species appeared to be sufficiently superior to warrant consideration for hybridising with P.tuberosa. Such interspecific hybridisation does not seem to offer much prospect for improving the overall usefulness of P.tuberosa for two reasons.

1. Hybrids between P.tuberosa and the annuals P.canariensis and P.minor have reduced perenniality; even under the relatively mild climatic conditions of Palmerston North, neither of the P.tuberosa x minor hybrids survived the summer. The P.tuberosa x canariensis hybrids, which have in fact been selected for perenniality (Oram, personal communication) survived well at Palmerston North but are not as drought resistant as P.tuberosa (Oram 1970 and personal communication). A decrease in drought resistance or perenniality of P.tuberosa - one of its special virtues under Australian conditions (e.g. Biddiscombe 1964; Axelsen and Morley 1968; Morley, Bennett and McKinney 1969; Hutchinson 1970) - would greatly reduce the agronomic value of this species, which does not readily re-establish from seeds falling on the soil surface (Campbell 1968; Dowling, Clements and McWilliam 1971).

2. The P.tuberosa strains examined in this experiment were expected from our earlier results (Clements, Oram and Scowcroft 1970) to vary markedly in herbage digestibility and protein content, and this proved to be the case. In particular, the Algerian ecotype CPI 19280, previously found to be relatively high in IVDOM at Canberra, was again superior. Furthermore, this ecotype was at least equal to (nonsignificantly greater than) all the P.tuberosa x canariensis hybrids for both IVDOM and %N. Although CPI 19280 is not an agronomically acceptable strain in other respects, it appears to be a useful source of herbage quality within P.tuberosa. Also, %N values for the high N and low N experimental strains, which were derived from a P.tuberosa breeding population (McWilliam and Latter 1970; see experiment 3) show that selection for this character within P.tuberosa would probably give changes in %N equal to those obtainable by interspecific hybridisation.

However, there are agronomic deficiencies other than low herbage quality in P.tuberosa, such as weak seedling vigour, poor seed retention and the presence of toxins which can cause staggers and sudden death

in sheep. If for some reason it was decided to produce interspecific hybrids, then the present results suggest that those between P.tuberosa and most annuals (and possibly P.californica) should have superior digestibility to P.tuberosa. It would be necessary to chose strains of the annual parent that were also high in %N (e.g. P.minor strains CPI 19197 and 19195).

The relationship between herbage quality and heading date deserves some comment. Many authors have described a similar relationship for several temperate grasses such as Lolium perenne, Dactylis glomerata, and Phleum pratense (Dent and Aldrich 1963; Mowat et al 1965b; Breese and Thomas 1966; Walters et al 1967; Christie and Mowat 1968), i.e., within a species, earlier maturing genotypes or strains have higher digestibility at comparable growth stages than later maturing lines. A weak to moderate negative association between heading date and IVDOM of several plant parts at heading was observed among seven predominantly Mediterranean strains of P.tuberosa (Clements, Oram and Scowcroft 1970), but there was no relationship between heading date and IVDOM of mature herbage. In the present experiment, heading dates of the eight P.tuberosa strains differed by up to 4 weeks, but the relationships with IVDOM and %N were small and nonsignificant. However, measured over all species and strains the multiple (curvilinear) correlations between earliness and IVDOM (R=0.75) and earliness and % N (R = 0.63) were highly significant.

Part of this relationship is no doubt due to small changes in the environment. Temperature, light intensity, moisture stress and soil nitrogen levels are known to affect one or both characters. Under field conditions it is difficult to isolate the effect of any one of these factors from that of another. However, Deinum et al (1968) and Minson and McLeod (1970) have recently shown that, under conditions of adequate moisture and nutrient supply, herbage dry matter digestibility decreases at a rate of about 1.14 units per 1°C increase in "mean" ambient temperature. In the present experiment, "mean" temperatures (average of daily maximum and minimum) calculated for the 4-week period prior to sampling each strain (i.e., as derived by Minson and McLeod 1970) increased from 10°C for the earliest strain to 15.5°C for the latest strain, which differed by 16 digestibility units. Temperatures calculated in this way and averaged for the annual and perennial groups differed by only 2°C between groups, while unadjusted group means for IVDOM differed by 8 digestibility units.

However, adjustment of IVDOM and %N values on the basis of heading date would be expected to correct for changes in the environment with

time. When values were adjusted, there were still marked differences between annual and perennial species. Whether there is some fundamental biological relationship between herbage quality and the annual habit is a matter for speculation, but the present results suggest that this may well be the case. Such a possibility has been recently recognised for Lolium species by Corkill (1965). It is significant that the annual and short-rotation ryegrasses in New Zealand are superior in nutritive value to the more perennial cultivars, and that animal liveweight gains are higher on pastures containing the short-lived types (many relevant experiments are reviewed by Butler, Rae and Bailey 1968).

Finally, it must be emphasised that the herbage quality survey described here was confined to a single growth stage in one year. Because of the number of strains examined and the marked differences between species and groups of species, it is unlikely that the general conclusions are misleading, but the possibility that differences at other growth stages and in other years would be smaller cannot be rejected.

EXPERIMENT 2

THE INHERITANCE OF HERBAGE DIGESTIBILITY AND NITROGEN CONTENT IN AN ALGERIAN ECOTYPE OF PHALARIS TUBEROSA

INTRODUCTION

In the previous experiment, some alternative methods of generating base populations for subsequent selection in cross-pollinating pasture grasses were described. The simplest procedure is to choose an existing variety or ecotype. Normally, such a variety would be a commercial or agronomically acceptable cultivar. Alternatively it could have potential value, subject to improvement by plant breeders. However, useful information can also be obtained by choosing an ecotype already superior to existing commercial cultivars for a character of interest, and examining the potential for achieving still higher levels by selection.

Phalaris tuberosa is a predominantly cross-pollinating species, most plants being highly (but not completely) self-incompatible (McWilliam 1962). Although it is a tetraploid species (2n=28), inheritance is disomic (McWilliam 1962). Ecotypes vary considerably in herbage digestibility and protein content (Clements, Oram and Scowcroft 1970; experiment 1). One ecotype, CPI 19280 from Algeria appears to have superior herbage digestibility at heading and later growth stages and to have relatively high crude protein content at heading in comparison with the Australian cultivar. In this experiment, the inheritance of these two characters in this ecotype at two different growth stages will be described.

MATERIALS AND METHODS

Sixty-five 2-year old plants, taken at random from a larger spaced-plant population of P. tuberosa CPI 19280, were crossed to provide an array of full-sib and half-sib families using the "design 1" mating system of Comstock and Robinson (1948). Thirteen plants were randomly assigned as pollen parents, and each pollen parent was mated with 4 ovule parents, providing a total of 52 full-sib matings in 13 sire groups. Crosses were made using the technique of mutual pollination under glassine bags. Subsequently, 13 families were rejected (no seed;

suspicion of pollen contamination; suspicion of selfing; insect damage). Ten-week-old seedlings from the remaining 39 families were transplanted from seed boxes to the field at 60 x 60 cm spacings in autumn 1968 (May 2). The site was immediately adjacent to that of the previous experiment and soil type and conditions were the same. Each family was represented by an 8-plant row in each of 3 replicates in a randomised block design, and the plants were maintained without defoliation (other than sampling) during the experiment. Seedling vigour was scored on a 1 (low)- 5 (high) scale 2 weeks after transplanting.

Herbage samples were collected at two growth stages. Rows were sampled individually as each group of 8 plants reached the heading stage (defined as for experiment 1). Only tillers at the defined stage were collected and an equal number was taken from each plant in the row and bulked to give a single row sample. The second harvest was taken in late summer (February 21, 1969), all rows being sampled on the one occasion. At this time, all leaves, inflorescences and higher internodes were dead (internodes to a height of 30-40 cm were usually green), seeds were dispersed and both heads and leaves were disintegrating. All plants were apparently dormant (i.e., no growing vegetative tillers were visible). This will be subsequently referred to as the mature stage. As before, special care was taken in the sampling of herbage, and an equal number of tillers was taken from each plant in the row.

Harvested tillers from each 8-plant row were dried in a forced-draught oven, weighed, ground in an Apex cutter mill to pass through a 1 mm screen and analysed for Kjeldahl nitrogen content (%N, dry matter basis) and *in vitro* digestibility of organic matter (IVDOM). The analytical techniques are described in appendices 1 and 2.

Statistical analysis of the data was carried out as detailed in Appendix 5. The 8 characters examined (all having normal distributions) were % N (heading), % N (mature), IVDOM (heading), IVDOM (mature), $\log_{10}$ tiller weight (heading), $\log_{10}$ tiller weight (mature), heading date (days after 31.10.1968) and seedling score (square root transformation of scores summed over all plants in the row).

RESULTS

A description of the variation between families for each character is given in table 10. The difference between families at the extremes of the herbage IVDOM range was 8.8 digestibility units at heading and 11.3 units at the mature stage. There was significant variation in % N of mature herbage, but families did not differ significantly at the earlier stage. Families differed by up to 11.5 days in mean heading date and up to twofold in tiller weight, but there was relatively little variation for seedling vigour. There were large genetic maternal effects on seedling vigour, and there was some evidence of similar effects (or alternatively, of non-additive genetic effects) on % N at heading. The heritability estimate of the former character (table 10) is essentially zero when the maternal genetic effect is removed, while the estimate for % N at heading as given in the table is probably biased upwards. The remaining heritability estimates are all high. It must be remembered that these estimates are for family means, and would be expected to be higher than those for single plants. Replication and careful sampling, and some inevitable phenotypic assortative mating for flowering date all contributed to some degree to these values.

Although families differed significantly in herbage quality at each growth stage, the same families were not necessarily superior at both stages. On the contrary, there were significant family x stage interactions for both % N and IVDOM (table 11). This can also be seen in the low phenotypic correlations between family mean values for each character at the two stages (0.303 and 0.036 respectively for IVDOM and % N; table 12). However, for predicting correlated responses in one character (or at one stage) resulting from selection for another character (or at another stage), estimates of genetic correlations between characters are necessary. These are given in table 12. The genetic correlation between IVDOM at the 2 stages was low but positive, while the corresponding correlation for % N was (nonsignificantly) negative. Also of interest are genetic correlations between IVDOM and % N; it can be seen that IVDOM at one harvest is negatively related genetically to % N at the other, but less closely related to % N at the same harvest.

The genetic correlations between IVDOM and % N measurements, and the heritability estimates and standard deviations from table 10,

have been combined to produce estimates of correlated responses to selection (table 13). In this table, two variables - % N and IVDOM of mature herbage - have been designated "main characters", and the relative increase in their levels after a single cycle of family selection for any of the 4 herbage quality measurements is given. For the purposes of this table, nonsignificant heritability estimates and genetic correlations have been taken at face value. Several conclusions can be drawn. First, the relative increase in each main character from a cycle of selection is greatest when selection is based on the character itself; correlated responses following selection for an alternative character are much smaller and occasionally negative. Second, even assuming that generation interval would be two years if quality had to be measured on mature herbage (compared with one year if measurements could be made before flowering), the selection response in the main characters per unit time would still favour direct rather than indirect selection. Furthermore, since herbage quality measurements require considerable time and labour, it is likely that many more families or genotypes could be measured at the mature stage than would be possible during the short time between heading and anthesis. Higher selection intensity for the main character would further increase the superiority of direct selection. Third, selection for IVDOM at either stage would have relatively little effect on % N, and vice versa. Should both characters need to be improved, selection must be applied for both; should only one character be chosen for improvement, the other could be easily maintained near the existing level.

Despite the magnitude of the interaction between families and growth stages for both IVDOM and % N, it should be possible to produce populations having relatively high levels of each character at each stage. Such a conclusion can be reached from theoretical considerations, but is also apparent from a close inspection of the families described here. As an example, the values of the 39 families for IVDOM (heading) are plotted against their values for IVDOM (mature) in figure 2. It is clear from this figure that 2 families had high values at both stages. For all other characters measured these families, which were unrelated, were intermediate in value.

Some of the remaining relationships between characters are of interest. First, heading date was negatively related to IVDOM at heading (but not at maturity) and positively related to % N at

at maturity (but not at heading). Second, tiller weight was negatively related to % N; the genetic correlations were quite high. Tiller weight showed a low but significant positive association with IVDOM. Third, neither IVDOM nor % N was closely correlated phenotypically with seedling vigour. However, genetic correlations could not be calculated. Fourth, heading date was negatively related to tiller weight.

TABLE 10

STATISTICAL DESCRIPTION OF THE VARIATION IN 8 QUANTITATIVE CHARACTERS AMONG 39 FULL-SIB FAMILIES OF PHALARIS TUBEROSA (CPI 19280). ALL DATA REFER TO FAMILY MEANS BASED ON 3 REPLICATIONS

Character and growth stage	Mean	Range	Standard deviation (σp)	Significance of effect, (a) sires (b) dams in sires		Heritability (h^2) ± S.E.
% N, heading	1.263	1.08-1.42	0.077	NS	NS	0.270 ± 0.198 (NS)
% N, mature	0.511	0.38-0.66	0.066	*	*	0.586 ± 0.119 (***)
% IVDOM, heading	64.2	59.3-68.1	1.96	NS	*	0.545 ± 0.124 (**)
%IVDOM, mature	33.1	27.5-38.8	2.56	***	*	0.782 ± 0.071 (***)
Log_{10} tiller wt. (gx10), heading	1.36	1.18-1.49	0.069	*	***	0.825 ± 0.052 (***)
Log_{10} tiller wt. (gx10), mature	1.32	1.11-1.49	0.075	***	***	0.830 ± 0.057 (***)
Heading date (days after 31/10/68)	20.6	15.8-27.3	2.77	***	***	0.930 ± 0.025 (***)
Normalised seedling score	5.76	5.3 -6.1	0.205	NS	**	0.000 §

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant. §best estimate, based on sire variance only.

TABLE 11

ANALYSIS OF VARIANCE OF IVDOM AND % N AT TWO GROWTH STAGES IN 39 FULL-SIB PHALARIS TUBEROSA FAMILIES

Character	Mean squares and significance of effects due to.....			Interaction sum of squares as a percentage of families sum of squares.
	Growth stages	Families	Families x stages	
IVDOM	56597.3400***	20.1384***	11.0155**	55
% N	33.065442***	0.016036*	0.014914*	93

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

TABLE 12

PHENOTYPIC AND ADDITIVE GENETIC CORRELATIONS AMONG 8 CHARACTERS MEASURED ON 39 FULL-SIB PHALARIS TUBEROSA FAMILIES. CORRELATIONS ABOVE THE DIAGONAL LINE ARE PHENOTYPIC; THOSE BELOW ARE GENETIC. ALL DATA REFER TO FAMILY MEAN VALUES, BASED ON 3 REPLICATIONS.

(H1 and H2 refer to characters measured at heading and mature stages respectively)

Character and growth stage	IVDOM (H1)	IVDOM (H2)	% N (H1)	% N (H2)	Heading date	Tiller wt. (H1)	Tiller wt. (H2)	Seedling score
IVDOM (H1)		0.303	-0.055	-0.167	-0.465**	0.127	0.143	0.031
IVDOM (H2)	0.397*		-0.207	0.235	0.100	0.134	0.090	0.106
% N (H1)	-0.232	-0.552*		0.036	-0.105	-0.076	-0.165	-0.074
% N (H2)	-0.366*	0.217*	-0.271		0.293	-0.395*	-0.339*	-0.132
Heading date	-0.570*	0.111*	-0.079	0.386*		-0.403*	-0.451**	-0.042
Tiller wt. (H1)	0.180*	0.172*	-0.220	-0.535*	-0.475*		0.856***	0.321*
Tiller wt (H2)	0.192*	0.260*	-0.545*	-0.596*	-0.547*	0.967*		0.212

Phenotypic correlations: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Genetic correlations: following McWilliam and Latter (1970), a genetic correlation is taken as significant (indicated *) if the ratio of r_g to its S.E. exceeds 2.0.

TABLE 13

RELATIVE INCREASE IN TWO MAIN CHARACTERS FROM A SINGLE CYCLE OF FAMILY SELECTION FOR EACH OF SEVERAL ALTERNATIVE CRITERIA

Main Character	Increase in main character, relative to response from selecting for main character= 100, when selection of equal intensity is based on.....			
	% N, heading	% N, mature	IVDOM, heading	IVDOM, mature
% N, mature	-18	100	-35	25
IVDOM, mature	-32	19	33	100

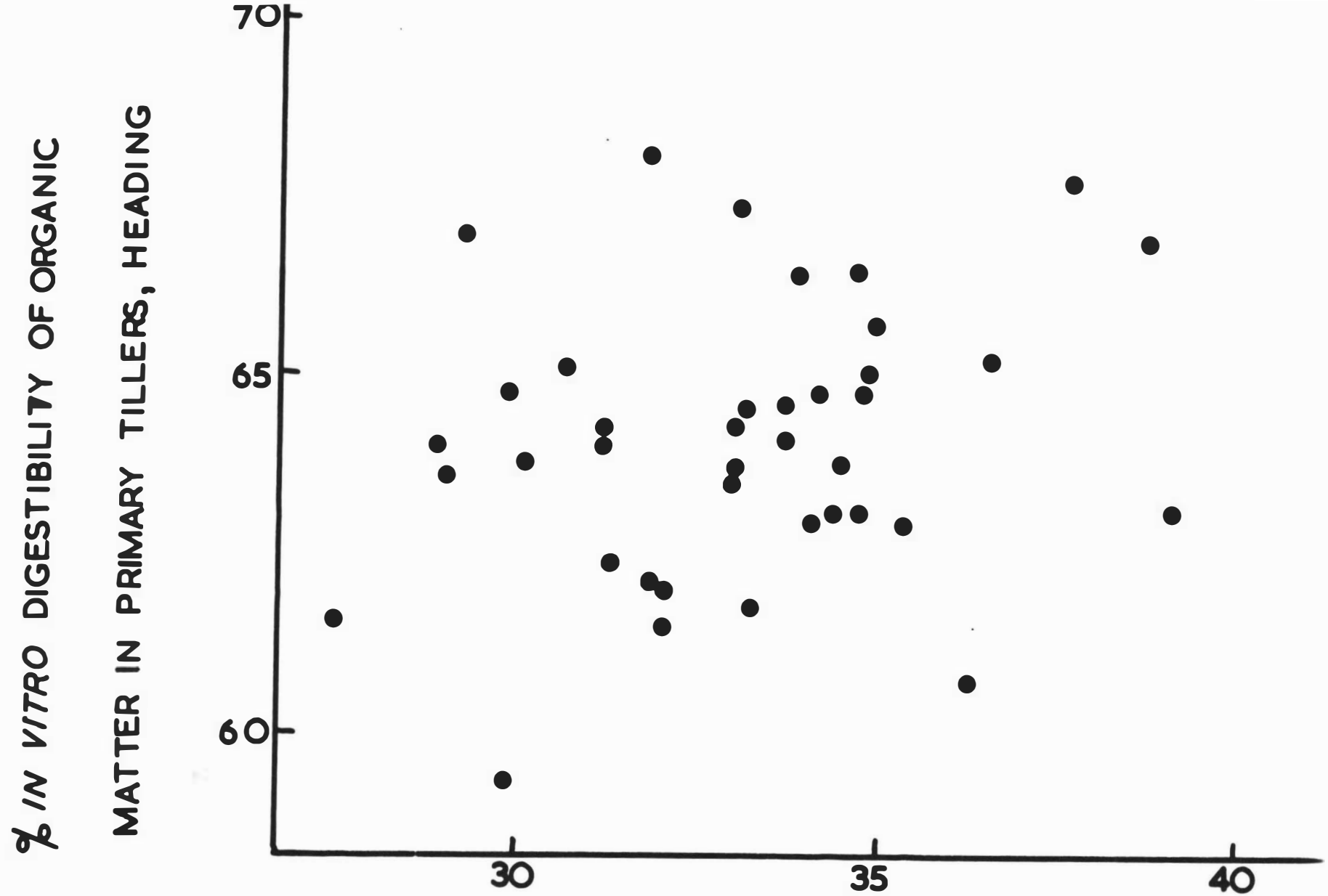

Figure 2. - IVDOM (heading) plotted against IVDOM (mature) for 39 P. tuberosa families.

DISCUSSION

The extensive variation for IVDOM at both the heading and mature stages, together with the high heritability estimates indicate that selection would produce a marked improvement in these characters. Providing the heritabilities and standard deviations remain constant, selecting the best 10% of the families should raise IVDOM of the subsequent generations by 1.87 and 3.50 digestibility units respectively for each cycle of selection. The range in IVDOM between families at heading (59.3-68.1, or 8.8 units) is greater than the range between P. tuberosa strains observed in experiment 1 (61.0-67.7, or 6.7 units). Selection for IVDOM at heading would probably also alter the heading date (although some improvement could be achieved holding heading date constant), and selection for IVDOM of mature herbage might reduce % N at heading. It will be shown later (experiment 4) that in another population, selection for % N at heading did in fact reduce IVDOM of mature herbage. Both of these likely correlated responses would need to be kept in mind in a breeding program. The mean % N level of the population at heading (1.26) was not high.

Prospects for increasing % N by selection are less promising. At heading, heritability of % N was low (0.27), but at the mature stage the heritability was much higher (0.59). A cycle of 10% family selection should raise levels in the subsequent generation by 0.04 and 0.07 % N at the heading and mature stages respectively. These expected gains are small; about 7-8 generations of selection would be required to raise the nitrogen concentration of the mature herbage to 1%, a figure often taken as the minimum dietary requirement for ruminants (see earlier literature review). If each selection cycle required 2 years - as seems likely, since family performance for % N of mature herbage could not be predicted in advance of flowering - increasing the % N of mature herbage to an acceptable level could take up to 15 years. However, it must be remembered that in P. tuberosa pastures animals select herbage with a higher % N than the mean of the available pasture (Arnold 1960a, b). Thus even a small improvement in % N of mature herbage might mean the difference between a positive and a negative nitrogen balance for the grazing animal.

In this ecotype, % N is negatively correlated with tiller weight and therefore possibly with plant and pasture dry matter yield. In the following experiments it will be shown that such a relationship is also found in other P.tuberosa populations, and that selection for

% N results in correlated decreases in tiller weight. The present data support our earlier results (Clements, Oram and Scowcroft 1970) which showed negative phenotypic correlations between % N (heading) and yield at heading and preceding stages among 21 P. tuberosa strains. However, previously we had not observed close phenotypic relationships between % N of mature herbage and dry matter yields at maturity or preceding stages. In the present experiment, the phenotypic correlations between % N and tiller weight are low, but genetic correlations are comparatively high. Similar negative associations between nitrogen content and herbage yield have recently been reported in other species (see literature review).

There are no comparable estimates of heritability of mature herbage quality in the literature. Christie and Mowat (1968) reported broad-sense heritability of in vitro dry matter digestibility (IVDDM) of Bromus inermis at anthesis to be 0.73, 0.60, 0.62 and 0.73 for leaves, stems, vegetative tillers and whole plants respectively. At earlier stages, heritabilities of crude protein content and IVDDM (or IVDOM) have been estimated as 0.14 to 0.75 and -0.29 to 0.69 respectively in Lolium perenne (Cooper 1961; Cooper et al 1962; Rogers and Thomson 1970); 0.53 to 0.69 and 0.52 to 0.53 respectively in Dactylis glomerata (Cooper 1961; Cooper et al 1962); and 0.19 to 0.41 and 0.06 to 0.66 respectively in Phalaris arundinacea (Asay et al 1968; Carlson et al 1969). Other authors have reported significant general combining ability for one or both characters in Dactylis glomerata (Knight and Yates 1968) and Medicago sativa (Lamprecht and Stevens 1964; Lamprecht et al 1965; Gil et al 1967).

The highly significant family x growth stage interactions for % N and IVDOM agree with the P.tuberosa ecotype x stage interactions reported earlier (Clements, Oram and Scowcroft 1970). It is possible to deduce from other published work that similar interactions may well occur in B. inermis (Pickett 1950), M.sativa (Troelsen and Campbell 1969), D. glomerata (Mowat et al 1965b; Julén and Lager 1966) and other species. Such interactions could conceivably be caused by regular differences between genotypes in proportions of leaf and stem. It is now well known that stem digestibility is initially higher than leaf digestibility, but declines more rapidly with time (Pritchard et al 1963; Terry and Tilley 1964; Mowat et al 1965a; Walters et al 1967; Dent and Aldrich 1968). However, in P. tuberosa, digestibility and % N at both the heading and ripe seeds stages are higher for leaves

than stems (Clements, Oram and Scowcroft 1970), so that both characters may well have been negatively correlated with stem: leaf ratio at each sampling stage in the present experiment. The family x stage interactions could therefore be due to changes in the relative ranking of families for stem: leaf ratio between harvests (e.g. reflecting differences in dry leaf retention), or to other factors quite unrelated to this ratio.

The present experiment was restricted to a single environment and only one year. Furthermore, families were grown as spaced plants and were not defoliated. However, the results agree remarkably well with those previously obtained for both spaced plants (Clements, Oram and Scowcroft 1970) and swards (Oram, Clements and McWilliam, unpublished) in a different environment and in two different years.

EXPERIMENT 3

SELECTION FOR HIGH AND LOW NITROGEN CONTENT IN AN ADVANCED PHALARIS TUBEROSA BREEDING POPULATION

INTRODUCTION

Many of the important temperate and Mediterranean pasture species have been subjected to intensive selection by plant breeders for such characters as herbage yield, seedling vigour, persistence and adaptation. Consequently, existing commercial or experimental cultivars and advanced breeding populations are likely to have high levels of selected characters. Plant breeders may be understandably reluctant to sacrifice the proportion of this superiority which would be lost if selected material was crossed with relatively inferior material in order to incorporate other characters into the breeding population. It is often possible to select instead within existing elite populations or cultivars and thus (in the absence of unfavourable correlated responses) maintain current levels of previously selected characters while improving other traits.

Such an alternative is of interest for Phalaris tuberosa. The existing breeding populations in Australia were established in the late 1950s and have since been intensively selected for yield, seedling vigour, seed retention and adaptation (e.g. McWilliam 1963; McWilliam et al 1965; McWilliam and Latter 1970; Oram unpublished). The relatively nutritious ecotype described in experiments 1 and 2 has good seedling vigour but is inferior in this and especially other characters to the breeding populations. Furthermore, the wide genetic base of CSIRO breeding material suggests that many of the populations may contain considerable genetic variability for herbage nutritive value, and significant variation has in fact been found in one of the populations (Oram, Clements and McWilliam, unpublished). Heritability of crude protein content (%N x 6.25) at about the heading stage in full-sib family swards was 0.54 (for a family mean based on 4 replicates). However, although %N was positively genetically correlated with digestibility (r_g = 0.48), it was negatively correlated with yield, particularly with winter yield in the year of sowing (r_g = - 0.55). This latter correlation was thought to reflect a negative genetic association between % N and seedling vigour.

The present experiment describes the effects of selection for % N of P.tuberosa plants measured at heading. The material chosen was a broadly-based, advanced breeding population (McWilliam and Latter 1970) which had previously been selected for yield and seedling vigour. The same population at an earlier evolutionary stage had provided the unpublished data previously referred to. Selection was carried out in controlled environments to shorten the generation interval and reduce the environmental variation, and hence increase the accuracy of selection and the rate of response. The heading stage was chosen because it was considered to be the latest distinct growth stage in the reproductive cycle at which a reasonably large plant population could be analysed for % N in advance of flowering.

MATERIALS AND METHODS

These experiments were conducted in the Controlled Environment Research Laboratory (CERES) at Canberra. Plants were grown in perlite or in perlite-vermiculite mixtures, and were watered twice daily, once with Hoagland's solution and once with water.

(a) Selection for Crude Protein Content

A representative sample of seeds taken from this base population was prechilled at 4°C for 5 days and germinated at 25° in the dark. A random sample of 140 seedlings was planted in 5 by 5 cm peat cups and grown at an alternating temperature (21/16° day/night) in natural light with the photoperiod extended to 16 hr by low intensity incandescent light (40 f.c.) until all plants had produced at least three fully emerged leaves. Seedlings were then vernalized for 5 weeks in short days (8 hr) at 9/4° in natural light. Following cold treatment, plants were transferred to 4-in. plastic pots and grown at 24/19° in long days as before. Elongating tillers were harvested at ground level at heading, i.e. as soon as the inflorescence emerged clear of the flag leaf. This occurred 3-7 weeks after transferring plants from the cold treatment. Harvested tillers were dried for 24 hr at 80° in a forced-draught oven, weighed, and ground. Sampled material from the earliest tiller on each plant was analysed for nitrogen content, except where the first tiller was obviously abnormal. Occasionally more than one tiller was sampled, especially in later generations. Total nitrogen content was measured by the Kjeldahl technique (Appendix 1).

The 10 plants with the highest and the 10 with the lowest percentages of nitrogen were selected as parents for the high and low selection lines respectively, and 10 plants were taken at random to establish a control (unselected) line. The flowering of plants within each line was synchronized by using cool and warm glasshouses; lines were recombined in isolation at 24/19° and seeds were produced by random pollination within each line.

From the seed produced, equal numbers of progeny from each ovule parent were planted to provide c. 100 first selected generation (S_1) plants in each of the high (H), low (L), and control (C) lines.

Later generations of plants were grown and measured in an essentially similar manner. The vernalization period was increased to 6 weeks after the S_0 generation, and a Technicon autoanalyser was used to measure nitrogen content in the S_2 generation. The generation interval was 5-6 months, allowing two cycles of selection per year.

Realized heritabilities were calculated as described by Falconer (1960), after homogeneity of variances within lines and generations was demonstrated, and selection differentials were weighed for maternal parent contribution to the generation concerned (Latter, personal communication). In estimating heritabilities, corrected selection differentials were measured as deviations from random control differentials, and the regression lines of selection response on selection differential were corrected to pass through zero after appropriate tests. Coefficients of inbreeding were estimated as described by Falconer (1960).

(b) Measurement of Correlated Responses

In each cycle of selection, weights of harvested tillers and of their leaves, and time from the end of cold treatment to heading, were recorded for each plant. During the fourth (S_3) cycle additional measurements were made on each plant; these included tiller number, length of harvested tillers, and number of leaves per harvested tiller (see table 15).

Equal weights of ground herbage from each plant were bulked within lines in each generation to provide material for other chemical and biological analyses. These included *in vitro* dry matter digestibility (IVD), normal acid fibre (NAF)(ap Griffith and Thomas 1955), and water-soluble carbohydrate (WSC) by a modified anthrone method (K.R. Christian, personal communication).

In the S_3 generation seedling characters were examined in replicated experiments. Seedlings were grown in 3-in. plastic pots at 15/10° in natural light and watered daily with Hoagland's solution. They were measured for leaf lengths and widths; leaf area, measured at the three-leaf stage by length x width regression (appendix 7) and at the five-leaf stage with an airflow planimeter (Jenkins 1959); rate of leaf appearance from fourth to sixth leaf; and dry weight of tops and roots at both three-leaf and five-leaf stages. All measurements were made on 30 seedlings per line, 5 randomly chosen half-sib families in each line being used. The measurements allowed relative growth rate, relative rate of leaf area expansion, and specific leaf weight (weight per unit area; Hunt and Cooper 1967) to be calculated.

RESULTS

(a) Response to Selection

Three generations of selection for high and low percentage nitrogen resulted in a highly significant divergence of selected population means (3.3 and 2.7% N respectively) from the control mean of 3.0% (table 14, fig. 3). Although the S_3 distributions of percentage nitrogen for the three populations overlapped, the total range of values slightly increased.

The higher corrected selection differential in the high direction (fig. 3) was due to random fluctuations in the selection differential in the control population (see table 14) and was not due to lower phenotypic variation in the low line. In fact over all generations the variation in all populations was similar, and there was no evidence of any decline with selection.

Realized heritabilities and the regression equations from which they were calculated are presented in table 14 and figure 3 respectively. Since the realized heritabilities in each direction were not significantly different, the data were pooled to calculate a combined estimate, $h^2 = 0.221$.

The inbreeding coefficients presented in Table 14 are probably underestimates. Robertson (1961) and Gill and Clemmer (1966) have shown that selection and linkage each increase the rate of inbreeding, so that the true F values may be higher in the selected than in the control population. However, the increase in inbreeding due to population size is similar in all lines.

(b) Correlated Responses in Characters Measured at Heading

Values of a number of morphological and physiological characters measured at heading in selected lines deviated from the control mean (table 15, fig 4). Tiller length and weight increased in the low line and leafiness (percentage leaf) steadily decreased. In the high line, tiller weight fell in the first two selected generations and leafiness increased (see fig 4), but in the S_3 generation these two characters did not differ significantly from control values. Over all lines and generations, there was a consistent and highly significant negative relationship between % N and tiller weight (table 16).

Of special interest are changes in characters associated with nutritive value (fig. 4). Correlations between these characters and % N, estimated over all lines and generations, are tabulated (table 16). Significant differences occurred in WSC and IVD, but not in NAF, between selected lines in each generation. Over all generations, % N was significantly correlated with IVD ($r = 0.815$) and NAF ($r = -0.670$) but not with WSC ($r = -0.133$). The latter lack of correlation probably reflects the susceptibility of WSC to temporary environmental influences.

(c) Correlated Responses in Seedling Characters

Selection resulted in marked changes in seedling characteristics of both the high and the low lines (table 17). The overall effect was a reduction of seedling vigour in the high line and an increase in the low line. There were significant negative correlations between % N and almost all indicators of seedling vigour. In addition, the selection lines differed significantly in relative growth rates and specific leaf weight (control lines being consistently intermediate) and % N was negatively correlated with these characters (table 17).

The % N in S_2 seedlings at 4 weeks did not differ between lines (data not presented). Similar seedling measurements have since been made in the S_3 and S_4 generations (see experiments 6 and 7), and lines have been found to differ.

TABLE 14

RESPONSE TO SELECTION FOR HIGH AND LOW TOTAL NITROGEN CONCENTRATION: S_3 GENERATION LINE MEANS, ACCUMULATED SELECTION DIFFERENTIALS AND INBREEDING COEFFICIENTS, AND REALIZED HERITABILITIES

Line	Percentage of nitrogen ± SE	Coefficient of inbreeding (F)	Accumulated Selection Intensity (i)*	Realized Heritability (h^2) ± SE	
High (H)	3.323 ± 0.037	0.1452	4.696	0.246 ± 0.032	0.221 ± 0.049†
Control (C)	3.007 ± 0.029	0.1361	-0.742		
Low (L)	2.727 ± 0.031	0.1370	-4.654	0.196 ± 0.090	

*$i = \frac{\text{Mean of selected parents - mean of population from which they came}}{\text{Standard deviation of population}}$ (Falconer 1960).

† Combined estimate (see text).

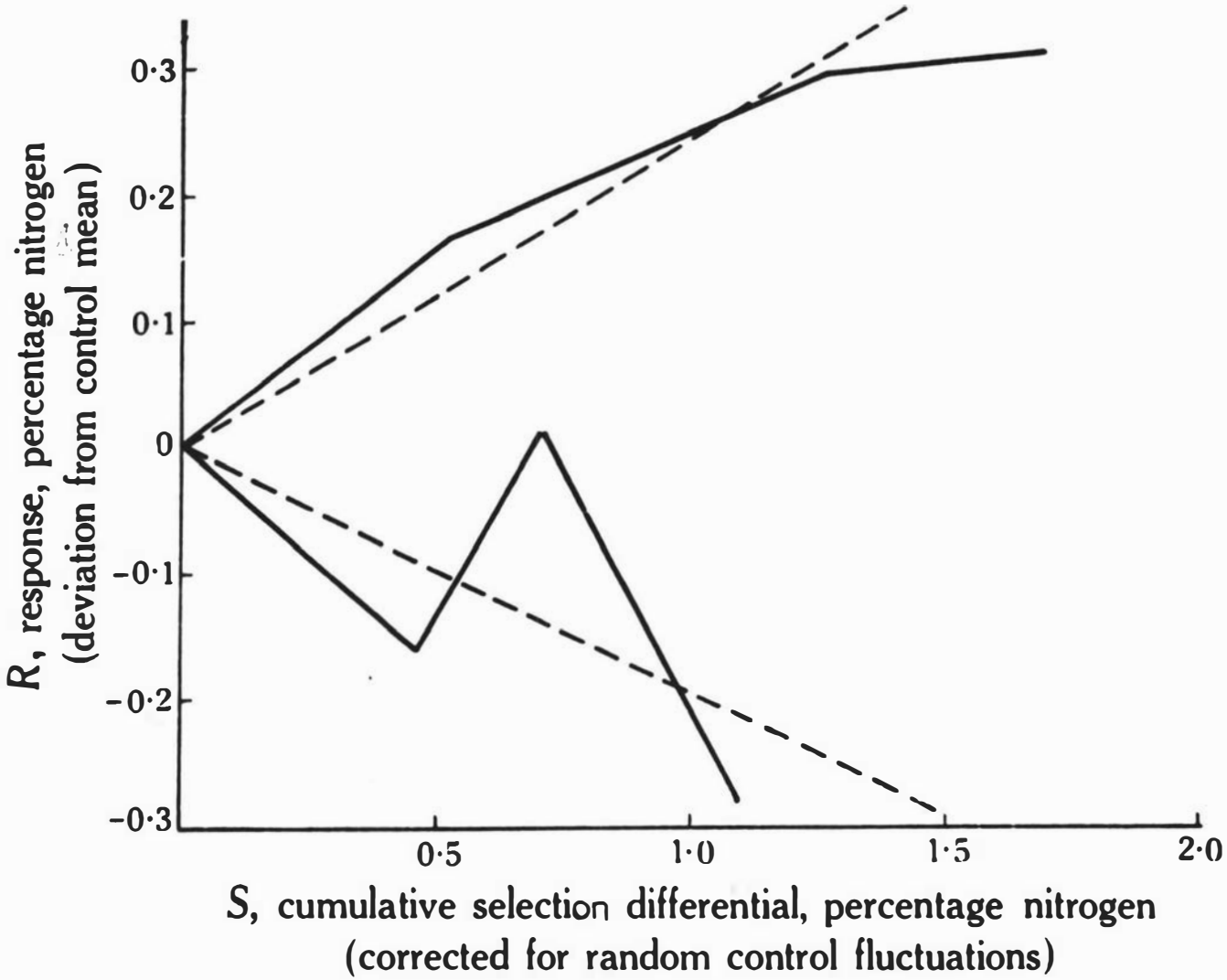

Fig. 3.—Response to selection for high and low percentage nitrogen in whole tillers at heading.

——— Observed response.

– – – – Fitted line.

Equations for fitted lines:

High nitrogen : $R = 0{\cdot}246S$.

Low nitrogen : $R = -0{\cdot}196S$.

TABLE 15

DIFFERENCES BETWEEN S_3 LINES FOR PLANT CHARACTERS MEASURED AT HEADING

Character	Line Mean Values ± SE			Line Diffs. Significant at $P < 0.05$
	High	Control	Low	
Days from cold treatment to heading	29.1 ± 0.5	26.7 ± 0.6	25.4 ± 0.6	H > C,L
Length of reproductive tillers (cm)	54.5 ± 1.1	52.6 ± 1.1	60.6 ± 1.2	L > C,H
No. of reproductive tillers per plant	12.9 ± 0.6	9.8 ± 0.3	10.8 ± 0.2	H > L > C
No. of vegetative or new tillers per plant	7.6 ± 1.0	7.6 ± 0.7	5.4 ± 0.7	C > L
No. of leaves per reproductive tiller	7.8 ± 0.2	7.0 ± 0.1	7.6 ± 0.2	H,L > C
Leaf weight per reproductive tiller (mg)	324 ± 15	346 ± 14	447 ± 16	L > H,C
Weight per reproductive tiller (mg)	885 ± 39	860 ± 35	1192 ± 44	L > H,C
Percentage leaf per reproductive tiller	39.6 ± 0.4	40.7 ± 0.4	37.0 ± 0.7	H,C > L

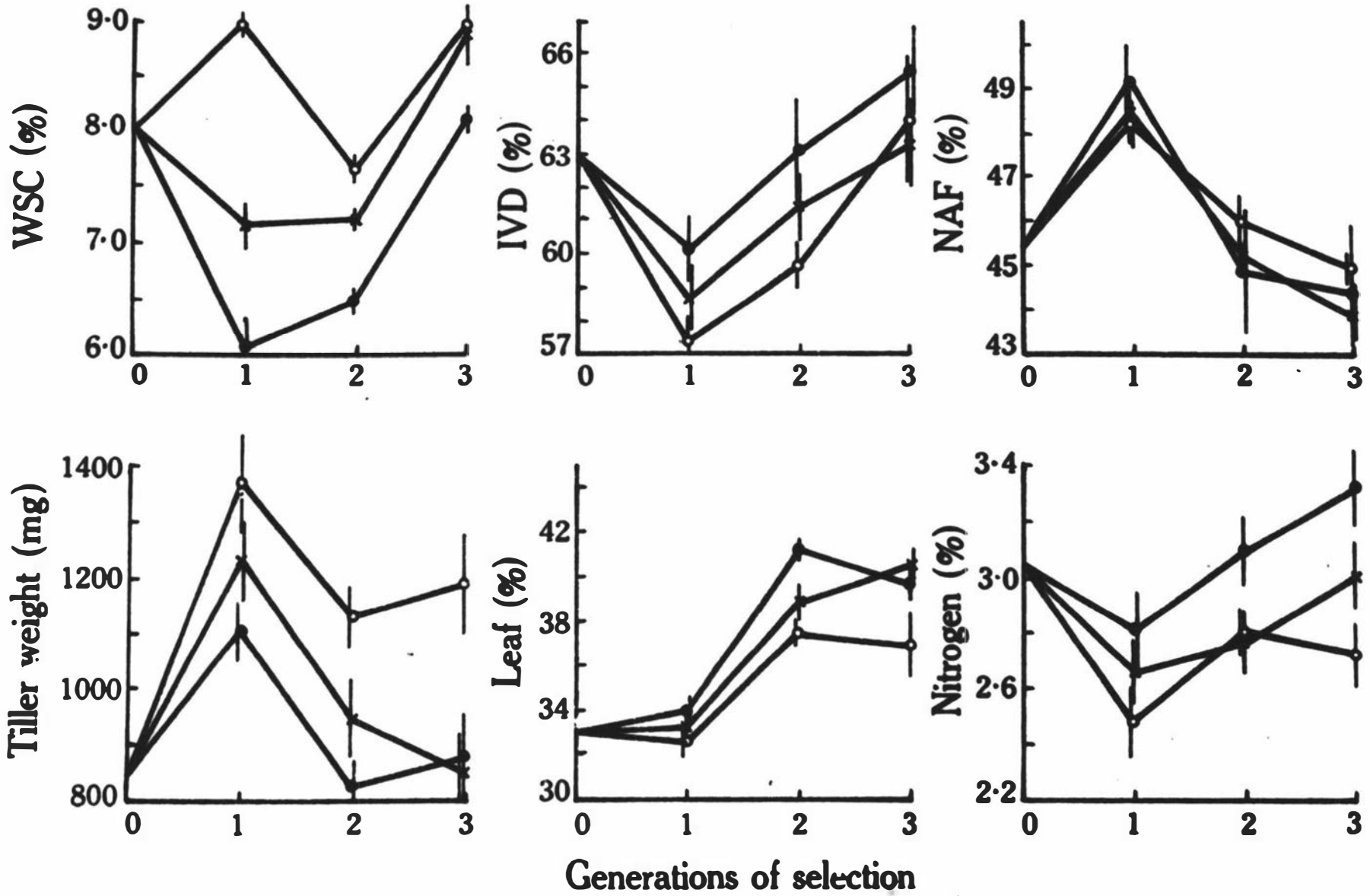

Fig. 4.– **Changes in percentage nitrogen and associated characters per cycle of selection. Vertical lines indicate ±2SE. ○ Low line; × control line; ● high line.**

TABLE 16

CORRELATIONS AMONG CHEMICAL AND MORPHOLOGICAL CHARACTERS AT HEADING OVER ALL LINES AND GENERATIONS

Total of 10 populations

Character	IVD	WSC	NAF	Leafiness	Tiller Wt.
% Nitrogen	0.815**	-0.133	-0.670*	0.444	-0.883***
IVD		0.165	-0.838**	0.534	-0.753*
WSC			-0.371	-0.058	0.195
NAF				-0.734*	0.733*
Leafiness					-0.597

* $P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

TABLE 17

CORRELATIONS BETWEEN SEEDLING CHARACTERS AND PERCENTAGE NITROGEN AT HEADING, S_3 GENERATION

Character	Correlation
Relative growth rate of roots	-0.59*
Relative growth rate of tops	-0.58*
Relative growth rate, whole plants	-0.60*
Rate of leaf appearance	0.25
Log plant weight, 3-leaf stage	-0.54*
Log plant weight, 5-leaf stage	-0.71**
Relative rate of leaf area expansion	-0.39
Specific leaf area	-0.61*

* $P < 0.05$. ** $P < 0.01$.

DISCUSSION

In this experiment, selection for high and low %N in whole tillers at heading resulted in significant responses, which were accompanied by changes in other characters. Realized heritabilities were similar in the high and low lines.

The overall benefit from selection for any character depends not only on improvement of that character but also on correlated changes in other desirable or undesirable attributes. Thus in these selection lines, improvement in crude protein content must be considered against a reduction in seedling vigour and tiller weight when evaluating agricultural merit. Low seedling vigour is a major weakness of P. tuberosa as a pasture plant, and seedling vigour is one of the selection objectives in current breeding programmes.

In this breeding population, these results and those of Oram, Clements and McWilliam (unpublished data) demonstrate a consistent negative correlation between seedling vigour and nutritive value characters. In experiment 2, a negative genetic correlation between %N and tiller weight was observed in an Algerian ecotype of P.tuberosa, and negative phenotypic correlations between %N and yield have been observed among 21 strains grown as spaced plants (Clements, Oram and Scowcroft 1970). Scurfield and Biddiscombe (1966) examined 54 introduced lines of P. tuberosa during the seedling stage and concluded that percentage nitrogen was not related to the yield of individual lines. However, their results demonstrate a close negative association. Thus, unfavourable relationships between %N and various indicators of plant vigour have been demonstrated in several different populations and it seems reasonable to suggest that this is a general phenomenon in this species. Further evidence will be given in experiments 4-7. There are indications that similar relationships may occur in other species; this has been discussed elsewhere (see literature review) and need not be reiterated here.

The results of this experiment allow some speculation on the possible reasons for variation in seedling vigour and tiller weight. Levels of inbreeding are similar, at least in the selected lines, and inbreeding depression can therefore be discounted. Some variation in vigour was probably due to seed weight differences which may in turn have been due to a maternal genotype effect or some environmental component. The change in the time from the conclusion of vernalization to heading in these lines (table 15) suggests that heading and

flowering date may have been changed by selection. Date of ear emergence is negatively correlated genetically with seed weight in Australian Commercial P. tuberosa (r_g = -0.24; Latter 1965).

Some variation in seedling vigour in this experiment, however, was due to differences between lines in relative growth rates. There was in addition a great deal of variation in relative growth rates between half-sib families within lines, which suggests that there is considerable scope for further correlated changes (or for selection in breeding programmes). The variation in relative growth rate of whole plants was correlated with specific leaf weight (r = 0.5). and there are indications that the latter character is associated with chlorophyll concentration per unit leaf area (Hunt and Cooper 1967).

The relationship between %N and seedling vigour is described in detail in experiments 6, 7 and 9. The association between %N and tiller weight, and its consequences in terms of herbage yield are further examined in experiments 4, 5 and 8.

The fluctuations in control line mean values (fig.4) for nutritive value characters (including %N) in each generation deserve comment. They are thought to be largely due to variation in light intensity at different times of the year, since this was the only major environmental factor that was not controlled. Changes in WSC, %N, IVD and NAF are known to result from variation in light intensity (see, for example, Jones 1961; Alberda 1965; Deinum 1966; Nowakowski and Cunningham 1966; Deinum et al 1968).

Correlated responses in nutritive value characters (fig. 4) were generally as expected. Oram, Clements, and McWilliam (unpublished data) found negative and positive genetic correlations respectively between % N and NAF (r_g = -0.48) and between % N and IVD (r_g = 0.48) in this breeding population, and Cooper (1961) has reported negative genetic correlations between % N and WSC in Lolium perenne and Dactylis glomerata. In the present experiment, there were no significant changes between selected lines for NAF (although the difference between C and L lines in the S_3 generation just reached significance) but changes in WSC and IVD occurred in the expected directions in the S_1 and S_2 generations. Over all generations and lines, a close relationship (r_p = 0.815) between % N and IVD was found. However, in the S_3 there were no significant line differences in IVD, and line differences in WSC were markedly lower than those for earlier generations. It will be shown in experiments 4 and 5 that the early (S_1 and S_2) correlated

changes in IVD were not maintained in the S_3 and a later (S_4) generation under field conditions. In experiment 2, three of the four genetic correlations between % N and IVDOM were negative (one nonsignificantly) and only one was positive. Other workers have usually not found % N and digestibility to be closely related genetically (Gil et al 1967; Burton et al 1967; Roth et al 1970; Rogers and Thomson 1970). The result here in the S_1 and S_2 generations may reflect transient linkage between genes affecting the two characters.

The breeding population which was used for this experiment clearly contains genetic variation for % N and responds readily to selection for this character. However, there are obvious dangers in extrapolating from controlled environment to field situations and from one growth stage to another. In the following two experiments the field performance of the lines will be described.

EXPERIMENT 4

FIELD STUDIES ON SPACED PLANTS OF PHALARIS TUBEROSA SELECTION LINES GENETICALLY DIFFERENT IN NITROGEN CONTENT

INTRODUCTION

In the previous experiment, the development of selected lines of Phalaris tuberosa with high, low and "random" (control) percentage nitrogen in whole tillers at heading was outlined. The present experiment was designed to examine the performance of the selection lines grown as spaced plants in the field. In particular, information was sought on aspects of plant morphology and physiology suspected of contributing to line and plant differences in %N; on differences between lines with respect to nitrogen concentration of plant parts (leaf blade, leaf sheath, stem and head fractions); on differences between lines in %N and herbage digestibility at different plant growth stages; and on the nature of the relationship between %N and tiller weight. In addition to these major aims it was intended to estimate the residual genetic variability of %N within the high and low lines. Variation in digestibility of individual plants at the heading stage was assessed, and its relationship with %N and other characters was examined.

MATERIALS AND METHODS

(a) Design and Establishment of the Experiment

Nine-week-old seedlings from the S_4 generations of the high N, low N and control N selection lines were transplanted from seed boxes to the field in autumn 1968 (May 5). The site was adjacent to those used for experiments 1 and 2, and soil type and conditions were the same. Each line was represented by a 20-plant row in each of ten replicates in a randomised block design. Plants were spaced at 60cm intervals within rows 100cm apart, and were maintained without defoliation (other than sampling) during the experiment. Care was taken to ensure that, within lines, progeny of each maternal parent were included in each replicate, and the maternal

parentage of each plant in the experiment was recorded.

(b) Sampling Procedures and Data Collection

Plants were sampled at 5 stages during their first year of growth. At the first and last stages (referred to as the vegetative and mature stages), all plants were sampled on September 1, 1968 and March 23, 1969 respectively. The remaining samples were taken successively from each plant as it reached a defined stage in the reproductive growth phase. These were the preheading stage (defined as the date of flag leaf appearance, which occurred on average 21, 22 and 23 days prior to heading in the high, control and low N lines respectively); the heading stage, as defined in previous experiments; and anthesis, which occurred on average 23, 21 and 22 days after heading in the high, control and low N lines respectively. The mature stage was similar to that described in experiment 2; leaves, heads and upper internodes were dead, but lower leaf sheaths and internodes were green and sappy. New vegetative growth was usually negligible, i.e. most plants were dormant.

At each sampling, several early tillers (enough to provide 10-30 gm dry matter) at the defined growth stage were taken from each plant. Tillers were cut by hand into short segments (2-3 cm in length) either before (later stages) or after (earlier stages) drying in a forced-draught oven at 80°C and weighing. Except at the heading stage, material for subsequent analysis was prepared by bulking equal (weighed) amounts of dried, chopped herbage from each plant to provide a single sample for each line in each of the ten replicates. Bulk samples at these stages and samples from individual plants at the heading stage were ground in an Apex cutter mill to pass through a 1mm screen and analysed for Kjeldahl nitrogen content (%N, dry matter basis) as described in appendix 1. Bulk samples at the heading stage were prepared from the ground individual plant material, and the bulk samples from all 5 stages together with a number of single-plant samples at the heading stage were analysed for *in vitro* digestibility of organic matter (IVDOM) as described in appendix 2.

Additional tillers were collected from each plant at the heading stage. As before, only tillers at the defined stage were taken. Measurements on several tillers from each plant were pooled to provide most of the morphological and physiological data for tables 19-21, 23 and 26. Tiller length (including head) was

measured to the nearest centimetre, and the numbers of green leaves and elongated internodes (i.e. greater than 2 cm in length) were counted. Each tiller was then separated into leaf, sheath; stem and head portions. The lengths of the penultimate internodes were recorded. Widths of the penultimate and lowest elongated internodes were obtained by lying the internodes in each category from each tiller side by side and measuring the total width to the nearest millimetre. An "average" internode width was later computed as the mean of these two measurements, and the ratio of tiller length (cm) to average width (mm) was calculated. An "average" internode length was estimated from internode number and tiller length measurements.

Following separation of tillers into their component parts, separated material was dried at 80°C and weighed. Line bulk samples of each fraction were then prepared by taking an equal (weighed) amount of the fraction from each plant in the replicate. For each fraction, there were thus ten bulk samples of each line, and these were analysed for Kjeldahl nitrogen content (%N) as before.

(c) Statistical Analysis

Two aspects of the statistical analysis need some explanation. In relating %N of individual plants to the combined effects of the various morphological and physiological features measured, a multiple linear regression analysis was used. The "step down method" (Snedecor and Cochran 1967) was chosen; variables for which the partial regression coefficient with %N was least significant were successively eliminated until all remaining b values were significant at the 5% level.

The genetic analysis was complicated by inequalities in the subclasses and a significant replicate (block) effect on %N. Initially, two alternative models were fitted. The first model (designated model A) defined each individual measurement in terms of a population mean value, plus a specific dam effect, plus an individual error. The second analysis (model B) contained two additional components, a replicate effect and a dam x replicate interaction. Details of these models and the computations involved are given in appendix 6. A comparison of the two analyses is given in table 22; they gave similar heritability estimates for %N. Because replicate effects were not significant for any other character measured on individual plants, the simpler model (model A) was used for all subsequent genetic analysis.

RESULTS

(a) Nitrogen Content at Different Growth Stages

The high N line had higher %N than the low N line at each of the 5 growth stages (table 18). The superiority of the high N line was most marked at the heading and mature stages, when %N values exceeded those of the low N line by about one third. The control N line was always intermediate, but not always significantly so. The failure of one or other of the selected lines to differ from the control N line at some stages resulted in highly significant line x stage interactions. A result of particular interest was the lack of difference between the high N and control N lines at the vegetative stage.

(b) Morphological and Physiological Characters

At each growth stage, the low N line had heavier tillers than the high N line. Again the control N line was intermediate, but not always significantly superior to the high N line (tables 18, 19). At heading, weights of leaf, sheath, stem and head per tiller were greater for the low N line than other lines; stem weight in the high N line was less than half that in the low N line, but leaf weight per tiller was two thirds that in the low N line. It is clear that selection for high and low %N has produced lines differing markedly in the relative proportions of leaf and stem, the tissues with the highest and lowest %N respectively at this stage (e.g. table 24). The differences between lines in the weight and proportion of stem tissue have been brought about partly by differences in tiller length (which in turn have been caused by changes in the number and length of internodes) and partly by changes in stem thickness. Differences between lines in leaf weight per tiller reflect changes in both the number and weight of individual leaves; high N line plants had smaller and fewer leaves per tiller than low N line plants. Clearly the greater "leafiness" of the high N line on a unit tiller basis was due not to greater leaf production, but to a relatively greater decrease in stem production than leaf production. However, it must be emphasised that the data refer to individual tillers, not to whole plants.

Differences between the high N and low N lines in the proportions of sheath and head, though statistically significant, were negligible by comparison with differences between the selected and control lines. At this growth stage, %N of sheath is similar to that of whole tillers

(table 24), so that selection for high and low %N of whole tillers would not be expected to change the relative proportion of this component except to compensate for changes in other fractions. However, among individual plants of each of the selected lines, %N was more closely correlated with the proportion of sheath than with any other of the morphological/physiological characters examined (table 20).

The relationships between %N and other characters presented in table 20 are informative. First, they show that no one character accounted for more than 25% of the between-plant variation in %N. Differences in %N between the high and low N lines were highly significant after values were adjusted for any of the characters individually. Second, the average within-line correlations were usually smaller in magnitude than the correlations obtained when line groupings were ignored. Since there was much less variation for all characters within lines than in the larger, pooled population, this result is not surprising. Third, the within-line linear regressions of %N on the various characters did not differ significantly between lines for any character. However, the average within-line regression coefficient was often of lower magnitude than the regression obtained when line groupings were ignored. This occurred particularly in equations relating %N (whole tillers) to the weights and proportions of tiller components, and was caused by between-line differences in %N of the components (table 24). Such an interpretation can most easily be understood by considering the two groups of characters which must (in the simplest analysis) account for any changes in %N of whole tillers, i.e. the proportions and %N of the tiller components. Selection for high and low %N (whole tillers) has produced populations which differ partly because of stable relationships between %N (whole tillers) and the proportions of tiller components having different %N values. For each line, linear equations relating %N (whole tillers) to tiller proportions have the same slope but different intercepts, the difference between intercepts reflecting differences in %N of the various components. Wherever the distributions of values for both the dependent and independent variables differ between lines, regressions obtained when line groupings are ignored can be expected to differ from the within-line regressions. In all of these cases, the within-line correlations and regressions have more meaning than those estimated ignoring groups. However, none of the phenotypic correlations in table 20 are of value in predicting likely changes in the characters as a result of continued selection for %N (whole tillers). For this purpose genetic correlations are required.

Multiple linear regression analyses (table 21) showed that a combination of all the morphological and physiological characters measured accounted for only 37% and 50% of the variation in %N (whole tillers) in the high and low N lines respectively, or 54% ignoring line groupings. The remaining variation was presumably due to individual plant differences in %N of tiller components and to errors in measurement. This is discussed later. The significant variables in table 21 were not the same for each line, but since many of the variables were related this is not surprising. However, the fact that at least one third of the 18 variables contributed significantly to each multiple correlation is of interest, illustrating the complex origin of individual plant %N values.

(c) Genetic Analysis

A description of the genetic variation for %N at heading in the high and low N lines is given in table 22. The standard deviation was lower, but the heritability was (nonsignificantly) higher, in the low N line. Expected response to a further cycle of 10% individual selection is therefore similar for both lines (0.100 and 0.124 %N respectively in the high and low N lines, assuming heritabilities to be 0.21 and 0.34 respectively). Although little reliance can be placed on these predictions because of the large standard errors of the heritability estimates, due in turn to the small numbers of dams used to generate the S_4 populations, the heritabilities obtained here are in close agreement with those obtained in earlier generations (experiment 3). Furthermore, dam effects were significant in each line. It seems reasonable therefore to expect some further response to selection in these lines, and the possibility of further correlated changes in the various morphological and physiological characters previously described can be examined. Such associated changes can be predicted if the heritabilities and standard deviations of the correlated characters, and their genetic correlations with %N, are known. The necessary information is given in table 23 for each line. As before, the estimated heritabilities and genetic correlations have large sampling variances; four of the heritability estimates are negative, and little confidence can be placed in any individual statistic. However, the agreement between lines, and within different categories of characters (e.g. weights and proportions of tiller components) is excellent. If the statistics are taken at face value, then it is clear that further changes in %N in these populations will be accompanied

by opposite changes in the weight of all tiller components, since these characters have the required combination of moderate to high heritabilities (0.20 to 0.68), negative genetic correlations with %N (-0.04 to -0.16) and high phenotypic variability (coefficients of variation from 35 to 50%; c.f. tables 19 and 23). Tiller length can be expected to show further correlated response; presumably the components of tiller length, i.e. internode length and number, would also change. It cannot be assumed that the remaining characters would remain at their present levels, but there is little evidence that further changes would occur. Of particular interest is the apparently low heritability of tiller composition (proportions of leaf, sheath and stem, but not head) and its negligible genetic correlation with %N of whole tillers.

(d) Nitrogen Concentration in Tiller Components at Heading

As previously noted, the lines differed in %N of each tiller part at heading (table 24). The high N line had the highest %N values for all four parts, and the low N line had the lowest values for all but the head fraction. The lack of difference between the control and low N lines for %N (head) caused a highly significant line x component interaction for %N (table 25). There was relatively less difference between lines for nitrogen concentration in head than in other parts; in relative terms, nitrogen concentrations in leaf, sheath, stem and head in the high N line exceeded values in the low N line by 19, 23, 37 and 11% respectively.

Among tiller components, leaf and head had the highest %N values and stem and sheath the lowest. Leaf and head, which together accounted for less than one third of tiller weight at this growth stage, contained 55-60% of the total nitrogen in the tillers. Individual tillers in the low N line contained about 50% more total nitrogen than those in the high N line at the heading stage.

(e) In vitro Digestibility

Table 18 shows the IVDOM values of the three lines at five stages of growth. Although its superiority was not always significant the low N line had the highest IVDOM values at each stage. There was very little difference between any of the lines before the heading stage, but from heading onwards the control N line was regularly inferior to both selected lines. The largest - and most nutritionally significant - line differences occurred at the mature stage, when IVDOM of the low N

line was 4.6 and 6.3 digestibility units higher than that of the high and control N lines respectively. During the experiment, IVDOM declined from over 80% in early spring to about 40% in late summer/ early autumn and was significantly lower at each successive growth stage.

In table 26, the relationships between IVDOM at heading and ten other variables are given for a subpopulation of 43 individual plants. The plants were not a completely random sample; instead, the 10 plants with the highest %N values were taken from the high N line, the 10 with the lowest values were chosen from the low N line, and the remainder were taken at random from both lines. For subsequent statistical analysis of these data, the line groupings of the plants were retained, although an overall correlation analysis (ignoring groups) was also carried out. The values of the 43 plants ranged from 56 to 76% IVDOM, with an overall mean of 64% and a standard deviation of 4% IVDOM.

The interpretation of the results in table 26 is as follows. First, the two groups of plants differed in %N and IVDOM. After adjusting to a common %N, the group IVDOM values no longer differed significantly although the adjusted IVDOM values were almost identical with the unadjusted values. The conclusion is that a negative relationship between %N and IVDOM contributed to the observed IVDOM values. Second, despite the negative relationship between %N and IVDOM, some variables were correlated similarly with both of these characters. For example, tiller length and proportion of stem were each correlated negatively with both %N and IVDOM within groups, while proportion of leaf was correlated positively. However, when groups were ignored, the negative relationship between %N and IVDOM masked many of the real, underlying associations between IVDOM and other characters, and the correlations were much lower. The effect can also be seen in the adjusted group means, where "correcting" for many variables actually increased group differences in IVDOM. Third, an inspection of the adjusted group means and the various correlations shows that only 2 of the 10 independent variables (i.e. the proportions of sheath and head) were unrelated to IVDOM. Differences <u>between</u> the groups were closely related to ash content and less closely correlated with heading date and %N. <u>Within</u> the groups, high IVDOM was associated with short, thin stems, high leaf: stem ratios, early maturity and low ash content. Tiller length alone accounted for 40% of the within-group variation in IVDOM, and the partial correlation between IVDOM and tiller length

among the 43 plants with %N held constant was -0.581***. Many of these relationships would not have been apparent from a simpler analysis (e.g. from the correlations with groups disregarded).

TABLE 18

MEAN %N, % IVDOM, % ASH AND DRY WEIGHT OF TILLERS SAMPLED AT FIVE GROWTH STAGES FROM HIGH N (H), LOW N (L) AND CONTROL N (C) SELECTION LINES

Growth stage and selection Line		Selection line mean values (S.E.)			
		% N	% IVDOM	% ASH	Tiller wt. (g)
Vegetative	H	3.32 (0.12)	82.4 (0.3)	7.45 (0.50)	-
	C	3.22 (0.10)	82.4 (0.4)	7.65 (0.24)	-
	L	2.86 (0.10)	84.0 (0.7)	6.47 (0.29)	-
Pre-heading	H	2.19 (0.06)	75.3 (0.5)	8.71 (0.20)	2.42 (0.08)
	C	1.82 (0.04)	76.0 (0.2)	7.02 (0.15)	2.61 (0.07)
	L	1.72 (0.04)	76.9 (0.4)	7.24 (0.15)	4.02 (0.09)
Heading	H	1.42 (0.03)	67.3 (0.6)	8.01 (0.17)	3.49 (0.11)
	C	1.32 (0.04)	65.1 (0.8)	6.60 (0.12)	4.04 (0.11)
	L	1.09 (0.02)	68.9 (0.4)	6.28 (0.14)	6.83 (0.13)
Anthesis	H	1.15 (0.02)	56.2 (0.6)	8.45 (0.17)	4.51 (0.16)
	C	1.05 (0.02)	53.6 (0.6)	6.74 (0.11)	4.76 (0.25)
	L	0.93 (0.01)	56.5 (0.6)	6.99 (0.07)	7.96 (0.12)
Mature	H	0.44 (0.01)	38.0 (0.4)	13.70 (0.43)	2.88 (0.10)
	C	0.41 (0.01)	36.3 (0.7)	10.30 (0.36)	3.24 (0.15)
	L	0.32 (0.01)	42.6 (0.9)	11.11 (0.29)	6.18 (0.16)
Analysis of variance:					
Data transformation		log	nil	log	log
Line differences		***	***	***	***
Growth stage differences		***	***	***	***
Lines x stages		***	***	NS	***

*** $P < 0.001$ NS = not significant

TABLE 19

DIFFERENCES BETWEEN HIGH N, LOW N AND CONTROL (UNSELECTED) LINES IN SEVERAL CHARACTERS MEASURED AT HEADING

(Statistics are based on replication mean values)

Variable	Line mean values §			Significance of line effect	Line differences significant at P < 0.05 §
	H	C	L		
Leaf weight per tiller (gm)	0.86	0.84	1.35	***	L > H,C
Sheath weight per tiller (gm)	0.91	0.96	1.75	***	L > C,H
Stem weight per tiller (gm)	1.39	1.73	3.22	***	L > C > H
Head weight per tiller (gm)	0.33	0.51	0.58	***	L > C > H
Percent leaf	25.2	21.1	20.0	***	H > C > L
Percent sheath	26.1	23.7	25.5	***	H > L > C
Percent stem	39.3	42.6	45.8	***	L > C > H
Percent head	9.5	12.6	8.7	***	C > H > L
Heading date (days after 30/10/68)	55.5	59.8	51.6	***	C > H > L
Penultimate internode length (cm)	16.3	17.0	21.1	***	L > C,H
"Average" internode length (cm)	17.4	20.3	21.0	***	L > C > H
Tiller length (cm)	82.6	95.1	108.4	***	L > C > H
Number of internodes per tiller †	4.8	4.7	5.2	***	L > H,C
Width (mm) at base of tiller	4.6	4.9	5.6	***	L > C > H
Width (mm) of penultimate internode	3.6	3.4	4.4	***	L > H > C
"Average" tiller width (mm)	4.1	4.2	5.0	***	L > C,H
Ratio length: average width	20.2	23.0	21.7	***	C > L > H
Number of green leaves per tiller	15.2	14.5	17.0	***	L > H > C

*** P < 0.001

§ H, C and L denote high N, control N and low N lines respectively

† This refers to elongated internodes (see text)

TABLE 20

LINEAR RELATIONSHIPS BETWEEN % N (WHOLE TILLERS) AND OTHER CHARACTERS MEASURED ON INDIVIDUAL PLANTS AT THE HEADING STAGE

(These statistics are based on measurements of 161 individual plants in the high N line, ignoring replicates, and 193 plants in the low N line)

Character	Average within-line regression of %N on character §	Regression with lines ignored	Average within-line correlation between %N and character §	Correlation with lines ignored	Significance of line differences in %N after fitting regression
Leaf weight per tiller (gm)	-0.0498	-0.2076***	-0.098	-0.377***	***
Sheath weight per tiller (gm)	-0.0789***	-0.1960***	-0.200***	-0.496***	***
Stem weight per tiller (gm)	-0.0134	-0.0754***	-0.074	-0.417***	***
Head weight per tiller (gm)	0.0216	-0.4222***	0.018	-0.341***	***
Percent leaf	0.0093**	0.0310***	0.138**	0.468***	***
Percent sheath	-0.0258***	-0.0182***	-0.317***	-0.178***	***
Percent stem	-0.0012	-0.0195***	-0.024	-0.387***	***
Percent head	0.0322***	0.0499***	0.272***	0.343***	***
Heading date (days after 30/10/68)	-0.0045*	0.0027	-0.133*	0.065	***
Penultimate internode length (cm)	0.0092***	-0.0066*	0.204***	-0.130*	***
"Average" internode length (cm)	0.0087**	-0.0127***	0.140**	-0.182***	***
Tiller length (cm x 10^{-1})	-0.0059	-0.0455***	-0.051	-0.382***	***
Number of internodes per tiller †	-0.1091***	-0.1847***	-0.300***	-0.427***	***
Width (mm) at base of tiller	0.0182	-0.0567***	0.082	-0.229***	***
Width (mm) of penultimate internode	0.0457**	-0.0436**	0.174**	-0.147**	***
"Average" tiller width (mm)	0.0323*	-0.0550***	0.129*	-0.198***	***
Ratio length (cm): average width (mm)	-0.0207***	-0.0353***	-0.257***	-0.360***	***
Number of green leaves per tiller	-0.0015	-0.0225***	-0.018	-0.222***	***
Degrees of freedom	351	352	351	352	1 and 351

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

§ Within-line regression and correlation coefficients did not differ significantly between lines for any character.

† This refers to elongated internodes (see text)

TABLE 21

MULTIPLE LINEAR REGRESSION OF %N (WHOLE TILLERS) OF INDIVIDUAL PLANTS ON THE VARIABLES LISTED IN TABLE 20.

(Partial correlations (r^1) and partial regression coefficients (b^1), when the partial regression coefficients of all remaining variables in the multiple regression equation are significant at $P < 0.05$)

Significant variable	High N line		Low N line		All plants (lines disregarded)	
	r^1	b^1	r^1	b^1	r^1	b^1
Leaf weight (gm) per tiller	0.43	-0.58	0.41	-0.20	0.43	-0.51
Stem weight (gm) per tiller					0.26	0.11
Head weight (gm) per tiller					0.22	-0.42
Percent leaf	0.45	0.04				
Percent sheath			0.54	-0.04	0.55	-0.06
Percent stem			0.45	-0.02	0.52	0.06
Percent head	0.26	0.03				
Heading date (days after 30.10.68)			0.29	-0.01		
Penultimate internode length (cm)	0.39	0.03	0.31	0.01	0.13	0.01
Total tiller length (cm)					0.12	0.003
Width (mm) at base of tiller	0.22	0.10	0.33	0.10		
"Average" tiller width (mm)					0.32	0.15
Number of green leaves per tiller	0.20	0.02				
R (multiple correlation)	0.590***		0.683***		0.733***	
R^2 (coefficient of determination)	0.348		0.467		0.537	
Ftest for significance of regression	13.7***		27.2 ***		50.2 ***	
Degrees of freedom	154		186		345	
Standard error of estimate	0.199		0.138		0.186	
Y intercept	-0.6		3.0		4.6	
R (multiple correlation) with all 18 variables included	0.610***		0.710***		0.737***	

*** $P < 0.001$

TABLE 22

STATISTICAL DESCRIPTION OF THE VARIATION IN %N (WHOLE TILLERS) OF INDIVIDUAL SPACED PLANTS WITHIN HIGH N AND LOW N SELECTION LINES IN THE S_4 GENERATION AT THE HEADING STAGE

Statistic	High N line	Low N line
Population size (individuals measured)	161	193
Population mean, %N ± SE	1.422 ± 0.019	1.091 ± 0.013
Phenotypic standard deviation, %N	0.244	0.187
Range of individual values, %N	0.95 — 2.13	0.65 — 1.55
Range of dam family means, %N	1.20 — 1.53	0.95 — 1.15
F ratio and significance of dam effect, model A	2.226*	3.293**
Coefficient of inbreeding, F	0.1955	0.1820
Heritability (h^2) ± SE, model A	0.200 ± 0.187	0.327 ± 0.217
Heritability (h^2) ± SE, model B	0.222 ± 0.197	0.363 ± 0.230

TABLE 23

GENETIC ANALYSIS OF THE WITHIN-LINE VARIATION IN CHARACTERS MEASURED ON INDIVIDUAL PLANTS AT THE HEADING STAGE (These statistics† are based on measurements of 161 individual plants in the high N line, and 193 plants in the low N line, analysed according to model A (see text), appendix 6)

Character	High N line				Low N line			
	Dam effect	σp	h^2	r_g	Dam effect	σp	h^2	r_g
Leaf weight per tiller (gm)	*	0.37	0.24	-0.16	***	0.47	0.38	-0.12
Sheath weight per tiller (gm)	**	0.42	0.32	-0.14	***	0.63	0.50	-0.11
Stem weight per tiller (gm)	**	0.67	0.32	-0.13	**	1.49	0.33	-0.10
Head weight per tiller (gm)	*	0.15	0.20	-0.14	***	0.20	0.68 §	-0.04
Percent leaf	NS	3.35	0.01	0.07	*	3.03	0.16	0.05
Percent sheath	NS	2.50	0.07	-0.05	NS	2.74	0.03	-0.05
Percent stem	NS	3.70	0.09	0.01	NS	4.67	-0.01	—
Percent head	**	1.75	0.32	-0.00	***	1.87	0.43	0.08
Heading date (days after 30/10/68)	*	7.10	0.22	-0.04	***	5.58	0.72 §	-0.08
Penultimate internode length (cm)	NS	4.92	-0.02	—	NS	4.62	0.06	-0.09
"Average" internode length (cm)	NS	4.01	0.05	-0.13	*	2.90	0.22	-0.10
Tiller length (dm)	NS	1.90	0.11	-0.11	***	1.83	0.55	-0.10
Number of internodes per tiller	*	0.54	0.18	-0.04	***	0.63	0.38	-0.08
Width (mm) at base of tiller	NS	1.09	0.07	-0.19	NS	0.85	0.06	-0.16
Width (mm) of penultimate internode	NS	0.86	-0.07	—	*	0.77	0.17	-0.04
"Average" tiller width (mm)	NS	0.96	-0.01	—	NS	0.77	0.09	-0.10
Ratio length (cm):average width (mm)	**	2.50	0.31	-0.02	***	2.79	0.43	-0.09
Number of green leaves per tiller	NS	2.31	0.12	0.05	*	2.66	0.19	-0.03

† dam effect = significance of variation due to dams; σp = phenotypic standard deviation; h^2 = single-plant heritability; r_g = genetic correlation with % N. *P < 0.05, ** P<0.01, ***P < 0.001 (NS not significant).

A dash indicates a non-estimable r_g value (due to negative h^2 estimate). For simplicity, standard errors of h^2 and r_g are omitted; in only 2 instances (§) was the ratio of either h^2 or r_g to its SE greater than 2.0.

TABLE 24

DIFFERENCES BETWEEN HIGH N, LOW N AND CONTROL (UNSELECTED) LINES IN %N OF WHOLE TILLERS AND TILLER COMPONENTS AT HEADING

Tiller component	%N in component			Significance of line differences	Line differences significant at P < 0.05 §
	high N line	Control N line	Low N line		
Whole tillers	1.420	1.317	1.091	***	H > C > L
Leaf	2.673	2.425	2.253	***	H > C > L
Sheath	1.049	0.963	0.855	***	H > C > L
Stem	0.879	0.740	0.643	***	H > C > L
Head	2.326	2.004	2.094	***	H > L > C

*** P < 0.001

§ H, C and L denote high N, low N and control N lines respectively

TABLE 25

ANALYSIS OF VARIATION IN %N OF TILLER COMPONENTS AND WHOLE TILLERS AT HEADING. DATA WERE TRANSFORMED USING LOGARITHMS

$(X^1 = 100 \log_{10} (X + 1.1))$

Source	Degrees of freedom	Sums of squares	Mean Squares	F ratios
Replications	9	197.175	21.9083	10.913***
Selection lines (L)	2	572.704	286.3520	142.634***
Tiller components (C) §	4	18106.802	4526.7005	2254.782***
L x C interaction	8	86.638	10.8298	5.394***
Error	126	252.961	2.0076	
Total	149	19216.280		

*** $P < 0.001$

§ All tiller components were significantly different in %N. The mean de-transformed values were as follows:

leaf	2.43
sheath	0.95
stem	0.75
head	2.14

TABLE 26

RELATIONSHIPS BETWEEN IVDOM OF WHOLE TILLERS AND SEVERAL OTHER CHARACTERS AMONG 43† INDIVIDUAL PLANTS AT THE HEADING STAGE

Variable ϕ	Correlation with IVDOM		Group mean values		Significance of differences between group means, for....		
	Average within-group §	Disregarding groups	High N group	Low N group	(i) Variable	(ii) IVDOM after adjusting for regression on variable (brackets: adjusted high N and low N group means resp.)	
IVDOM	—	—	62.1	65.5	**	—	—
% N	-0.03	-0.33*	1.73	1.01	***	NS	(62.3,65.3)
% leaf	0.42**	-0.01	26.1	19.2	***	***	(60.1,67.0)
% sheath	-0.05	-0.06	25.5	27.1	*	*	(62.1,65.5)
% stem	-0.38*	0.02	38.4	45.2	***	***	(60.3,66.8)
% head	0.03	-0.12	10.0	8.5	***	*	(62.1,65.5)
Heading date (days)	-0.24	-0.39*	58.4	50.0	***	NS	(62.8,65.0)
Tiller length (cm)	-0.63***	-0.27	76.2	102.0	***	***	(60.1,67.0)
Average tiller width (mm)	-0.47**	-0.13	3.74	4.87	***	***	(60.5,66.7)
Ratio length: width	-0.32*	-0.27	20.5	21.0	NS	**	(62.0,65.6)
% ash	-0.34*	-0.50***	9.5	6.4	***	NS	(63.9,64.2)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant.

† 2 groups, containing 18 plants from the high N line and 25 plants from the low N line respectively (see text)

ϕ see earlier tables for details of these characters.

§ within-group regression and correlation coefficients did not differ significantly between groups for any character.

DISCUSSION

The main results of this experiment were as follows:

(1) %N differences between lines selected for high and low %N (whole tillers) at heading in an artificially controlled environment were maintained in the field under spaced plant conditions at a wide range of growth stages and in different tiller components. The relative differences between these two lines varied to some extent, depending on the growth stage and tiller fraction.

(2) Nitrogen concentration in whole tillers at the heading stage in the high N line was approximately 30% higher than that in the low N line after 4 cycles of selection and crossing. There was considerable residual genetic variation for this character in each line and further response to selection could be expected. Heritability estimates were similar to the realised heritabilities obtained in earlier generations.

(3) Changes in both the proportions and %N of tiller components contributed to the selection response in each selection line. The main difference in tiller composition was a change in the leaf: stem ratio. This was caused by disproportionate changes in the weights of leaf and stem per tiller in the high and low N lines relative to the control N line.

(4) The weight of whole tillers and all tiller components was negatively related to %N. Selection for %N resulted in opposite changes in tiller weight, and further selection would result in further correlated changes.

(5) The low N line was marginally higher in IVDOM than the high N line at most growth stages, and markedly superior for IVDOM of mature herbage. The control N line was lower in IVDOM than both selected lines from the heading stage onwards.

(6) Among individual plants at the heading stage there was a small but significant negative correlation between IVDOM and %N. High IVDOM was associated with short, thin stems, low ash content, early maturity and high leaf: stem ratios. Variation in IVDOM between plants was extensive.

The finding that differences in %N between the high and low N lines were maintained at different growth stages and in different tiller components is important. Previous results (Clements, Oram and Scowcroft 1970; experiment 2) have indicated highly significant genotype x stage interactions for both %N and IVDOM in P.tuberosa. In this experiment, line x stage interactions were again highly significant for both characters, and line x tiller fraction interactions were significant for %N. However, most interactions involved the control N line and one or other of the selected lines. The relative ranking of the high and low N lines for these characters was stable, although differences were not always significant. The lack of a significant difference between the high and control N lines for %N of vegetative herbage agrees with the results obtained in experiment 5.

The ranking of the 3 lines for %N of tiller parts was the same for leaf, sheath and stem fractions but not for head. This result is in agreement with the correlations between %N in the same plant parts described by Clements, Oram and Scowcroft (1970), which showed that nitrogen concentrations in the head portion tend to vary independently of those in other fractions at this growth stage.

Although heritability estimates for %N in each selected line have large standard errors, they agree very well with the realised heritabilities described in experiment 3. The large standard errors are due to the small numbers of parents in each line. The mating system was quite inefficient for estimating heritabilities, but this was not a major aim of the experiment. There were other possible sources of bias. Completely random mating was assumed, and although flowering dates of the parents were synchronised there could have been a small amount of self-pollination. In addition, because of the small numbers of parents (8 and 9 in the high and low lines respectively), the polycross families would have included both half and full sibs. Furthermore, because the field environment was very different to the controlled environment described in experiment 3, genotype x environment interaction could have contributed to the heritabilities estimated here. All

these sources of bias would tend to inflate heritability values, so that the four negative estimates (see table 23) almost certainly reflect random sampling errors due to the small numbers of parents (Gill and Jensen 1968).

The negative genetic relationship between %N and tiller weight was expected (See experiments 2 and 3). The data show that the relationship holds for all tiller components and at all growth stages during the reproductive phase. Although negative associations between %N and plant weight (or herbage yield) have frequently been described (see literature review), the relative contribution of the number of tillers per plant and the weight per individual tiller to these relationships does not seem to have been examined. Lazenby and Rogers (1965) measured %N and tiller weight of 6 Lolium perenne clones grown as spaced plants under a range of nitrogen fertiliser levels for 2 years. Within years, their data can be shown to indicate a weak negative relationship between %N and tiller weight. However, because of the small number of genotypes examined, and because they found %N and yield of spaced plants to be positively related (in conflict with most other results that have been obtained), this particular aspect of their results must be accepted with some caution.

The data in tables 19 and 24 can be manipulated to provide a crude estimate of how much of the between-line variation in %N of whole tillers at heading resulted from differences in the proportions of tiller components and how much was due to differences in %N of these components. As an example of the calculations required, assume that the %N values of the tiller components had not changed with selection, i.e. that the control line values for these components had been maintained. From the observed changes in tiller composition in selected lines, an increase of 0.027%N in the high N line and a decrease of 0.073%N in the low N line, relative to the control value of 1.317%N (whole tillers), would then have been expected. However, the observed changes were 0.103 %N and 0.226 %N respectively, i.e. the expected changes are only 26.2% and 32.3% of the observed changes. The remainder of the difference between %N (whole tillers) of the control and each selected line (73.8% and 67.7% respectively) is due to changes in %N of the tiller components. Comparisons of the expected and observed differences between the high and low N lines can be made similarly by holding %N of tiller components constant, first at

(say) the low N line values and then at the high N line values, calculating the expected differences between the lines resulting from tiller composition differences, and comparing the expected differences with those observed. Alternatively, tiller composition can be held constant instead and the contribution of observed changes in %N of the components can be estimated. When the various estimates obtained in this way are averaged, it is found that about 25% of the difference between the high and low N lines was due to changes in tiller composition and 75% was due to changes in %N of the components. Because the tillers providing the composition data were not the same tillers used for %N determinations, these estimates are only approximate, but they suggest that changes in the proportions of tiller components have had a relatively small effect on whole tiller nitrogen concentration.

Several other pieces of evidence support this conclusion. First, differences between selected lines were maintained in vegetative herbage (table 18; see also experiments 6 and 7) where tiller composition would not be a factor. Second, except for the head fraction (which accounted for only a small proportion of tiller weight), relative differences between lines for %N of tiller components and whole tillers were similar (table 24). Third, phenotypic correlations and regressions (table 20) and genetic correlations (table 23) relating %N to tiller composition indicate that these relationships are weak. Taken as a whole, the results indicate clearly that the main contributing factor to the observed line differences in %N (whole tillers) was the difference between lines in ability to concentrate nitrogen in all tissues. This ability or inability to concentrate nitrogen may possibly apply also to some other elements, since the line differences in % ash (table 18) at each growth stage are to some extent correlated with %N differences. However, more evidence is needed on this possibility before any conclusions are possible.

The line differences in ability to concentrate nitrogen in the tissues were not primarily due to changes in the total amount of nitrogen present in the tillers. A comparison of the high and low N lines showed that tillers in the low line actually contained 50% more nitrogen than those from the high line. In fact, the variation in nitrogen concentration per unit dry weight seems more truly to reflect differences between lines in dry weight production per unit of nitrogen in the tillers. Evidence to

support this interpretation is described in experiments 5-8.

The relationship between %N and IVDOM in these lines is of special interest. The results indicate that the positive genetic correlation between these characters during the early evolutionary history of the lines (experiment 3) has not persisted in later generations. The weak negative association between %N and IVDOM observed here agrees well with the results of experiments 1 and 2. However, among the 43 individual plants examined for IVDOM at the heading stage there were some genotypes with relatively high values for both characters, suggesting that it would be possible to produce populations which also had higher levels of both characters. A similar conclusion was reached in experiments 1 and 2. It is curious that the negative relationship between %N and IVDOM occurred despite the fact that other variables such as leaf: stem ratio and tiller length had similar effects on each of the nutritive value characters.

Mowat et al (1965 a, 1967) found no relationship between stem diameter and digestibility of Bromus inermis and Medicago sativa herbage, while Allinson et al (1969) observed that a thick-stemmed Phalaris arundinacea cultivar was more digestible than a thin-stemmed ecotype. In the present experiment, there was a highly significant negative correlation (r= -0.47**) between average tiller width (i.e. stem diameter) and IVDOM at the heading stage within the high and low N lines, but the overall correlation was nonsignificant (r=-0.13). The partial correlation between the characters, holding %N constant, was also nonsignificant (r=-0.28), suggesting that the within-line correlation gave a misleadingly high estimate of the relationship between tiller width and IVDOM. Tiller length on the other hand was clearly related negatively to IVDOM; the within-line correlation (r=-0.63***) and the partial correlation between these characters holding %N constant (r=-0.58***) were in good agreement. Tiller length is also negatively related to the leaf: stem ratio (table 19), and at the heading stage the digestibility of leaf is greater than that of stem (Clements, Oram and Scowcroft 1970). These factors may account for the correlations observed here. In addition, Pritchard et al (1963) showed that in the species Dactylis glomerata, Bromus inermis and Phleum pratense digestibility was higher in basal stem segments, and that, above 60-70 cm, stem digestibility decreased markedly. Average

tiller length (i.e. height) among the 43 plants measured for IVDOM at heading here ranged from 51 to 158 cm, but although the two extreme plants also showed very large differences in leaf: stem ratio (1.00 and 0.38 respectively) they were similar in IVDOM (63.8% and 60.4% respectively). It is perhaps significant that they differed widely in %N (1.85% and 0.80%).

EXPERIMENT 5

A COMPARISON OF THE YIELD AND NUTRITIVE VALUE OF HERBAGE FROM SWARDS OF *PHALARIS TUBEROSA* LINES PREVIOUSLY SELECTED FOR HIGH AND LOW HERBAGE NITROGEN CONCENTRATION

INTRODUCTION

Most pasture plant breeding programs include an initial phase of evaluation and selection of genotypes grown as spaced plants, based on the assumption that the performance ranking of individual genotypes as spaced plants and in swards will be similar. For some important characters such as disease resistance this is likely to be true. In a highly variable breeding population, it is unlikely that the genotypes with highest herbage yields as spaced plants will yield poorly in swards, but in less variable populations (or in highly selected material) it has been repeatedly shown that sward performance cannot be reliably predicted from yields of widely-spaced plants (see, for example, the short review by Lazenby and Rogers 1962). Consequently it cannot be assumed that differences in herbage nutritive value between genotypes grown as widely spaced plants would be maintained in swards under any given management system. Although most experiments to date have shown little evidence of significant changes in the herbage quality ranking of genotypes grown under different spacings, levels of applied fertiliser and defoliation treatments (see literature review), there are a number of contradictory results in the literature. In addition, the relative nutritive value of genotypes in pure swards and mixtures with other species seems not to have been studied at all.

The present experiment was designed specifically to compare the yield and herbage quality of the *Phalaris tuberosa* lines selected for high and low %N levels (experiments 3, 4). For this purpose small swards of each line were established and the performance of the lines was examined under two quite different defoliation treatments and two levels of applied nitrogen fertiliser. These treatments are not claimed to represent or even to approximate agricultural practices. The amount of seed available was inadequate for studies of the behaviour of the lines under different grazing intensities and managements. Instead, the treatment combinations were chosen to provide an array of environments between which the yield and nutritive value of *Phalaris*

herbage could be expected to differ considerably.

MATERIALS AND METHODS

Seeds for this experiment were obtained by polycrossing the parents which produced the S_3 generation in experiment 3. When seeds for experiment 3 had been collected, these parents were cloned and transplanted to the field at 100 x 100 cm spacing at Canberra on August 3, 1967. Clones were liberally irrigated and fertilised during the spring and transferred to the Canberra Crossatron (McWilliam 1964b) just prior to anthesis (November 20, 1967). One Crossatron cell was allocated to each selection line, and clones of each of the 12 parents of the line were randomly positioned within each of 4 replicates within the cell. The seeds collected from these parents were bulked in each line to provide the quantities necessary for this experiment.

Small swards were established at Palmerston North in spring (October 1) 1968 on a carefully prepared seedbed. Four plots (each 1 x 4 m, or 0.001 ac. in size) of each of the high, low and control N lines were sown in each of 3 replicates by hand-broadcasting and lightly raking a seed/sawdust mixture. The sowing rate of the control N line was 11.2 kg per hectare (10 lb per acre) of viable seed, and sowing rates of the other lines were adjusted to an equivalent viable seed rate (750 seeds per sq. m, or 70 seeds per sq. ft). The site was adjacent to those of experiments 1, 2 and 4 and the soil type was the same (see experiment 1). A basal fertiliser dressing of 250 kg superphosphate, 250 kg calcium ammonium nitrate and 63 kg muriate of potash per hectare (2 cwt, 2 cwt and 0.5 cwt per acre respectively) was applied prior to sowing and a further 750 kg superphosphate and 250 kg potash per hectare was applied to all plots during the experimental period (20 months).

Establishment measured 6 weeks after sowing was uniformly high, averaging 64% on a viable seed basis. During the first 3 months the plots were regularly mown and sprayed to eliminate weeds but there was no attempt to prevent the growth of volunteer white clover (Trifolium repens). At the end of this period of preliminary growth the swards contained a mixture of 75% (by weight) P. tuberosa and 23% T. repens and were virtually weed free (less than 2%). There was no attempt to control weeds after this establishment phase.

Experimental treatments which commenced at the end of 1968 (December 31) consisted of two levels of applied nitrogen fertiliser and two defoliation frequencies. Frequently defoliated plots were cut 3 cm above ground level when the average height of the grass was about 15 cm and infrequently defoliated plots were cut similarly when the grass was approximately 30 cm high. Mown herbage was removed from the plots. The arrangement allowed all plots to be mown together each time the infrequently defoliated plots were cut. The nitrogen treatments were zero (N_0) fertiliser applied, and 56 kg N per hectare (50 lb N per acre) applied as urea (N_1), during the time interval between each mowing of the infrequently cut plots. The same total amount of urea fertiliser was applied to each N_1 plot, as a single dressing following mowing (infrequently defoliated plots) or as a split dressing after each mowing (frequently defoliated plots). The treatments and lines were combined factorially in each replicate, providing a total of 36 plots. In 1970, the frequent defoliation treatment was discontinued, but observations on the infrequently cut plots continued until the spring (September 4).

Pasture samples were cut by hand 3 cm above ground level from an area of 0.19 sq m (2 sq ft) in each plot immediately before mowing, for measurements of yield and botanical composition. Harvested material was separated into P.tuberosa, clover and other species and was dried at 80°C in a forced-draught oven. During 1969, the Phalaris herbage samples at each harvest were ground in an Apex cutter mill to pass a 1 mm screen and analysed for Kjeldahl nitrogen content (%N, dry matter basis) and in vitro digestibility of organic matter (IVDOM) as described in appendices 1 and 2. Tiller numbers were counted during the second half of the experiment on infrequently defoliated plots, with measurement techniques varying to suit the pasture conditions. Some counts in replicated quadrats were always made, although quadrat size varied, and additional information was occasionally obtained from soil core samples, giving a total measurement area of 0.19 sq m (2 sq ft) in each plot at each harvest.

For the purpose of statistical analysis, data from the individual harvests of the frequently defoliated plots were pooled for the time intervals between harvests of the infrequently cut plots. Data transformation was frequently necessary and the transformations are indicated in the tables.

The general features of the Palmerston North climate have been

described by various authors (e.g. Sears 1953). Rainfall during the experiment is given in appendix 8. An unusual feature was the marked drought from late December 1969 to mid-March 1970.

RESULTS

(a) Botanical Composition and Herbage Yield

A particular feature of this experiment was the inability of P.tuberosa to persist in the frequently cut swards (figure 5). After 12 months the proportion of Phalaris in these swards had declined to less than 10%, while the "weed" component (mainly Holcus lanatus) had increased to about 25%. All three selection lines were equally affected. This treatment was therefore discontinued after 1969 but the plots were subsequently maintained (without measurement) during 1970 under infrequent defoliation, and at the conclusion of the experiment a visual inspection showed that some recovery had occurred.

Differences in botanical composition between lines and nitrogen fertiliser treatments occurred during the experiment (table 27). The effect of nitrogen was expected and details are not presented. Nitrogen influenced only the balance of clover and Phalaris and had no effect on the proportion of weeds. The only harvests for which the nitrogen effects were substantial were those in the spring and early summer of 1969; at these times, application of nitrogen reduced the average proportion of clover from 38 to 26% (November 2 harvest) and from 60 to 47% (January 6, 1970). The selection lines showed no differences in susceptibility to weed infestation (table 27), but the low N line swards tended to contain a higher proportion of grass and lower proportion of clover than those of the other lines. Apart from this tendency, the botanical composition of the plots of all lines responded similarly to the various treatments, as indicated by the almost complete lack of interaction between lines and treatments (the single exception is indicated in table 27).

There were significant yield effects due to defoliation and nitrogen treatments in 1969 (tables 28 and 30; figure 6) and due to nitrogen treatments in 1970 (table 28; figure 6), but these results are not of immediate interest. Differences between the selection lines in annual and seasonal Phalaris yields are presented in detail in table 28 and figure 6. The low N line had the highest total Phalaris yields in both 1969 and 1970 and there were no significant line x treatment

interactions for annual Phalaris yield in either year. However, there were significant line x defoliation treatment interactions for Phalaris yield at individual harvests in 1969, due to the deterioration of the frequently cut plots in which there were no yield differences between lines after the September 6 harvest. At earlier harvests, differences between lines were similar in both frequently and infrequently defoliated plots. Therefore, Phalaris yields of the infrequently defoliated swards at all harvests during 1969/70 are shown in figure 6, from which it can be seen that the low N line had the highest yields in every season except spring 1969 (November 2). Although its superiority was not statistically significant at the individual 1970 harvests, its total yield of Phalaris in 1970 was significantly higher. In general, differences between the lines were smaller in 1970 than in 1969. The control and high N lines differed very little in total yields of Phalaris in either year, although there were differences at individual harvests.

Although at no stage was there a significant line x nitrogen level interaction, it was obvious that on several occasions the yield superiority of the low N line compared with the high N line was most marked when nitrogen fertiliser was not applied. After November 1969 the response to applied nitrogen was negligible for the low N line; during the remaining 10 months of the experiment, the average yield increase on these infrequently cut plots was only 1 kg dry matter for each 1 kg of applied N. During the same time, the yield increase from infrequently cut high N line plots was 4.5 kg dry matter per kg N. Thus, for example, in both winter periods the low line outyielded the high line by at least 30% in the absence of applied nitrogen, but only by 17% and 6% in the 1969 and 1970 winter periods respectively when nitrogen was added.

Differences between lines in the weight and number (density) of Phalaris tillers in infrequently cut swards are given in table 29. At each harvest at which these data were collected, tillers from the low N line plots were heavier than those from the high line plots. Average weight of control line tillers during the 10 months from 2.11.1969 to 4.9.1970 was intermediate between weights of the selected lines (151, 200 and 263 mg/tiller respectively for the high, control and low N lines), but at individual harvests this relationship was not always consistent. Usually, tiller density was greater in the high line plots although on one occasion (6.1.1970) the low N line was superior.

Tiller numbers on control plots were again inconsistent but the average density was similar to that on the low line plots (1688, 1235 and 1187 tillers/sq m respectively in high, control and low N line swards during the 10 months of measurement). The application of nitrogen increased tiller weights but had variable effects on tiller density at different harvest.

Data for total yield (i.e., from all species) in each year are presented in table 30. In both years, yields from the low N line swards exceeded those from other swards under infrequent cutting, but there were no line differences in the frequently defoliated plots. In 1969 there was a significant yield reduction due to frequent cutting and a significant response to applied nitrogen, but in 1970 there was no significant response to nitrogen.

(b) Nitrogen Concentration in Phalaris Herbage

At each sampling during 1969, Phalaris herbage from the low N line plots had lower %N values than high N line herbage (table 31; figure 6) but line differences were not always significant and were relatively smaller than differences previously observed in spaced plants (experiments 1, 3 and 4). At no harvest were there any line x defoliation frequency or line x nitrogen level interactions for this character. The lowest values were recorded at the January 6 (1970) harvest, when anthesis had occurred on the earliest tillers in the infrequently cut plots. This was the most advanced developmental stage in the normal reproductive growth cycle achieved by the swards under the defoliation systems imposed, and nitrogen content at this stage ranged from 1.25 - 1.43%N (figure 6).

There was no significant difference in %N of Phalaris from the high and control N plots (table 31) at any individual harvest, and differences between the control and low N line plots were significant at only 2 of the 5 harvests in 1969. However, when averaged over all 5 harvests, the high, control and low N lines contained 2.52, 2.43 and 2.31 %N respectively.

Nitrogen content was always higher in herbage from frequently cut than from infrequently cut plots. On two occasions there was a significant increase in %N of herbage from frequently cut plots due to the application of nitrogen fertiliser, but at most times the effect of applied nitrogen on this character was small.

(c) Yield of Nitrogen from Phalaris Herbage

There were significant differences between selection lines in yield of nitrogen from Phalaris at 3 of the 5 sampling dates in 1969 (table 32). On each of these occasions the low N line plots yielded the most nitrogen. Total nitrogen yields during 1969 were 175, 155 and 191 kg N/ha for the high, control and low N lines respectively. Recovery of applied nitrogen by the Phalaris was generally low, but it must be remembered that some nitrogen would have been recovered by the clover and weed components of the swards. Recovery was greatest during the winter in 1969, and this was also the period of greatest pasture response (in terms of herbage yield) to applied nitrogen (figure 6); at this time, there was an average yield increase of 40 kg Phalaris herbage per kg N applied. During 1969, the greatest recovery of applied nitrogen by the Phalaris occurred on high N line plots (average 36% recovery), with smaller amounts being recovered on low (27%) and control (21%) line plots, although this ranking was not always consistent at individual harvests.

(d) In vitro Digestibility of Phalaris Herbage

Phalaris herbage from the low N line plots was highest in IVDOM at each sampling in 1969 (table 33) and that from the control line was usually lowest in IVDOM. However, differences between the lines were small, at no stage exceeding 3 digestibility units. There were no line x treatment interactions for this character at any harvest. The lowest digestibility values were recorded at the 6.1.1970 sampling, when Phalaris herbage from infrequently cut plots averaged 52% IVDOM. Herbage from nitrogen fertilised plots was often higher in IVDOM than that from unfertilised plots (table 34) although the differences were never greater than 3 digestibility units. Herbage from frequently cut plots was more digestible than that from infrequently cut plots except during the winter period.

DISCUSSION

The aim of this experiment was to examine the agronomic performance of the high, low and control N selection lines under sward conditions, and the main interest is the comparative performance of the high and low lines. The results indicate that the low N line swards gave higher yields of Phalaris and total herbage, and that their superior Phalaris yields were mainly due to a tiller weight advantage. Low N line plots tended

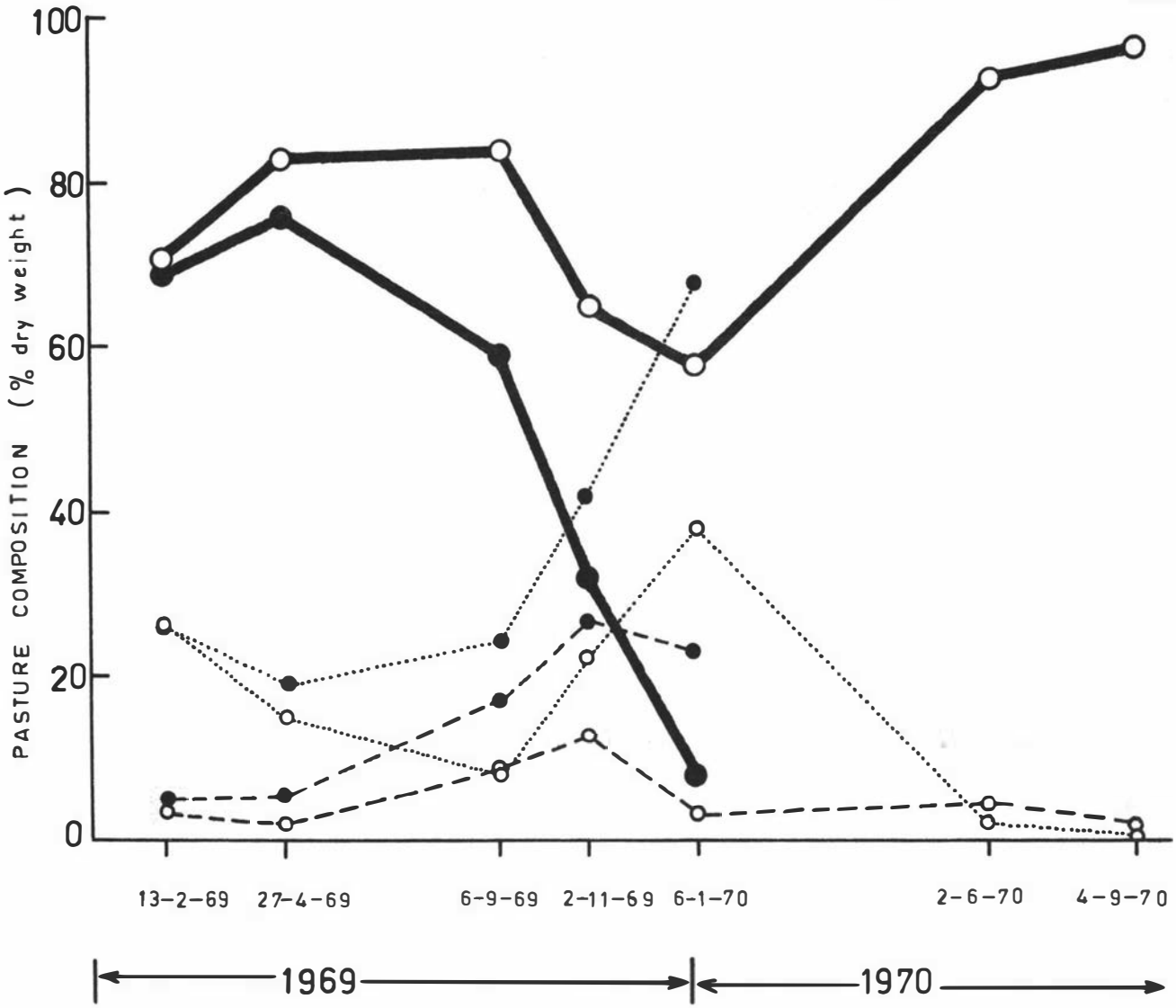

Fig.5 – Effect of frequency of defoliation on pasture botanical composition. After 13-2-69, differences between defoliation treatments are significant ($P < 0.001$) for each component.

Phalaris ..... clover – – – other spp.

● frequent defoliation ○ infrequent

TABLE 27

DIFFERENCES IN BOTANICAL COMPOSITION OF SWARDS SOWN WITH HIGH N, LOW N AND CONTROL N SELECTION LINES. (Line mean values in each harvest are averaged over treatments; analyses of variance were carried out after arcsin transformation of data).

Component and Selection line §		Proportions of components (% total dry weight) by harvests #				
		13-2-1969	27-4-1969	6-9-1969	2-11-1969	6-1-1970
Phalaris	H	68.3	75.2	73.1	48.5	31.3
	C	66.9	77.3	64.3	45.9	31.2
	L	74.3	85.2	76.8	50.6	37.0
Sig. differences		(NS)	(L > C,H)	(L,H > C)	(NS)	(L > C)
Clover	H	27.7	20.7	15.4	34.0	55.9
	C	28.3	19.1	20.2	33.0	57.2
	L	22.5	11.3	11.7	28.8	47.2
Sig. differences		(NS)	(NS)	(C > L)	(NS) †	(C,H > L)
Other spp.	H	4.0	4.1	11.5	17.5	12.9
	C	4.8	3.6	15.5	21.1	11.5
	L	3.2	3.5	11.4	20.6	15.9
Sig. differences		(NS)	(NS)	(NS)	(NS)	(NS)

§ H = high N line; C = control N line; L = low N line.

† Significant line x defoliation frequency interaction; with frequent defoliation, C > L; with infrequent defoliation, H > L.

In 1970, there were significant differences between lines at the 2-6-1970 harvest; however, since during 1970 the proportion of Phalaris did not fall below 90% on any plots, line differences were negligible and the data are not presented.

TABLE 28

EFFECTS OF SELECTION LINES, DEFOLIATION TREATMENTS AND LEVELS OF APPLIED NITROGEN ON ANNUAL PHALARIS YIELDS.

Year and treatment	Phalaris yield (kg dry matter/hectare)		
	High N line	Control N line	Low N line
1969			
Frequent defoliation N_o	4101	3714	4846
Frequent defoliation N_1	6448	4917	6668
Infrequent defoliation N_o	7571	8319	11015
Infrequent defoliation N_1	12162	12605	15353
1969 means*	7570a	7839a	9471b
1970			
Infrequent defoliation N_o	5242	5441	6839
Infrequent defoliation N_1	6923	6177	7056
1970 means * §	6083_{ab}	5809_{a}	6947_{b}

1969 treatment means: N_1 (9692) > N_o (6594), ($P < 0.001$).
Infrequent (11171) > frequent defoliation (5116), ($P < 0.001$).

1970 treatment means: N_1 (6719) > N_o (5841), ($P < 0.05$).

* In each year, means having the same subscript are not significantly different. There were no significant interactions between lines and/or any treatments for annual Phalaris yields.

§ Up to 4-9-1970.

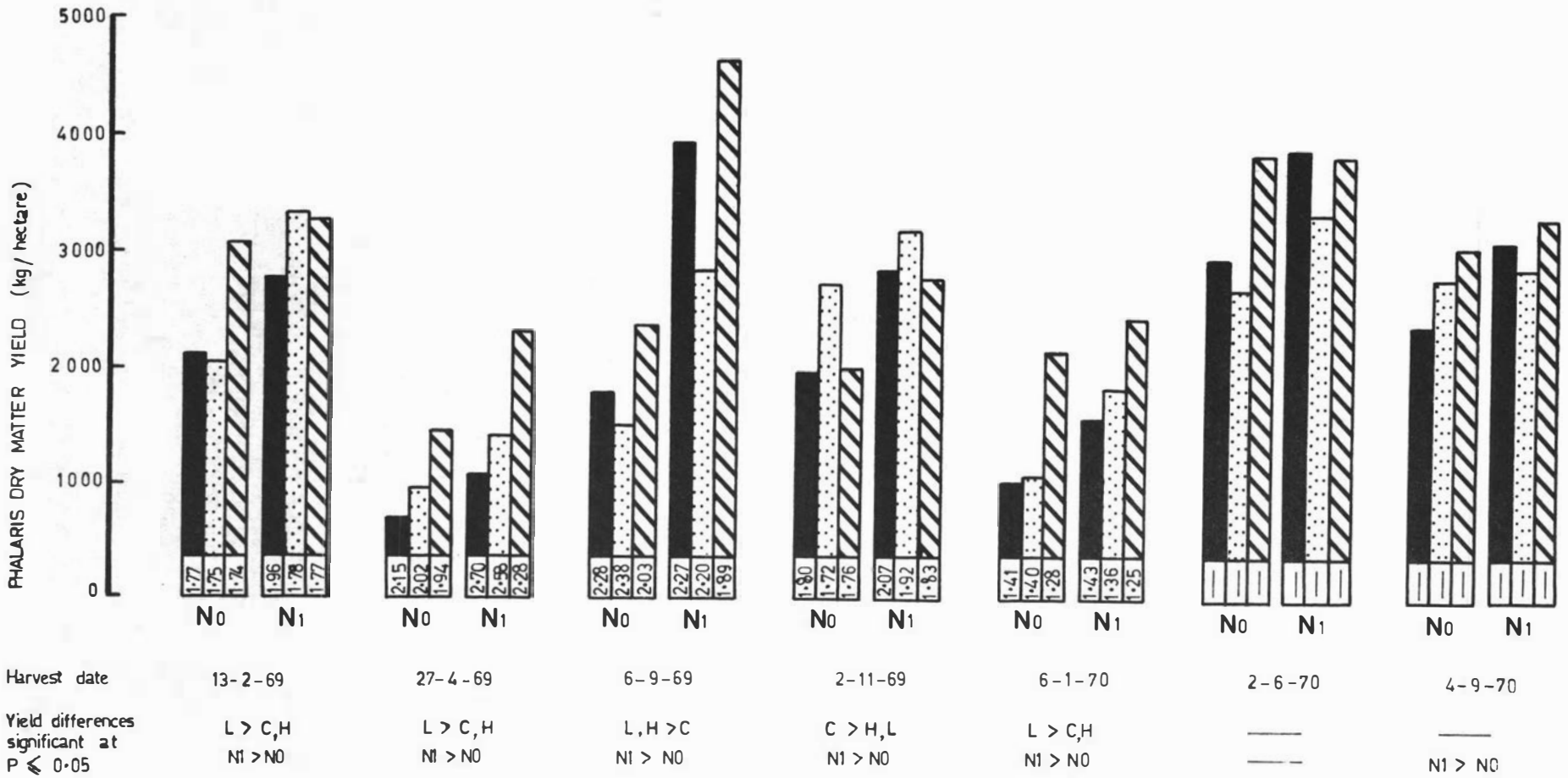

Fig. 6 .– Differences in seasonal Phalaris yields between lines previously selected for high (■), low (▨) or random (⁘) %N in whole tillers at heading. Yields refer to infrequently defoliated plots at two levels of applied nitrogen, N_0 and N_1 (see text). The figures within the columns are N concentrations (%N) of Phalaris herbage.

TABLE 29

DIFFERENCES BETWEEN SELECTION LINES AND N FERTILISER TREATMENTS IN THE DENSITY AND WEIGHT OF PHALARIS TILLERS IN INFREQUENTLY DEFOLIATED SWARDS.

Harvest date	Selection line mean values §				N fertiliser treatment means		
	High N line	Control N line	Low N line	Significance † of line effects	N_o		N_1
(a) Tiller density (tillers/m^2)							
6-1-1970	443_a	388_a	602_b	**	435	(*)	520
2-6-1970	1981_a	1546_b	1152_c	**	1621	(NS)	1499
4-9-1970	2641_a	1772_b	1808_b	**	2359	(**)	1789
(b) Tiller weight (mg/tiller)							
6-1-1970	287_a	373_b	374_b	*	313	(*)	376
2-6-1970	173_a	196_a	340_b	***	213	(NS)	259
4-9-1970	111_a	166_b	176_b	***	121	(***)	181

(Analyses of variance were carried out after log. transformation)

§ Within-harvest line means having the same subscript are not significantly different.

† Asterisks indicate significance of line and treatment effects:-
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant.

TABLE 30

EFFECTS OF SELECTION LINES, DEFOLIATION TREATMENTS AND LEVELS OF APPLIED NITROGEN ON TOTAL ANNUAL* DRY MATTER YIELD.

Year and treatment	Pasture yield (kg dry matter/hectare) †		
	High N line	Control N line	Low N line
1969			
Frequent defoliation	11695_a	11195_a	11657_a
Infrequent defoliation	14241_b	14950_b	17524_c
1970 §			
Infrequent defoliation	8863_a	8807_a	10753_b

§ Up to 4-9-1970

† Within each year, means having the same subscript are not significantly different. In 1969, the interaction between lines and defoliation frequency was significant ($P < 0.05$).

Effect of applied N. 1969: N_1 (14877) $> N_o$ (12208), $P < 0.001$.
1970: no significant differences overall or at any harvest.

* Yields at individual harvests are not presented. The ranking of the selection lines for total yield at individual harvests was essentially the same as their ranking for Phalaris yield at the same harvests in both years.

TABLE 31

DIFFERENCES BETWEEN SELECTION LINES AND TREATMENTS IN % N OF PHALARIS HERBAGE HARVESTED FROM SMALL PLOTS IN 1969

Date	Selection line mean values §				Treatment mean values §	
	High N line	Control N line	Low N line	Significance of line effect †	Defoliation: frequent	infrequent
13.2.1969	2.61_a	2.40_a	2.40_a	NS	3.14 (***)	1.79
27.4.1969	2.82_a	2.67_{ab}	2.54_b	**	3.07 (***)	2.28
6.9.1969	2.94_a	2.94_a	2.64_b	***	3.50 (***)	2.17
2.11.1969	2.47_a	2.41_a	2.31_b	**	2.95 (***)	1.85
6.1.1970	1.79_a	1.74_a	1.67_a	NS	2.11 (***)	1.36

Date	Treatment mean values §	
	N fertiliser: No	N_1 #
13.2.1969	2.39 (NS)	2.55
27.4.1969	2.50 (***)	2.85
6.9.1969	2.85 (NS)	2.82
2.11.1969	2.34 (***)	2.46
6.1.1970	1.74 (NS)	1.72

§ Analysis of variance was carried out log. transformation.

† Asterisks indicate significance of line or treatment effects.

a,b: within-harvest line means having the same subscript are not significantly different.

Note: the significant N fertiliser effects at 27.4.1969 and 2.11.1969 occurred on frequently defoliated plots only.

** $P < 0.01$ *** $P < 0.001$

TABLE 32

DIFFERENCES BETWEEN SELECTION LINES IN YIELD OF N FROM PHALARIS HERBAGE HARVESTED FROM SMALL SWARDS

Harvest date	Selection line mean values (kg N/hectare) § † #			Significance of line effect
	High N line	Control N line	Low N line	
13.2.1969	46.8_{ab} (33)	40.3_{b} (27)	51.5_{a} (11)	*
27.4.1969	25.0_{a} (23)	27.1_{a} (14)	35.3_{b} (37)	*
6.9.1969	55.2_{a} (70)	42.7_{a} (35)	55.6_{a} (52)	NS
2.11.1969	38.0_{a} (45)	36.4_{a} (24)	34.8_{a} (29)	NS
6.1.1970	9.6_{a} (10)	8.7_{a} (7)	13.3_{b} (5)	**

§ There were no significant interactions involving selection lines.

† Within-harvest line means having the same subscript are not significantly different.

Figures in parenthesis are the percentages of applied N recovered by the Phalaris.

* $P < 0.05$ ** $P < 0.01$ NS = not significant

TABLE 33

DIFFERENCES BETWEEN SELECTION LINES IN IVDOM OF PHALARIS HERBAGE HARVESTED FROM SMALL SWARDS

Harvest date	Selection line mean values (%IVDOM) † §			Significance of line effect
	High N line	Control N line	Low N line	
13.2.1969	69.5_{ab}	68.5_{a}	71.4_{b}	**
27.4.1969	70.9_{ab}	69.9_{a}	71.8_{b}	*
6.9.1969	77.1_{a}	78.6_{ab}	79.7_{b}	*
2.11.1969	77.8_{a}	76.6_{b}	78.4_{a}	**
6.1.1970	55.7_{a}	55.1_{a}	56.7_{a}	NS

§ Within-harvest line means having the same subscript are not significantly different.

† There were no significant interactions involving selection lines.

* $P < 0.05$, ** $P < 0.01$.

TABLE 34

EFFECTS OF DEFOLIATION FREQUENCY AND NITROGEN FERTILISER ON IVDOM OF PHALARIS HERBAGE HARVESTED FROM SMALL SWARDS

Harvest date	Treatment mean values (% IVDOM) §			
	Frequent defoliation		Infrequent defoliation	
	N_o	N_1	N_o	N_1
13.2.1969	73.5_a	74.9_b	64.3_c	66.5_d
27.4.1969	72.8_a	72.4_a	68.0_b	70.1_c
6.9.1969	77.9_a	79.7_a	78.3_a	78.0_a
2.11.1969	78.2_a	79.7_b	76.0_c	76.4_c
6.1.1970	58.4_a	61.6_b	51.4_c	51.9_c

§ Within-harvest treatment mean values (i.e. row means) having the same subscript are not significantly different

to contain a greater proportion of sown grass, but there were no differences between lines in susceptibility to weed infestation. Phalaris herbage from the low line swards was lowest in %N and highest in IVDOM. Although Phalaris from the high N line plots had the highest nitrogen concentration, the yield of nitrogen from the Phalaris was greater for the low N line. Phalaris in the high line plots recovered more nitrogen from the applied fertiliser and herbage yield response to applied nitrogen was greater, and in these senses the high N selection line was relatively "efficient". However at five of the seven harvests Phalaris yields on the unfertilised low line plots were at least as high as those from high line plots to which nitrogen fertiliser had been applied (figure 6). In other words, in order to produce a given yield of Phalaris herbage, the high line plots required more nitrogen than the low line plots and in this sense the high N selection line was relatively "inefficient".

The control N line was (on average) intermediate for nitrogen concentrations in the Phalaris herbage and for tiller weight. In most other characters it resembled the high N line more than the low line, with the exceptions of tiller density (similar to that of the low line), and Phalaris IVDOM and nitrogen yield (inferior to both the high and low N lines).

P. tuberosa is noted for its persistence under heavy stocking rates and in a variety of grazing management systems (Moore et al 1946; Willoughby 1959; Hutchings et al 1963; Biddiscombe 1964; Morley et al 1969; Hutchinson 1970; and many others). The failure of the Phalaris to persist under frequent cutting in this experiment might therefore seem surprising at first sight. However, as long ago as 1932 Richardson et al noted that..."the vigour and future growth of Phalaris tuberosa may be considerably impaired by too frequent defoliation", and suggested that cutting plants regularly at 5-weekly intervals (which corresponds to the average period between cuttings in the present frequent defoliation treatment) was "too drastic" because of its markedly deleterious effect on the root;shoot ratio. McKell et al (1966, 1970), Berry and Hoveland (1969) and Hoveland (1970) have shown that frequent cutting (especially during and shortly after the period when reproductive growth would normally be occurring) reduces carbohydrate reserves, plant survival and subsequent yield of P.tuberosa pastures. Grimmett (1967) found that regularly cutting P. tuberosa to a height of1.3cm resulted in greater plant mortality and yield

reduction than cutting to a height of 7.5 cm and that some plant mortality occurred under all frequent cutting systems. Observations by these workers are thus in agreement with the generally accepted notion that frequent, severe defoliation of individual plants or monoculture swards will reduce plant or pasture herbage yields (see, for example, reviews by Alcock 1964; Milthorpe and Davidson 1966; Davidson, 1968). In addition, many authors have noted that frequent, severe cutting or grazing of mixed (grass/legume) pastures results in a decrease in the proportion of grass and an increase in the proportion of legume; Brougham's (1959) work in the present environment is a good example of this trend.

However, as has frequently been pointed out (e.g. Moir 1959; Moore and Biddiscombe 1964; Davidson 1968) botanical composition of grazed pastures may be quite different to that of swards which are artificially cut or mown. In particular, pastures which are cut rather than grazed are reported to be more susceptible to weed infestation (e.g. Sears 1951, 1953; Lynch 1960). Although weed infestation - mainly by annual grasses - has been occasionally observed on very heavily grazed P. tuberosa/ Trifolium subterraneum or P. tuberosa/Trepens pastures (Morley 1966a; Reed 1970; Hutchinson 1970), the botanical composition of these pastures after the establishment phase is usually relatively stable[1] under economically optimal grazing pressures[2] in the south-eastern Australian environment (Moore et al 1946; Willoughby 1959; Axelsen and Morley 1968; Morley et al 1969). Phalaris pastures have been reported to be resistant to invasion by specific weeds, including various thistles (Silybum marianum, Michael 1968a; Onopordum spp. Michael 1968b; Cirsium vulgare, George et al 1970), barley grass (Hordeum leporinum; Oram and Hoen 1967), and Paterson's Curse (Echium lycopsis; Michael 1970) but their resistance

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1. "Stability" is used here in a sense roughly analagous to that of Morley (1966 b) and refers to the ability of P. tuberosa and the associated legume to persist "permanently" (i.e. for many years) as the major components of a productive pasture. No constancy of botanical composition is implied but many authors (e.g. Willoughby 1959; Morley et al 1969) have found that, at any given time, stocking rate has little effect on the grass/clover balance in these pastures.

2. Stocking rates within the range 8-15 merino ewes per hectare (3-6 per acre) appear to be most profitable for P.tuberosa pastures within the pastoral zones suitable for Phalaris in south-eastern Australia (Chisholm 1965; Morley and Ward 1966; Byrne 1968).

to Yorkshire fog (Holcus lanatus), the major weed in the present experiment, is not known. P.tuberosa persists in grazed pastures approximately 1 km (less than a mile) from the site of this experiment, but in a soil with superior drainage characteristics. It cannot therefore be concluded that the selection lines would not persist under normal grazing pressures in this environment.

The higher tiller weights from the low N line swards are in agreement with the results of experiments 2, 3 and 4, as are the lower tiller numbers (c.f. table 15), while differences in Phalaris yields between the low and high N lines agree with seedling vigour differences described in experiments 3, 6 and 7. McWilliam and Latter (1970) found high positive genetic correlations (r_g = 0.55 — 0.69) between seedling dry weight and herbage yield from swards in their second year of growth, among families from the base population from which these selection lines were derived. Tiller numbers and weights have not often been published for Phalaris pastures; more emphasis has usually been placed on plant numbers than tiller numbers per unit area. The relative importance of these two yield components in determining yield differences between genotypes or varieties of Lolium perenne has been carefully studied by Lazenby and Rogers (1962, 1964 a, 1965), who found that large differences were more closely related to tiller numbers than tiller weight. Yield differences between Dactylis glomerata strains also appear to result from tiller number differences during the first year of growth (Biddiscombe et al 1969) but in later years tiller weight is the most important determinant of yield differences (Knight 1961; Biddiscombe et al 1969). In the present experiment, the (usually) higher tiller density was insufficient to compensate for the lower tiller weights in the high N line swards, which regularly yielded less Phalaris than the low line plots, but the relative importance of the yield components could well be different in another environment or under other defoliation treatments.

Differences in %N of Phalaris herbage between selection lines in these swards were smaller than those previously observed in spaced plants (experiments 1, 3 and 4), but the ranking of lines for this character was the same. There was no indication of line x cutting frequency or line x nitrogen fertiliser level interactions for %N or IVDOM. All of these results are in good agreement with those of Lazenby and Rogers (1964a, 1965) for Lolium perenne genotypes and varieties grown under a range of spacings and nitrogen levels. Genotype x cutting frequency interactions for %N and IVDOM have also been found to be unimportant in

Cynodon dactylon (Burton et al 1967) and Phalaris arundinacea (Asay et al 1968; Carlson et al 1969), while genotype x nitrogen level interactions for these characters appear to be of little significance in Dactylis glomerata (Dent and Aldrich 1968), Panicum virgatum, and Andropogon spp. (Newell 1968). However, Rogers and Thomson (1970) recently reported quite large and highly significant progeny x N level interactions for both %N and IVDOM in spaced plants of Lolium perenne. Further studies on %N of seedlings of these selection lines grown under a range of nitrogen levels are described in experiment 6.

One of the most interesting and agronomically significant results of this experiment is the difference between the high and low N lines in their nitrogen economy and efficiency of nitrogen uptake and usage. Genetic variation for these characteristics has previously been observed in Lolium perenne (Vose 1962; Vose and Breese 1964; Lazenby and Rogers 1965; Holmes 1966; Thomson and Rogers 1970) and other species. As noted earlier, classification of the selection lines as "efficient" or "inefficient" depends on the meaning of efficiency, which has been variously defined in terms of the relative ability of a genotype (or sward) to produce dry matter from absorbed nitrogen (Vose 1962; Vose and Breese 1964; Thomson and Rogers 1970) and in terms of the amount of nitrogen required by different genotypes to achieve the same yield (Lazenby and Rogers 1965). However, another aspect of efficiency is the relative ability of genotypes to increase dry weight in response to fertiliser nitrogen, which depends both on efficiency of recovery and utilisation of applied nitrogen. Further, ability to concentrate nitrogen in the tissues could be considered an index of efficiency in some circumstances, in a herbage nutritive value sense. These various aspects of nitrogen efficiency have been appreciated by the authors cited, who have in every case clearly stated the sense of their definition, but they are described here in order to provide a basis for comparing the nitrogen economy of the high and low N lines.

In the first sense, as pointed out by Vose and Breese (1964), genotypes with low %N values will automatically tend to be efficient nitrogen users. Thus, the low N line in the present experiment utilises absorbed nitrogen more efficiently than the high line. It is also more efficient in the sense of requiring less nitrogen for a given herbage yield, as already mentioned. The low N line had the ability to extract more nitrogen (i.e. non-fertiliser nitrogen) from the soil, but recovered less of the applied nitrogen. Lazenby and Rogers (1965) observed that genotypes of Lolium perenne which did not differ in incremental herbage

yields per unit of applied nitrogen did differ in their ability to extract non-fertiliser nitrogen from the soil and in their recovery of fertiliser nitrogen. In the present experiment, the low N line was inefficient in its response to applied nitrogen, and it may be that at very high rates of nitrogen application the yield differential between the lines would be reduced or even reversed.

Because relevant information is also presented in experiments 6, 7 and 8 a more complete analysis of differences between lines in nitrogen uptake and utilisation will be given in the discussion of those experiments.

EXPERIMENTS 6, 7 AND 8

AN EXAMINATION OF THE SEEDLING VIGOUR, NITROGEN ECONOMY AND APPARENT PHOTOSYNTHETIC RATE OF PHALARIS TUBEROSA LINES PREVIOUSLY SELECTED FOR HIGH OR LOW HERBAGE NITROGEN CONCENTRATION

INTRODUCTION

In the course of producing experimental selection lines of Phalaris tuberosa having different concentrations of nitrogen (%N) in the herbage, it was observed that seedling vigour of maternal half-sib families was negatively correlated with %N at the heading stage (experiment 3). From a preliminary examination of this phenomenon, it was suggested that differences in seedling vigour were due to differences in both seed weight and relative growth rate between families. In experiments 4 and 5 it was found that the low N line had heavier tillers under both spaced - plant and sward conditions and that this superiority resulted in higher herbage yields from swards.

Low seedling vigour is a major limitation to the value of P.tuberosa as a pasture grass, resulting in poor establishment and low herbage yields during the first few months after sowing (see review by Cameron 1963). High herbage yields, especially during seasons when low pasture growth limits animal production (e.g. winter; Willoughby 1959), are usually required of any pasture grass. Improved seedling vigour and autumn-winter yields are therefore specific and major objectives in current Australian breeding programs for this species (e.g. McWilliam and Latter 1970). Although crude protein content (%N x 6.25) of herbage limits animal production under certain circumstances (see literature review), negative relationships between %N and seedling vigour or yield might well outweigh any advantages due to increased protein content.

The experiments described here were designed to examine several aspects of these relationships. The specific aims were as follows:-

(i) to confirm the existence of a negative relationship between %N and seedling vigour;

(ii) to examine the extent of seedling vigour differences between selection lines irrigated with nutrient solutions containing different levels of nitrogen;

(iii) to define the major causes of observed variation in seedling vigour;

(iv) to examine the relationship between %N and the rate of apparent photosynthesis;

(v) to investigate the relationships between %N and the rates of nitrogen uptake and utilisation.

The main criterion of vigour was taken to be seedling dry weight, but several other criteria were also examined.

MATERIALS AND METHODS

Experiments 6 and 7 were conducted in a glasshouse during the late autumn and winter of 1969. Glasshouse temperatures are shown in figure 7. Seeds were germinated on moistened filter paper in petri dishes, and seedlings were subsequently transplanted to individual 6 x 6 cm plastic pots containing perlite. Nutrient solutions containing either 2.8 (weak), 28 (medium) or 280 (strong) ppm nitrogen were applied daily and the pots were flushed regularly with water to prevent any accumulation of nitrogen in the perlite. The composition of the nutrient solutions is given in appendix 3; sodium nitrate was the nitrogen source in the weak and medium nutrient solutions, while the strong solution contained additional nitrogen in the form of ammonium nitrate. The concentrations of other essential elements were identical in each solution, so that "strength" refers only to differences in nitrogen content.

(a) Experiment 6

Seedlings from the S_3 and S_4 generations of the high and low N selection lines were used in this experiment. The S_3 seedlings originated from the bulk seed samples described in experiment 5, while seeds from each maternal parent of the S_4 generations were germinated separately to produce representative S_4 seedling populations. Seedlings from each of the 4 populations (i.e. 2 selection lines x 2 generations) were grown in pots supplied with nutrient solution containing 2.8, 28 or 280 ppm nitrogen. A total of 48 randomly positioned seedlings (12 from each population) was grown at each nutrient level in each of 2 replicates giving a total of 288 seedlings, half of which were harvested at the 3-leaf stage and half at the 5-leaf stage. Each plant was harvested individually as it reached one or other of these stages (i.e. on the day on which the tip of either the fourth or sixth leaf respectively first appeared within the enclosing sheath of the previous leaf). Rate of leaf appearance (days between the appearance of the fourth and sixth leaves) was measured on all seedlings harvested at the second stage. Plants harvested at each stage were washed free of perlite, dried at 80°C, weighed and temporarily stored over silica gel in the dark. At the conclusion of harvesting, the individual seedlings within populations

and treatments at the 5-leaf stage were bulked and analysed for Kjeldahl nitrogen content (%N, dry matter basis) as outlined in appendix 1.

Whole-plant relative growth rates (RGR) were calculated from the formula

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} ,$$

where W_1 and W_2 are seedling dry weights (mg) at times t_1 and t_2 respectively, and $t_2 - t_1$ is the time (days) between the 3-leaf and 5-leaf stages.

Since seedlings were harvested at fixed developmental stages rather than at a fixed point in time, dry weights were adjusted by means of RGR values to a common harvest date (6 weeks from transplanting) prior to analysis of variance of seedling weight.

(b) Experiment 7

Seedling growth of S_4 polycross (i.e. maternal half-sib) families in the high and low N lines was examined in this experiment. The seeds of each family were obtained from the final cycle of selection and crossing in the Canberra phytotron (see experiment 3); thus, seeds were from the same source as those used for experiment 4 and the S_4 generation in experiment 6. There were eight families in each of the high and low N lines. Seed samples were stored over silica gel for some weeks prior to weighing replicated 50-seed lots from each family. Twelve seedlings from each of the 16 families were grown in each of 2 replicates and were irrigated with the medium strength nutrient solution. Within replicates, seedlings were grouped in batches of 16 (1 from each family) to minimise bias due to position effects in the glasshouse. Half of the 384 seedlings were harvested at the 3-leaf stage and half at the 5-leaf stage, stages being defined as in experiment 6. Rate of leaf appearance (days between the appearance of the fourth and sixth leaves) was measured on all seedlings harvested at the second stage. As in experiment 6, each plant was harvested individually as it reached one or other of the developmental stages. Harvested seedlings were washed free of perlite, and length and maximum breadth (mm) of each leaf were recorded to allow subsequent calculation of seedling leaf area (see appendix 7). Seedlings were then dried at 80°C and separated into root and shoot (top) fractions which were weighed separately and temporarily stored over silica gel in the dark. When harvesting was completed, the dried

material from each family in each replicate was analysed for Kjeldahl nitrogen content, both roots and tops being analysed separately at each stage.

From the data collected, relative growth rates (RGR) of tops, roots and whole plants were computed as for experiment 6, and relative rates of leaf area expansion between the 3-leaf and 5-leaf stages were calculated in a similar manner. Average net assimilation rates (NAR) between the two stages were estimated from the formula

$$NAR = \left[\frac{W_2 - W_1}{t_2 - t_1}\right] \quad \left[\frac{\text{Log}_e A_2 - \log_e A_1}{A_2 - A_1}\right] ,$$

where W_1 and A_1 are seedling dry weights (mg) and leaf areas (cm^2) respectively at the 3-leaf stage, W_2 and A_2 are corresponding values at the 5-leaf stage, and $t_2 - t_1$ is the time (days) between the stages. Average leaf area ratios (LAR) between the stages were calculated similarly as

$$LAR = \left[\frac{A_2 - A_1}{W_2 - W_1}\right] \quad \left[\frac{\log_e W_2 - \log_e W_1}{\log_e A_2 - \log_e A_1}\right].$$

In addition, LAR at each stage was calculated directly as the ratio of leaf area (cm^2) to total plant weight (mg).

Following Williams (1939) and Keay et al (1970), the rate of seedling dry weight increase per unit of absorbed nitrogen between the stages per day (N_U) was calculated as

$$N_U = \left[\frac{\log_e N_2 - \log_e N_1}{t_2 - t_1}\right] \left[\frac{W_2 - W_1}{N_2 - N_1}\right] ,$$

where N_1 and N_2 are the total nitrogen contents (mg) per seedling at the 3-leaf and 5-leaf stages respectively. N_U, the rate of nitrogen utilisation, is a measure of the efficiency with which absorbed nitrogen is used in the production of dry weight.

The rate of nitrogen absorption (N_A) per unit dry weight of root per day between the stages was calculated as suggested by Williams (1948) from the formula,

$$N_A = \left[\frac{\log_e R_2 - \log_e R_1}{t_2 - t_1}\right] \quad \left[\frac{N_2 - N_1}{R_2 - R_1}\right] ,$$

where R_1 and R_2 are root dry weights per seedling (mg) at the 3-leaf and 5-leaf stages respectively.

Seedling dry weight differences between families were examined after adjusting weight data to a common harvest date, as in experiment 6. In addition, weight data at the 5-leaf stage are presented for comparison. Some other variables (see table 39) measured at the 5-leaf stage are also included.

(c) Experiment 8

The aim of this experiment was to examine the relationship between %N and net photosynthetic rate in a controlled environment. The number of plants that could be examined was limited by the small size of the available growth cabinets and the time-consuming nature of the measurements. Large, variable seedling populations were therefore not suitable for this study. Instead, plants known to have either high or low %N values under field conditions were used. It was assumed that these extreme genotypes were representative of the populations which would eventually result from many generations of selection for high and low %N at heading.

From the spaced-plant populations described in experiment 4, five genotypes having high %N were chosen from the high N line, and five having low %N were taken from the low N line. The %N values of these ten genotypes under field conditions are described elsewhere (table 43, experiment 9). In the autumn of 1969 (May 25), when the plants were completely vegetative, they were dug up and transferred to a glasshouse. Individual tillers at a similar stage of development were taken from the centre of each plant. The culm to which a tiller was attached was cut at a height of 6 cm above the point of insertion of the tiller, the top portion was discarded and the remaining material was washed free of soil. From a large number of single tillers thus obtained, twelve from each plant were selected on the basis of uniformity of development and apparent integrity of the root system of the developing tiller. As much as possible of the root system of the attached culm was removed, and the selected tillers were planted individually in perlite in 6 x 6 cm plastic pots and irrigated with the medium strength nitrogen nutrient solution. In this way, twelve tiller units (ramets) of each of the ten genotypes (clones) were provided for this experiment.

The ramets were grown for 5 days in the glasshouse, after which they were defoliated to a uniform height (6cm) and transferred to two growth cabinets. A constant temperature of 20°C was maintained, and in each cabinet a combination of tungsten and fluorescent lights provided a measured light intensity of 80 watts/sq. m. (approximately 1600 f c)

of visible radiation at the level of the plants during a 12-hour photoperiod each day. This light intensity was low, but was the maximum possible in the cabinets. In each cabinet, 6 ramets of each clone were grown, grouped into 6 replicates, and all plants were irrigated twice daily with the medium strength nitrogen nutrient solution.

Measurements commenced 26 days after ramets had been transferred to the growth cabinets. From the 12 ramets of each clone, 3 were selected according to the following criteria:

(i) morphologically uniform and representative of the clone;

(ii) conforming to the following description: possessing at least 3 fully expanded, apparently healthy leaves on a single tiller (i.e. no new tillers visible at the time of measurement).

These 30 ramets were used for measurements of photosynthetic rate and chlorophyll concentration. They were divided into 3 groups, each containing 1 ramet of each clone, and the groups were measured on 3 successive days (days 26, 27 and 28 respectively). On each day, 9 hours after the commencement of the light period, the youngest fully-expanded leaf from each ramet to be measured was detached at the ligule and its area was determined (see appendix 7). From the middle of each leaf a small segment (25mm in length) was taken, and its rate of apparent photosynthesis was determined by the manometric method described by Wilson et al (1969). In this method, the rate of gas evolution per unit leaf area is measured under controlled conditions in a Warburg apparatus. The leaf segment is placed in the centre well of a Warburg unit, with one cut edge immersed in 0.1 ml distilled water, and its upper (adaxial) surface facing a light source. A small quantity (2.0ml) of a suitable buffer solution is pipetted into the outer well to maintain a constant CO_2 concentration, the unit is gently (usually mechanically) rocked back and forth to hasten and maintain equilibrium between the gas and liquid phases, and the increase in the volume of gas in the system is calculated from gas pressure changes which are in turn measured on an attached manometer. It is assumed that the gas produced is oxygen. In the present experiment, the buffer consisted of 0.1 molar $NaHCO_3$ and 0.1 molar Na_2CO_3 in the ratio 9/1 ($^v/_v$). A temperature of $20 \pm 0.5^{\circ}C$ was maintained by immersing the Warburg flasks in a large waterbath and the flasks were mechanically rocked sideways 40 times per minute. A light intensity of 120 watts/sq m (approximately 2500 fc) of visible radiation was provided by mercury vapour lamps. After allowing 30 min for equilibration, the change in gas pressure in the system was recorded during 3 consecutive 20 min intervals, and the apparent rate of oxygen evolution per minute

was calculated from these readings by means of the flask constants. The values so obtained were corrected for slight changes in waterbath temperature using values obtained from two control units (i.e. units not containing leaf segments). Twelve units were available, allowing each group of 10 leaf segments and 2 controls to be measured simultaneously.

On the same days on which rates of apparent photosynthesis were measured, chlorophyll concentrations were determined. For each ramet, the leaf immediately below that used for measuring photosynthetic rate was taken, and its area was recorded. Preliminary analyses had shown that, with slight modifications, the technique of Hunt and Cooper (1967) could be adapted to measure the chlorophyll content of individual leaves with a high degree of accuracy. Chlorophyll was extracted by grinding each leaf in a mortar with pure acetone. The extract was centrifuged for 10 min at 2500 rpm and made up to a suitable volume with acetone, and its optical density was determined at 645 mμ and 663 mμ using a Beckman spectrophotometer. Concentrations of chlorophyll a, chlorophyll b and total chlorophyll were then determined by means of the equations derived by Arnon (1949).

The remaining 9 ramets of each clone, which were considerably more variable than those used for measurements of photosynthetic rates and chlorophyll concentrations, were used to establish a leaf weight: area relationship (i.e., specific leaf weight) for each clone (7 ramets), and to measure %N of tops (6 ramets) and roots (2 ramets). Because of the variability between the individual ramets of each clone, the %N values of tops fluctuated markedly (e.g. from 1.72 to 3.50 %N among the ramets of one clone and from 1.88 to 3.32 %N for another clone) and little confidence can be placed in these estimates; however, they are included in the results for completeness. Variability between ramets of a clone for %N of roots and specific leaf weight was much lower, and the clone mean values had relatively low standard errors. Using the leaf weight:area relationship it was possible to express the photosynthetic rates, chlorophyll concentrations and nitrogen concentrations on both a leaf area and a leaf weight basis, though with little confidence in the case of nitrogen.

RESULTS

(a) Experiment 6

The analysis of variance of seedling characters measured in experiment 6 is given in table 35. The effect of nutrient solution strength

(nitrogen level) was significant for all characters. Seedlings from high and low N selection lines had significantly different dry weights, %N and rates of leaf appearance. There were no significant interactions between lines and treatments for any character, and the main effects of the lines have therefore been summarised in table 36. Low N line seedlings were nearly twice as heavy on average as high N line seedlings and, although they had lower %N values, they contained larger total quantities of Kjeldahl nitrogen than high line seedlings; the nitrogen contents of seedlings 6 weeks after transplanting were approximately 0.90 and 0.70 mg respectively in the low and high N lines. The high N line seedlings had the faster rate of leaf appearance. There was no evidence that lines differed in RGR.

As the nitrogen concentration of the nutrient solution increased, seedling dry weight, %N and RGR also increased, while the time interval between the appearance of successive leaves decreased (table 37). Seedlings irrigated with the strong nitrogen solution were five times as heavy, and contained more than ten times as much Kjeldahl total nitrogen, as those irrigated with the weak solution.

An interesting result was the lack of any line x solution strength interaction for %N (table 35). The %N values of the selection lines grown at the different levels of nitrogen are presented in table 38, from which it can be seen that values for the high N line were always markedly greater than those for the low N line. Seedling nitrogen concentrations ranged from the very low value of 1.8 %N to the extremely high value of 6.5 %N.

(b) Experiment 7

The results of experiment 7 are presented in tables 39 and 39A. Low N line seedlings had lower nitrogen concentrations (%N) in both tops and roots than high N line seedlings, but contained greater total quantities of nitrogen. Families within lines were not significantly different in any of these characteristics. The low N line seedlings were also larger than the high N line seedlings; they had heavier tops and roots and, although they had the highest root:shoot ratios, they had larger leaves and a greater leaf weight per unit leaf area. Although the high N line seedlings had the highest LAR values they were not significantly superior in RGR of whole seedlings because NAR was higher in the low N line. Thus, highly significant differences in LAR were balanced by smaller and statistically non-significant, but presum-

ably real, changes in NAR and RGR. The interpretation of the results is complicated by the fact that LAR decreased in the low N line between the 3-leaf and 5-leaf stages (from 0.39 cm^2/mg to 0.30 cm^2/mg), while LAR did not change in the high N line; however, the consequent overestimation of NAR in the low N line is less than 5% (Coombe, 1960). Thus, during this period, the high N line allocated proportionately more assimilate to leaf growth and less to root growth than did the low N line, and this was reflected in both the superior leaf area expansion rate of the high N line and in its greater RGR of tops (which just failed to reach the conventional 5% significance level; P = 0.064).

The data in table 39 allow an assessment of the relative contributions of RGR and seed weight to seedling dry weight differences between families. Seed weight was markedly superior in the low N line, and correlations between this character and seedling dry weight and leaf area were high (0.76-0.84). Seedling dry weight at 6 weeks (i.e. at a common point in time) was taken to be the most appropriate criterion of seedling vigour. The partial correlation between this criterion and seed weight, holding RGR constant, was 0.77***, while the partial correlation between RGR and dry weight at 6 weeks, with seed weight held constant, was only 0.04. Clearly seed weight differences were a major cause of the variation in seedling vigour among families in this experiment. It is therefore of some interest to examine the relationship of seed weight to the remaining variables in table 39. Correlations with %N of seedlings were significantly negative. Apart from negative correlations with LAR (r = -0.59***) and rate of leaf area expansion (r = -0.34*), seed weight was unrelated to RGR and its components.

Nitrogen concentrations of whole seedlings, roots and tops were closely correlated, and were negatively related to seedling dry weight, leaf area, root: shoot ratio, specific leaf weight and rate of leaf appearance. The %N of tops was negatively correlated with NAR and positively correlated with LAR, and consequently there was no significant relationship between %N and RGR.

Line differences in N_A and N_U, the "efficiencies" of absorption and utilisation of nitrogen respectively, are summarised in table 39A. N_A and N_U are relatively crude estimates of efficiency, requiring assumptions that are unlikely to be met; for example, N_A assumes that nitrogen is absorbed uniformly over the entire root system, and N_U assumes that %N does not change during the time interval during which N_U is measured. However, provided one accepts that the biases are similar

for each selection line, the estimates of N_A and N_U can be taken to indicate the relative efficiencies of the lines. Thus, the results indicate that the high N line absorbed nitrogen at a much greater rate per unit root weight than the low line, but used the absorbed nitrogen less efficiently in the production of dry weight. Seedlings having high %N values had high N_A and low N_U values, as indicated by the highly significant correlations between these characters (table 39A), while seedlings originating from heavy seeds tended to absorb nitrogen relatively slowly and utilise it more efficiently.

(c) Experiment 8

The form of the analysis of variance of the data obtained in experiment 8 is shown in table 40, and a summary of the results is presented in table 41. Clones which had high %N values under field conditions had significantly higher rates of apparent photosynthesis and smaller leaves than the low N group of clones. The high N group had significantly higher %N levels in the roots and (nonsignificantly) higher %N values for tops; as noted earlier, the latter estimates have high sampling variances. There were no differences between groups (=lines in table 41) for nitrogen concentration per unit area, specific leaf weight, chlorophyll concentration or the proportions of chlorophylls a and b, although there were highly significant differences among the 10 clones for each of these characters. Broad-sense heritability values of characters other than %N, specific leaf weight and N/unit leaf area were high (0.60-0.96); the low heritabilities occurred for characters measured on the more variable ramets of the clones.

In table 42, simple correlations between the characters measured in experiment 8 are presented. Each correlation coefficient is based on ten clone mean values. Photosynthetic rates per unit leaf area and per unit leaf weight were highly correlated with %N of roots but not with %N of tops; no other correlations involving %N were significant. Photosynthetic rates on a unit chlorophyll, leaf area or leaf weight basis were all closely related and were negatively correlated with leaf size. Nitrogen and chlorophyll concentrations per unit leaf area, and specific leaf weight, were positively related to each other.

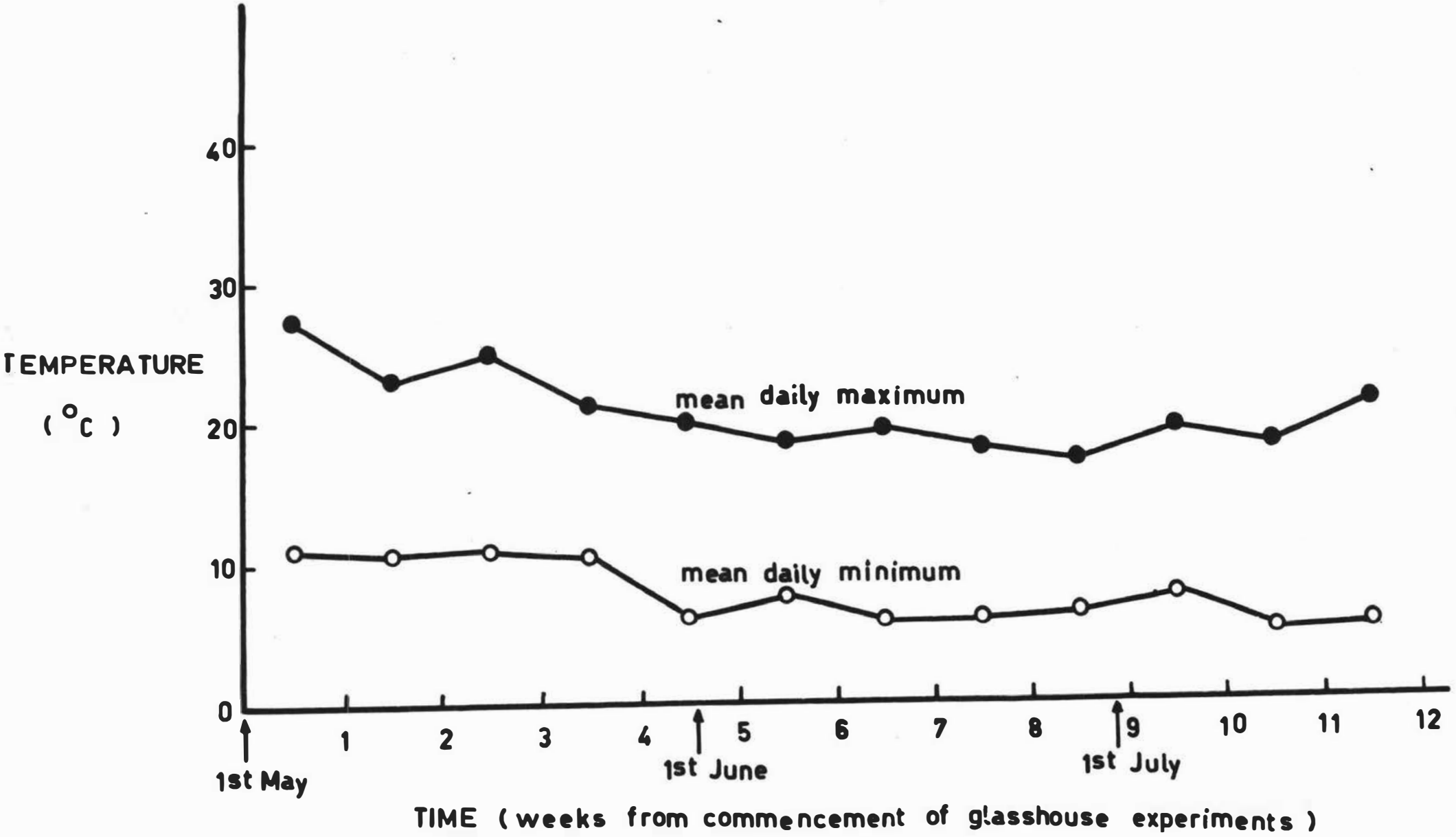

Fig.7.– Daily maximum and minimum glasshouse temperatures (weekly averages) during experiments.

TABLE 35

ANALYSIS OF VARIANCE OF SEEDLING CHARACTERS MEASURED IN EXPERIMENT 6

Source	Degrees of Freedom	Mean squares and significance of variation due to source			
		Rate of leaf appearance	Relative growth rate	Percent nitrogen	$\log_e$ seedling dry weight after 6 weeks
Replications	1	1.3920	549.7008	0.0002	0.000009
N levels (N)	2	635.5915*	12439.3941*	27.7070*	5.884166**
Error A	2	25.5449	178.3513	0.4275	0.058696
Lines (L)	1	112.4068**	154.2294	6.5836***	1.543308***
Generations (G)	1	2.7472	87.0204	0.1585	0.215462*
L x G	1	0.0182	5.9800	0.6369	0.159414
L x N	2	35.8880	249.4598	0.0142	0.077251
G x N	2	0.9613	91.3178	0.0984	0.065995
L x G x N	2	1.7024	34.7514	0.0738	0.021893
Error B	9	9.7261	144.1456	0.1605	0.034477
Total	23				

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

TABLE 36

DIFFERENCES BETWEEN SELECTION LINES IN SEEDLING CHARACTERS MEASURED IN EXPERIMENT 6. THE MEANS ARE AVERAGED OVER GENERATIONS, NITROGEN LEVELS AND REPLICATIONS

Character	High N line	Low N line	LSD (P=0.05)
Rate of leaf appearance (days 4-6 leaf)	22.99	27.32	2.88
Relative growth rate (1000 x mg/mg/day)	55.10	60.17	11.09 (NS)
% N, whole seedlings, 5-leaf stage	4.49	3.45	0.37
Log_e seedling dry weight at 6 weeks (mg) §	2.753 (15.7)	3.260 (26.1)	0.171

NS = not significant

§ values in brackets are means reconverted to mg.

TABLE 37

EFFECTS OF THE CONCENTRATION OF NITROGEN IN THE NUTRIENT SOLUTION SEEDLING CHARACTERS MEASURED IN EXPERIMENT 6

Character	Nitrogen level in solution (ppm)			LSD (P=0.05)
	low (2.8)	medium (28)	high (280)	
Rate of leaf appearance (days 4-6 leaf)	31.09	29.47	14.91	10.87
Relative growth rate (1000 x mg/mg/day)	36.01	33.73	103.14	28.73
%N, whole seedlings	2.31	3.62	5.98	1.41
Log_e seedling dry weight at 6 weeks (mg) §	2.334 (10.3)	2.714 (15.1)	3.972 (53.1)	0.521

§ Values in brackets are treatment means reconverted to mg.

TABLE 38

DIFFERENCES IN % N (WHOLE SEEDLINGS) OF SELECTION LINES GROWN UNDER THREE LEVELS OF NITROGEN IN NUTRIENT SOLUTION.

Nitrogen level in solution (ppm)	% N, high N line	% N, low N line	Mean
Low (2.8)	2.79	1.83	2.31
Medium (28)	4.18	3.06	3.62
High (280)	6.52	5.45	5.98
Mean	4.49	3.45	

5% LSD (line means) = 0.37

5% LSD (N level means) = 1.41

5% LSD (lines within N levels) = 0.64

TABLE 39

VARIATION IN SEEDLING VIGOUR CRITERIA BETWEEN AND WITHIN HIGH N AND LOW N SELECTION LINES, AND LINEAR CORRELATIONS OF VIGOUR CRITERIA WITH SEED WEIGHT AND SEEDLING % N. SEED WEIGHT REFERS TO SEEDS PRODUCING THE SEEDLING POPULATIONS

Character	Significance of effects due to.....			Line mean values		Range of family means within...		Phenotypic correlation with ...	
	Lines	Families within lines	Families ignoring lines	High N line	Low N Line	High N Line	Low N line	% N, tops	Seed Wght
%N, tops, 5-leaf stage	**	NS	*	5.05	4.09	4.7-6.0	3.5-5.3	—	-0.50
%N, roots, 5-leaf stage	***	NS	**	2.16	1.50	1.8-2.6	1.4-1.7	0.74	-0.60
%N, total, 5-leaf stage §	***	NS	***	4.12	3.06	3.6-5.1	2.6-3.7	0.94	-0.60
N yield/plant ($\log_e$ (10xmgN)), 5-leaf stage §	***	NS	**	2.39	2.74	2.1-2.6	2.6-3.1	-0.16	0.76
$\log_e$ seedling weight (mg) at 6 weeks ϕ	***	***	***	2.47	3.02	2.1-2.8	2.8-3.2	-0.44	0.76
$\log_e$ top weight (mg), 5-leaf stage §	***	*	***	2.88	3.41	2.6-3.1	3.3-3.5	-0.58	0.84
$\log_e$ root weight (mg), 5-leaf stage §	***	*	***	2.06	2.94	1.8-2.5	2.8-3.1	-0.63	0.78
$\log_e$ total weight (mg), 5-leaf stage §	***	*	***	3.26	3.91	3.0-3.6	3.8-4.0	-0.60	0.83
Root: shoot ratio, 5-leaf stage §	***	NS	***	0.47	0.65	0.40-0.56	0.56-0.73	-0.50	0.54
Specific leaf ~~area~~ weight (mg/cm^2), 5-leaf stage §	***	NS	NS	1.79	2.11	1.6-2.2	1.9-2.2	-0.56	0.49
$\log_e$ leaf area (cm^2), 5-leaf stage §	***	**	***	2.33	2.68	2.0-2.6	2.6-2.8	-0.41	0.81
Rate of leaf appearance (days 3-5 leaf)	*	***	***	27.1	30.2	23-29	28-33	-0.42	0.44
Relative growth rate, tops, 1000 x (mg/mg/day)	NS	NS	*	39.4	33.6	33-51	28-36	0.15	-0.24
Relative growth rate, roots, 1000 x (mg/mg/day)	NS	NS	NS	43.6	43.2	30-55	32-55	-0.20	0.03
Relative growth rate, whole plants, 1000 x (mg/mg/day)	NS	NS	NS	40.8	36.9	33-53	30-42	0.00	-0.13
Average net assimilation rate ($mg/cm^2/day$)	NS	NS	NS	0.104	0.116	0.08-0.14	0.09-0.13	-0.32	0.24
Average leaf area ratio (cm^2/mg) †	***	*	***	0.403	0.327	0.38-0.46	0.29-0.36	0.62	-0.59
Relative rate of leaf area expansion (1000x $cm^2/cm^2/day$)	*	**	**	38.1	28.1	28-56	24-31	0.33	-0.34
Relative rate of N yield increase (1000xmg/mg/day)	NS	NS	NS	31.6	26.4	24-40	22-38	0.51	-0.18
Seed weight (mg/50 seeds)	**	—	—	77.0	98.4	61-92	93-104	-0.50	—

§ Similar results were obtained at the 3-leaf stage. † similar results were obtained at individual harvests.
ϕ seedling weights adjusted to common harvest date.

Significance of correlations: if $-0.31 \geqslant r \geqslant 0.31$, $P < 0.05$; if $-0.40 \geqslant r \geqslant 0.40$, $P < 0.01$; if $-0.50 \geqslant r \geqslant 0.50$, $P < 0.001$.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant.

TABLE 39A

VARIATION BETWEEN AND WITHIN HIGH N AND LOW N SELECTION LINES IN THE RATE OF NITROGEN UPTAKE AND UTILISATION.

	Nitrogen absorption rate (mg N/g dry root/day)	Nitrogen utilisation rate (mg dry wt/mg N/day)
Significance of effects due to...		
(a) lines	**	*
(b) families in lines	**	NS
(c) families ignoring lines	***	NS
High N line mean value	5.33	0.86
Low N line mean value	2.76	1.02
Range, high N family means	3.91 — 8.43	0.66 — 1.15
Range, low N family means	1.83 — 3.76	0.90 — 1.16
Correlation with %N, tops	0.721 ***	-0.546 ***
Correlation with seed weight §	-0.562 ***	0.357 *

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant.

§ seed weight refers to seeds producing the seedling populations.

TABLE 40

STRUCTURE OF THE ANALYSIS OF VARIANCE OF DATA FROM EXPERIMENTS 8 AND 9

Source	Degrees of freedom	Expected mean square
Replications (R)	r-1	
Clones (C):-	c-1	$\sigma^2 e + r\sigma^2 G$
(1) N lines	l-1	
(2) clones in lines	c-1	
Error (E)	(r-1) (c-1)	$\sigma^2 e$
Total	cr-1	

H (broad-sense heritability of an unreplicated measurement) is calculated from the following formula:

$$H = \frac{\sigma^2 G}{\sigma^2 G + \sigma^2 e}$$

(H sets an upper limit to h^2 (narrow sense heritability) and measures the proportion of the phenotypic variance due to genotype).

TABLE 41

VARIATION IN % N AND IN CHARACTERS RELATED TO PHOTOSYNTHESIS AMONG SELECTED CLONES FROM HIGH N AND LOW N SELECTION LINES

Character	% of total sum of squares, and significance of effects due to....		H †	Line mean values		Range of clone means
	N lines	Clones in lines		high N line	low N line	
% N, leaves	9.1	22.0*	0.357	2.42	2.14	1.70-2.60
% N, roots	38.1**	12.8	0.465	1.21	0.92	0.85-1.39
Leaf size (cm^2) ‡	44.9*	33.6**	0.741	14.6	22.8	12.5-28.1
N/unit leaf area ($mg/100cm^2$)	1.8	45.4***	0.517	7.30	7.79	6.47-10.83
Specific leaf weight (mg/cm^2)	16.8	28.2***	0.453	3.10	3.65	2.68-4.30
Chlorophyll (A+B)/unit leaf area (mg/dm^2)	6.9	83.9***	0.957	2.41	2.76	1.81-4.05
Chlorophyll (A+B)/unit leaf weight ($\mu g/mg$)	0.6	81.0***	0.765	7.73	7.52	4.84-9.41
Chlorophyll A as % chlorophyll (A+B)	3.4	52.7**	0.633	70.4	71.1	69.0-73.1
§ PS rate/unit leaf area ($\mu l\ O_2/cm^2/min$)	51.7***	16.5	0.614	0.945	0.677	0.597-1.029
PS rate/unit leaf weight ($\mu l\ O_2/mg/min$)	73.3***	7.7	0.765	0.310	0.187	0.150-0.336
PS rate/unit chlorophyll ($\mu l\ O_2/mg/min$)	31.5*	36.5*	0.603	40.6	26.8	16.0-47.2

§ PS = photosynthesis † H= broad-sense heritability * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

‡ Leaf size here refers to those leaves used to measure PS rates.

TABLE 42

LINEAR CORRELATIONS §† BETWEEN % N AND CHARACTERS RELATED TO PHOTOSYNTHESIS

	% N, roots	leaf size	N/unit leaf area	Specific leaf weight	Chlorophyll/ unit leaf area	Chlorophyll/ leaf weight	Ch.A,% of total chlorophyll	PS rate/ unit leaf area	PS rate/ leaf weight	PS rate/ unit chlorophyll
% N, leaves	0.586	-0.381	0.388	-0.389	0.198	0.492	-0.521	0.223	0.439	0.069
% N, roots		-0.616	0.029	-0.459	-0.062	0.268	-0.578	0.829	0.856	0.511
Leaf size			-0.127	0.098	0.015	-0.038	0.128	-0.748	-0.655	-0.557
N/unit leaf area				0.685	0.874	0.643	-0.429	-0.150	-0.342	-0.573
Specific leaf weight					0.680	0.184	0.039	-0.324	-0.681	-0.572
Chlorophyll/unit leaf area						0.842	-0.508	-0.264	-0.471	-0.792
Chlorophyll/unit leaf wght							-0.717	-0.087	-0.122	-0.644
Ch A as % total chlorophyll								-0.116	-0.125	0.299
PS rate/unit leaf area									0.903	0.748
PS rate/unit leaf weight										0.824

§ Correlations were calculated using clone mean values. Details of the variables are given in the previous table.

† If $-0.63 \geqslant r \geqslant 0.63$, $P < 0.05$; if $-0.76 \geqslant r \geqslant 0.76$, $P < 0.01$; if $-0.87 \geqslant r \geqslant 0.87$, $P < 0.001$.

DISCUSSION

The most important results of these experiments were as follows:-

(1) Seedlings from the high N selection line were smaller than those from the low N line, but had similar whole-plant relative growth rates. Differences in seedling weight between the lines were maintained when the level of nitrogen in nutrient solutions applied to the plants ranged from 2.8 to 280 ppm. Seedling dry weights were closely, and positively, correlated with the weights of the seeds from which they originated and were not significantly correlated with RGR.

(2) High N line seedlings were higher in %N of both tops and roots than low N line seedlings, but contained smaller absolute amounts of Kjeldahl nitrogen. They absorbed nitrogen more efficiently per unit weight of roots but utilised nitrogen less efficiently in seedling growth. Low N line seedlings had the highest root:shoot ratios (and the lowest leaf area ratios), and allocated proportionately more assimilate to root growth than high N line seedlings.

(3) Clones known to have high %N values under field conditions had higher rates of apparent photosynthesis than clones having low %N in the field. However, there did not appear to be a strong correlation between %N (tops) and photosynthetic rate.

Differences in seedling dry weight between the selection lines agree with those described in experiment 3 (see table 17). In that experiment, %N of families at heading was negatively correlated with RGR ($r = -0.60^*$), but in experiment 7 there was no such relationship ($r = 0.00$) and no difference in RGR between the lines. The environment in which experiment 3 was conducted was carefully controlled, and although the temperatures used (15°C day, 10°C night) were similar to those which occurred here (20.6°C average maximum, 7.8°C average minimum, per day), the light intensity was more favourable; seedlings in experiment 3 were grown during the spring (August - September), while those in the present experiments grew in the winter (May - July). Thus, RGR between the 3-leaf and 5-leaf stages was approximately 3 times as high in experiment 3 as in the present experiments. In experiment 3, the higher RGR values for the low N line seedlings were due to their superior NAR values (0.83 and 0.52 mg/cm^2/day respectively for low and high line seedlings), and not to LAR values, which were greater for the high line (0.17 and 0.23 cm^2/mg). Thus, in that experiment, NAR was negative-

ly correlated with %N at heading (r = -0.67**) and LAR was positively correlated with %N (r = 0.55*). In the present experiments, the corresponding correlations were -0.32* and 0.62*** respectively, although %N here refers to seedlings. The apparently different relationships between %N and RGR in the experiments merely reflect the extent to which variation in NAR and LAR was responsible for variation in RGR.

One other apparent discrepancy in the present results deserves comment. Although in both experiments 3 and 7 NAR was negatively correlated with %N, experiment 8 showed that genotypes with high %N values (under field conditions) may actually have higher rates of apparent photosynthesis than low N clones. This latter result must be accepted with some reservations, since the measurement of photosynthesis of detached leaves - particularly if manometric methods are used (e.g. comments by Heath 1969)- can give misleading results. Nevertheless the two observations are compatible; it is quite possible that a plant having a high nitrogen concentration could have a high rate of photosynthetic oxygen production yet use the energy so obtained inefficiently in terms of dry weight accumulation. The measurement of oxygen evolution per unit leaf area (or leaf weight, or leaf chlorophyll) is in fact a measurement of the net surplus of reducing power after allowing for leaf respiration. It cannot be assumed that the reducing power so obtained is used only to reduce CO_2 to carbohydrate, for a source of energy is also required to reduce nitrate to ammonia as a first step in the process of amino acid and protein synthesis. Energy for this purpose may be provided by both photosynthesis and respiration (Beevers and Hageman 1969), and leaf nitrogen concentration is linearly related to the rates of both processes (Murata 1969). In fact, CO_2 and nitrate may compete for reducing substances, so that leaves containing high levels of nitrate may fix only 20-30% as much CO_2 as those with low nitrate concentrations (McKee 1962, chapter 2). This competition for reducing power may partly explain the well-known negative correlation between the concentrations of nitrogen and soluble carbohydrate in plants (see figure 4 and, for example, Cooper 1961; Vose and Breese 1964). Assuming that nitrate and CO_2 are reduced to the oxidation levels of ammonia and carbohydrate respectively, twice as much energy will be required to reduce a molecule of nitrate (8 electrons) as to reduce a molecule of CO_2 (4 electrons). If it is assumed that the molecular weights of NH_3 and (CH_2O) indicate the proportional dry weight increments resulting from reduction and assimilation of nitrate

and CO_2 respectively, then by reducing nitrate instead of CO_2 a plant would expend twice as much energy for roughly half as much dry weight gain. On the other hand, as already noted, leaf nitrogen content is closely correlated with photosynthetic rate. The effect of an increase in %N on NAR will therefore reflect the balance between increased supply of reducing power and decreased efficiency with which it is used to produce plant weight.

Observations concerning the nitrogen economy of high and low N selection lines in these experiments are in agreement with the results of experiment 5 where it was found that, under field conditions, swards of the high line recovered greater quantities of applied nitrogen but used nitrogen less efficiently than those of the low line. In addition, it was observed in experiment 7 that low N line seedlings had higher root:shoot ratios and allocated proportionately more assimilate to root growth than to shoot growth, and that the consequent decrease in LAR was at least partially compensated for by a nonsignificant increase in NAR. Should these differences be maintained under field conditions, they might help explain one puzzling feature of experiment 5, i.e. the ability of the low N line swards to extract greater amounts of non-fertiliser nitrogen from the soil. Vose (1962) suggested that differences in herbage yield between 3 strains of Lolium perenne were mainly due to variation in root:shoot ratio. Yield differences were most pronounced when the strains were grown as widely-spaced plants in the field and were less apparent when the strains were grown in swards or as individual plants in solution culture. The strains differed little in %N (although there were indications that the high yielding strain had the lowest nitrogen concentration), but total nitrogen yield was closely correlated with herbage yield. Vose suggested that "a strain with the capacity for extensive root growth can only show to advantage when it is given unlimited space for development", and that superior root growth and efficient utilisation of nutrients could more than compensate for a reduction in LAR. In the present experiments, and during at least the early stages of experiment 5, the ability of the roots to exploit the total volume of nitrogen-containing medium would have been a factor affecting nitrogen uptake. On the other hand, the nitrogen fertiliser in experiment 5 was applied to the soil surface, and it is likely that the surface soil would be the zone in which complete or nearly complete exploitation of the soil volume by the roots would be most rapidly attained (Troughton 1957). Thus, in this zone the

superior ability of the high N line to absorb nitrogen per unit weight of root might have been an advantage. This argument agrees with the suggestion in experiment 5 that, had the nitrogen fertiliser levels been higher, the yield differential between the high and low N line swards may have been reduced or even reversed. Significantly, Hoener and DeTurk (1938), who grew Illinois high and low protein corn (Woodworth et al 1952) in nutrient solution culture with nitrate varied from 25 to 200 ppm, found that at 25 ppm plants of the low protein strain were heavier than those of the high protein strain (2.32 and 2.02 g/plant respectively), but at 200 ppm the weight ranking was reversed (3.08 and 3.76 g/plant respectively). The strains differed markedly in %N of herbage at all levels of available nitrogen in the solutions. In experiment 6, both seedling dry weight and %N differences between the selection lines were maintained over the range of solutions supplied, but in the present work the seedlings were not grown in solution culture but in perlite irrigated with nutrient solution.

Because of the difficulty in comparing the results of these experiments with those of experiments 3 and 5, much of this discussion has been speculative. However, one fact that has emerged very clearly is the relationship between seedling vigour (i.e. dry weight or leaf area at a given time or growth stage) and seed weight. If seed weight could be increased in the high protein line, seedling vigour should be improved. The physiological nature of the relationship between %N and seed weight is examined in experiment 9.

EXPERIMENT 9

THE RELATIONSHIP BETWEEN SEED WEIGHT AND HERBAGE NITROGEN CONCENTRATION IN PHALARIS TUBEROSA

INTRODUCTION

In previous experiments (3,6,7) a negative relationship between seedling vigour and herbage nitrogen concentration in a Phalaris tuberosa breeding population has been described. After four generations of divergent selection for high and low %N of whole tillers at heading, seedlings from the low N selection line were nearly twice as heavy as those from the high line. Although in one experiment (3) the superior seedling vigour of the low N line partly resulted from its higher net assimilation rate (unit leaf rate), later work showed that most of the difference in seedling vigour between families and lines was caused by variation in seed weight. The present experiment was therefore undertaken to examine the relationship between herbage nitrogen concentration and subsequent seed weight.

In cereals, it is generally accepted that grain carbohydrate comes mainly from photosynthesis after anthesis and that, for those cereals possessing a terminal inflorescence, most of the photosynthate found in the grain is produced by photosynthesising organs above the flag leaf node; the literature on this subject has been recently reviewed by Thorne (1966) and Wardlaw (1968). The relative contribution of the organs concerned depends on the species, the genotype and the environment. For example, Jennings and Shibles (1968) found that in one variety of oats photosynthesis by the panicle accounted for 38% of the total grain weight, while in another variety (with large glumes) the contribution by the panicle was 63%. Similarly, Evans and Rawson (1970) observed that photosynthate produced by the ear provided 20% of grain requirements of an almost awnless wheat variety and up to 33% in varieties with large awns. These and many other examples indicate considerable genetic diversity among the small-grain cereals in the extent to which organs above the flag leaf node contribute to grain carbohydrate.

By comparison, relatively little information on seed development in pasture grasses is available. In Phleum pratense (Williams 1964),

Phalaris tuberosa (McWilliam and Wardlaw 1965), the wild progenitors of cultivated wheat (Evans and Dunstone 1970), and probably in Lolium species (Ryle 1970), the pattern of translocation of photosynthetic products during the reproductive phase appears to qualitatively resemble that of the small-grain cereals. Thus, in all these species, the inflorescence itself is an important source of carbohydrate for the developing seed, while at least some photosynthate is translocated to the inflorescence from lower organs such as the flag leaf. Evans and Dunstone (1970) have shown that ear photosynthesis is probably sufficient for grain requirements of primitive (diploid) wheats, and that during evolution there has been an increase in the relative importance of the flag leaf and other organs as sources of grain carbohydrate.

McWilliam (1963) and McWilliam and Wardlaw (1965) have described the pattern of seed development in Phalaris tuberosa in considerable detail. The inflorescence (head) is a panicle having a central rachis with many side branches, on each of which are a large number of individual spikelets. The total number of spikelets per head may be quite small (e.g. less than 100 on small heads) or very large (probably more than 2000), but on the largest heads of spaced plants the number is about 500-1000. Each spikelet consists of two laterally compressed glumes, subtending a single fertile floret and two sterile florets; the latter are each reduced to a single small lemma.

In P.tuberosa, the parts of the culm above (and including) the exposed stem of the penultimate internode contribute some photosynthate to the developing seed (McWilliam and Wardlaw 1965). Although a quantitative description of the relative importance of each organ is not available, the experiments described by these authors suggest that the organ contributing most photosynthate to the seeds is the inflorescence itself, followed by the flag leaf internode, and that the loss of the flag leaf has no apparent effect on seed number or seed weight.

The experiment to be described constituted an attempt to measure the photosynthesising area "available" to each seed in the various organs (particularly those above the flag leaf node) of P.tuberosa genotypes known to differ in herbage nitrogen concentration, and to relate these areas to %N and subsequent seed weight.

MATERIALS AND METHODS

(a) Design of the Experiment and Collection of Data

From the spaced plant populations described in experiment 4, ten genotypes (five with high and five with low %N values at heading) were selected. These plants were dug and broken into propagules (clumps), and 4 propagules of each clone were established in the field at 75 x 75 cm spacings in the early winter (June 4) of 1969. The site was immediately adjacent to those of experiments 1, 2, 4 and 5. Each clone was represented by one propagule in each of 4 replicates in a randomised block design. Apart from trimming each clump to a uniform height (5 cm) at planting, the plants were maintained without defoliation (other than sampling) during the experiment.

Herbage samples were collected at 3 growth stages. The first samples were taken in the early spring (September 18), when all clones were still in the vegetative stage and the herbage obtained was dried in a forced-draught oven at 80°C, ground in an Apex cutter mill to pass a 1mm screen and analysed for Kjeldahl nitrogen content (%N, dry matter basis) as described in appendix 1. Subsequently, each propagule was sampled individually as it reached the heading stage (defined as in experiment 1) and the ripe seeds stage. At the latter stage, all seeds on the earliest tillers had ripened, and the spikelets and flag leaves on these tillers were brown, but lower leaves and all internodes were green. As in earlier experiments, care was taken to sample only tillers at the defined growth stage, and on each occasion sufficient material was harvested to provide 10-30 g dry matter.

At the heading stage, four representative tillers were selected from each propagule and the following measurements were made: length and maximum breadth of all green leaves; tiller height, including head (inflorescence); average tiller width, obtained by lying the four tillers side by side and measuring the total width midway along their length; number of elongated internodes (counted if greater than 3 cm in length); length and average diameter of the head; length and average diameter of the flag leaf sheath. From the centre of each of the four heads a small sample of spikelets (3-5) was taken. The tillers were then separated into leaf lamina (=leaf), leaf sheath, stem and head portions, which were dried separately at 80°C and weighed. One average head was selected from each propagule and the number of spikelets was counted. (Because of the number of spikelets per head

it was scarcely practicable to increase this sample size; merely to measure 40 heads required the dissection and counting of 35,000 spikelets). The herbage from each propagule was then ground and analysed for Kjeldahl nitrogen content as before.

Ten spikelets from the sample collected from each propagule (3 from each of 2 tillers, 2 from each of the remaining 2 tillers) were dissected into their component outer glumes and placed on a glass slide. A photographic enlarger was used to project enlarged images of these glumes (10 x magnification, i.e. areas magnified x 100) onto white paper. The outlines were traced and the area of the traced images so obtained was measured on an airflow planimeter (Jenkins 1959). Previous experience with this technique had shown that such a sample size and magnification gave a repeatable and accurate estimate of spikelet area; the clone values given in the results are thus the means for 40 spikelets.

At the ripe seeds stage, four representative tillers were selected from each propagule. Seeds were collected from the central portion of each of the four heads, and 50-seed samples from each head were bulked, dried at 100°C, and weighed. The herbage samples were dried at 80°C and ground as before, and herbage and seed samples were analysed separately for Kjeldahl nitrogen content.

The dates of heading and flowering (first anthesis) were recorded for each propagule, and the date on which ripe seeds were collected from a propagule was taken to be the date on which seed ripening was completed. The average dates for these 3 stages were November 14 and November 30, 1969, and January 1, 1970 respectively.

(b) Statistical Analysis

From the data collected at the heading stage, areas of photosynthesising organs were calculated. Leaf areas were estimated from length x breadth measurements as described in appendix 7. The flag leaf sheath and the remaining tiller stalk were assumed to be cylindrical, and their areas were calculated accordingly from measurements of their length and average diameter; the errors in estimation were considered to be small in comparison with gross differences in area from clone to clone. Average spikelet area was calculated directly from the measurements of enlarged outlines as previously described. Inflorescence area was calculated by multiplying the average area of the spikelets by the number of spikelets per head. Knowing the number

of spikelets per head, it was possible to express the areas of photosynthesising organs not only on an absolute basis but also in terms of the area available per spikelet, and thus per seed. Visual inspection showed that there were no gross differences among the clones in percentage seed set, which was uniformly high.

The form of the analysis of variance for the characters measured in this experiment has been given previously (table 40). Linear and multiple correlation and regression analyses were used to relate the areas of photosynthesising organs, alone, and in all possible combinations, to seed weight and to %N at heading. Using a computer, the effect on these relationships of differences between clones in the following characters was examined: time between heading and seed collection; time between flowering and seed collection; specific leaf weight; relative rate of apparent photosynthesis (from experiment 8). Finally, the effect of simultaneous variation in either specific leaf weight or relative photosynthetic rate and the time from heading to seed collection was examined.

Although the primary interest was in the relationships described above, the experiment offered an opportunity to re-examine many of the relationships between %N and characters measured in experiments 3 and 4. Since the results agreed with those already described in detail in the earlier experiments, only a brief summary of some of the important relationships is given. In addition, for simplicity, the following results have been omitted: details of analyses of variance; broad-sense heritability estimates; details of correlation and regression analyses except where required for discussion; most correlations between variables.

In the results and discussion of this experiment, seed weight always refers to the weight of an individual seed.

RESULTS

(a) Relationships between Seed Weight and Areas of Photosynthesising Organs.

The most important relationships between seed weight and the areas of photosynthesising organs are summarised in figure 8 and tables 43-45. The character most closely related to seed weight was spikelet area ($r = 0.82^{***}$). Both the absolute area of the flag leaf sheath ($r = 0.46^{**}$; table 43) and the photosynthetic area per seed provided by this organ ($r = 0.42^{**}$; figure 8) were correlated with seed weight, but the relationship was relatively weak. Neither the absolute area nor

the area per seed of either the flag leaf or the penultimate leaf was correlated significantly with seed weight; some details of the relationship between seed weight and flag leaf area will be described in a subsequent paragraph. A simple addition of the area per seed provided by either the flag leaf or the flag leaf sheath, or both, to that provided by the spikelet resulted in a very small (but statistically significant) improvement in the correlation between seed weight and photosynthetic area per seed (figure 8; table 44). A comparison of the linear correlations between seed weight and cumulative photosynthetic areas in different organs (figure 8) and the multiple correlations and partial regression coefficients given in table 44 indicated that a differential "weighting" of the areas in different organs gave a significant improvement in the relationship only when areas of the penultimate leaf and lower organs were included; the partial regression coefficients relating area per seed below the flag leaf node to seed weight (e.g. that for the penultimate leaf in table 44) were far lower than those for areas of organs above the flag leaf node. The partial regression coefficient for flag leaf area was slightly lower than that for spikelet area and flag leaf sheath area.

In summary, the correlation and regression coefficients presented in figure 8 and tables 43 and 44 are consistent with the hypothesis that seed weight is closely related to the photosynthetic area per seed above the flag leaf node, and, in particular, to that contributed by the spikelets and flag leaf sheath. Approximately 75% of the variation in seed weight could be accounted for by this relationship.

The effects of including the various modifications (time from heading to seed collection, time from flowering to seed collection, specific leaf weight, and relative photosynthetic rate) are not presented in the results since in no case did they improve the relationship between seed weight and area per seed in any organ or combination of organs. However, there was relatively little variation between clones in the first 3 chracters (e.g. high and low N clone groups did not differ significantly), and the clone differences in photosynthetic rates must be accepted with reservations (see experiment 8).

One result of particular interest was the large proportion of the total area per seed above the flag leaf node which was provided by the spikelets. On average, 82% of the area was in the spikelets, 4.5% was in the flag leaf and 13.5% was in the flag leaf sheath (table 44). Although the relative contribution of the spikelet to

the area per seed would have decreased as the peduncle elongated, it is clear that because of the large number of spikelets in each head this organ would continue to provide most of the area. There were no gross differences between the clone groups in the relative contribution by each organ (figure 8), but there were quite large differences between individual clones. For example, clone H155 had relatively small spikelets, large flag leaves and a low number of spikelets per head (table 43); the proportions of the area per seed contributed by the spikelet, flag leaf and flag leaf sheath were 73%, 12% and 15% respectively for this genotype. Clone L 184 had a large number of spikelets per head, and the proportions for this genotype were 88%, 2% and 10% respectively. A comparison of the clone mean seed weights and spikelet areas given in table 43 shows that clone H155 had heavier seeds than would have been expected from its spikelet size. These data suggest that the heavy seeds of clone H155 were the result of its large flag leaves and reduced number of spikelets per head.

(b) Nitrogen Concentration in Herbage and Seeds

There were considerable differences between clones in herbage nitrogen concentration at each of the 3 sampling stages. On each occasion the high N group of clones had significantly higher %N levels than the low N group, and at the heading stage the %N values of the clones of the two groups did not overlap (table 43). Although there was some overlapping at the other stages (data not presented), genotype X growth stage interaction was of minor importance, as indicated (for example) by the close correlation between %N of propagules at the heading and ripe seeds stages (table 46). However, there was not a close correlation between %N of seeds and %N of herbage at any stage so that, despite a highly significant range in %N of seeds between the clones (table 46), the high N and low N clone groups did not differ significantly in this character (3.29 and 3.15 %N respectively). There were significant negative correlations between %N and tiller length, average internode length, number of internodes per tiller, tiller width, and the weights of whole tillers and all tiller components. For all of these characters there was extensive variation among clones.

(c) Correlations between Seed Weight and %N of Herbage and Seeds

Two clones, H155 and L109 (which had the highest and lowest %N values respectively at the heading stage) deviated markedly from otherwise close negative relationships between seed weight and %N of herbage at the heading and ripe seeds stages; consequently the two observed correlations were small and nonsignificant (-0.15 and -0.21 respectively at the two stages). There was no indication of any significant association between seed weight and %N of seeds (r = 0.21).

(d) Relationships between %N of Herbage and Areas of Photosynthesising Organs.

The correlations in table 43 indicate that %N of herbage was negatively, but not closely, related to both spikelet area (r =-0.49**) and to the total area of the flag leaf sheath (r = -0.46**). The latter correlation was due in turn to a negative correlation between %N and the width of the flag leaf sheath (r = -0.63***; table 46), that between %N and the length of this organ being nonsignificantly positive (r = 0.22). However, %N was not significantly related to the area of the flag leaf (r = 0.26). There was a weak negative association between %N and the number of spikelets per head (r = -0.34*).

Some important correlations between the areas of photosynthesising organs are presented in table 45. Attention is drawn to the following relationships: (i) spikelet area and flag leaf sheath area were positively correlated; (ii) areas of the flag and penultimate leaves were positively correlated, but were not correlated with those of other organs; (iii) high numbers of spikelets per head were associated with large flag leaf sheaths but not with the size of the other organs listed in the table.

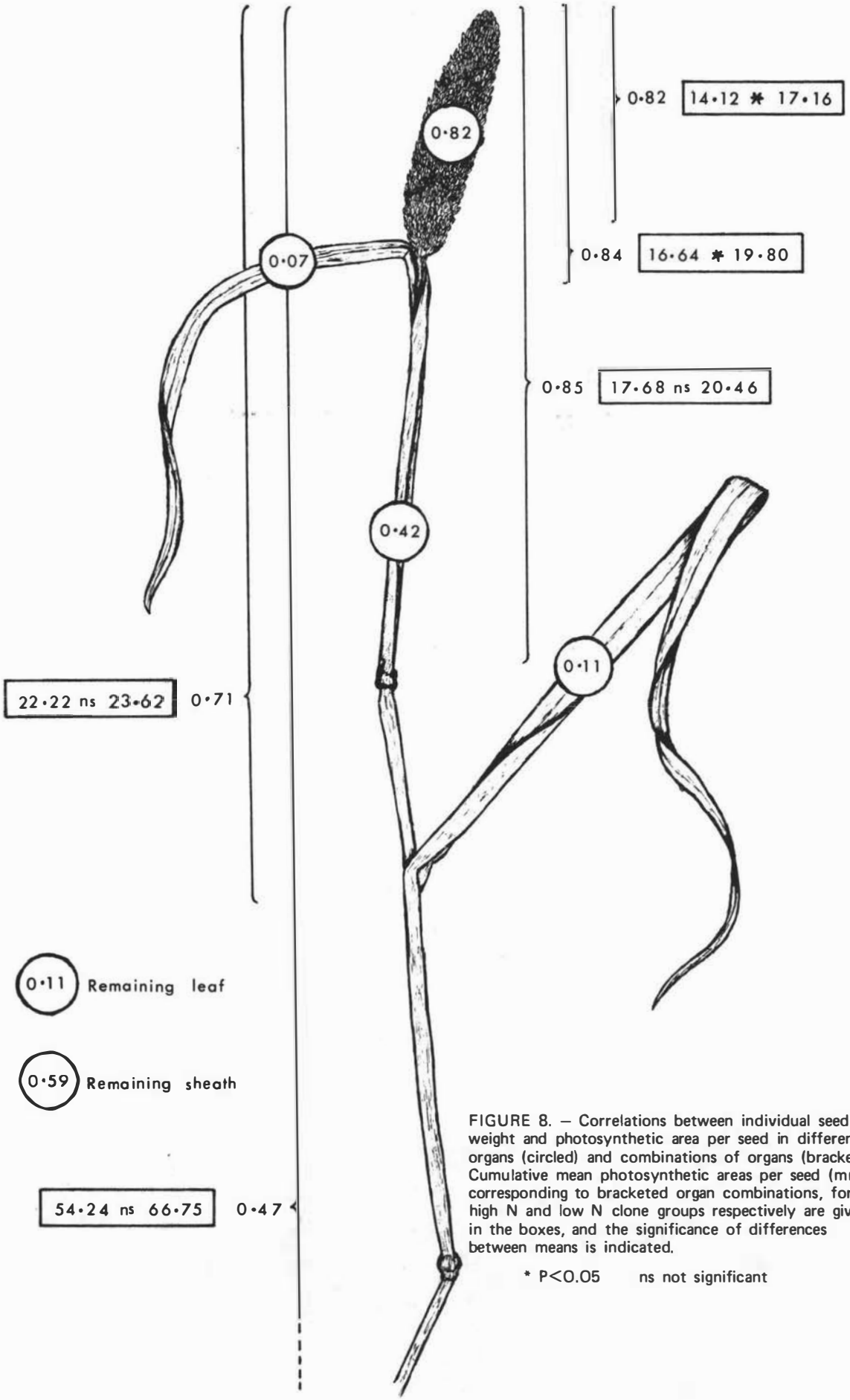

FIGURE 8. – Correlations between individual seed weight and photosynthetic area per seed in different organs (circled) and combinations of organs (bracketed). Cumulative mean photosynthetic areas per seed (mm^2), corresponding to bracketed organ combinations, for high N and low N clone groups respectively are given in the boxes, and the significance of differences between means is indicated.

* $P<0.05$ ns not significant

TABLE 43

CLONE VARIATION IN % N, SEED WEIGHT AND AREAS OF PHOTOSYNTHESISING ORGANS ABOVE THE FLAG LEAF NODE

(Figures are clone mean values ± standard errors)

Clone	% N, heading	Seed weight(mg)	spikelets/head	spikeletarea(mm^2)	Flag leaf area(cm^2)	Flag leaf sheath area(cm^2)
H155	2.10 ± 0.08	1.89 ± 0.04	510 ± 14	15.1 ± 0.2	12.3 ± 1.0	16.4 ± 1.0
H166	1.68 ± 0.10	1.80 ± 0.05	725 ± 96	14.7 ± 0.5	4.8 ± 1.4	21.4 ± 3.2
H17	1.76 ± 0.12	1.66 ± 0.03	665 ± 17	15.7 ± 0.3	3.7 ± 0.4	15.7 ± 1.3
H129	1.89 ± 0.12	1.41 ± 0.03	970 ± 126	10.3 ± 0.4	7.3 ± 1.0	19.0 ± 1.2
H99	1.81 ± 0.14	1.68 ± 0.03	949 ± 93	14.8 ± 0.3	6.8 ± 0.7	18.9 ± 1.9
High N group mean	1.85 ± 0.07	1.69 ± 0.02	764 ± 27	14.1 ± 0.1	7.0 ± 0.4	18.3 ± 0.5
L109	1.29 ± 0.07	1.72 ± 0.05	876 ± 46	16.7 ± 0.3	9.6 ± 1.4	2[illegible].6 ± 0.8
L187	1.57 ± 0.04	1.81 ± 0.03	962 ± 32	16.8 ± 0.3	4.1 ± 1.4	22.1 ± 0.8
L89	1.39 ± 0.11	1.91 ± 0.04	900 ± 135	16.8 ± 0.2	5.1 ± 1.0	25.7 ± 2.4
L165	1.43 ± 0.08	1.98 ± 0.06	852 ± 62	18.1 ± 0.4	6.0 ± 0.5	26.4 ± 1.5
L184	1.35 ± 0.09	1.86 ± 0.03	1260 ± 40	17.3 ± 0.1	6.0 ± 0.5	25.2 ± 2.3
Low N group mean	1.41 ± 0.03	1.86 ± 0.01	970 ± 20	17.2 ± 0.1	6.2 ± 0.6	24.8 ± 0.8
Correlation with % N	——	-0.15	-0.34*	-0.49**	0.26	-0.46**
Correlation with seed weight	-0.15	——	0.01	0.82***	0.02	0.46**
Significance of clone differences	***	***	***	***	***	**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 44

MULTIPLE LINEAR REGRESSION OF SEED WEIGHT ON AREAS (PER SEED) OF PHOTOSYNTHESISING ORGANS.
(Columns A,B,C and D show the partial correlations of the remaining variables with seed weight as least significant variables are successively eliminated)

Organ (brackets: average photosynthetic area (mm^2) available for each seed)		Partial correlations between area per seed and seed weight			
		A	B	C	D
X1 = spikelet area/seed	(15.64)	0.817***	0.826***	0.824***	0.816***
X2 = flag leaf area/seed	(0.85)	0.088	0.178	—	—
X3 = flag leaf sheath area/seed	(2.58)	0.243	0.338*	0.454**	—
X4 = penultimate leaf area/seed	(3.85)	0.051	—	—	—

Equations: (A) Seed weight = 0.60 + 0.064 X1 + 0.021 X2 + 0.054 X3 + 0.006 X4
(B) Seed weight = 0.61 + 0.063 X1 + 0.030 X2 + 0.061 X3
(C) Seed weight = 0.63 + 0.061 X1 + 0.076 X3
(D) Seed weight = 0.76 + 0.065 X1

Multiple linear correlation,	R	0.862***	0.862***	0.857***	0.816***
	R^2	0.743	0.743	0.734	0.667

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 45

CORRELATIONS AMONG VARIABLES CONTRIBUTING TO PHOTOSYNTHETIC AREA PER TILLER AND PER SEED ABOVE THE FLAG LEAF NODE

Character	Area of individual spikelet	Flag leaf area	Flag leaf sheath area	Penultimate leaf area	Total area above flag leaf node	Area per seed above flag leaf node
No. of spikelet/head	0.17	-0.17	0.59***	-0.05	0.86***	-0.14
Area of individual spikelet		-0.13	0.49**	-0.03	0.57***	0.90***
Flag leaf area			0.05	0.65***	0.00	0.18
Flag leaf sheath area				0.29	0.80***	0.43**
Penultimate leaf area					0.19	0.17
Total area above flag leaf node						0.36*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 46

DESCRIPTION OF CLONE VARIATION IN SOME PLANT CHARACTERISTICS CORRELATED WITH % N OF WHOLE TILLERS AT HEADING

Characteristic	Correlation with % N (r)	Range of clone means and significance of differences
%N of seeds	0.42	2.86 *** 3.61
%N, whole tillers, ripe seeds stage	0.81	0.76 *** 1.12
Tiller length (cm)	-0.66	74.9 *** 118.2
Mid-length tiller width (mm)	-0.50	4.94 *** 7.72
Number of elongated internodes/tiller	-0.59	4.1 *** 5.8
Length of flag leaf internode (cm)	0.22	13.4 ** 20.0
Width of flag leaf internode (mm)	-0.63	2.9 *** 5.8
Weight of leaf per tiller (gm)	-0.55	0.69 *** 2.10
Weight of sheath per tiller (gm)	-0.70	0.81 *** 2.56
Weight of stem per tiller (gm)	-0.64	1.14 *** 3.86
Weight of head per tiller (gm)	-0.58	0.41 *** 0.94
Length of stem (cm)	-0.66	63.8 *** 107.3
Mean internode length (cm)	-0.34	14.7 ** 19.9
Tiller weight (gm)	-0.68	3.15 *** 8.66
% leaf	0.48	16.4 *** 29.1
% sheath	-0.29	23.8 ** 29.5
% stem	-0.46	32.3 *** 48.6
% head	0.31	8.4 *** 16.1

** $P < 0.01$, *** $P < 0.001$.

If $-0.31 \geqslant r \geqslant 0.31$, $P < 0.05$; if $-0.40 \geqslant r \geqslant 0.40$, $P < 0.01$; if $-0.50 \geqslant r \geqslant 0.50$, $P < 0.001$.

DISCUSSION

The results of this experiment suggest that the photosynthetic area per seed could be maintained (and consequently a satisfactory seed weight might be retained) during continued selection for %N by concurrently selecting for large flag leaves and a reduced number of spikelets per head. Such a strategy should maintain a satisfactory photosynthetic area per seed above the flag leaf node despite expected further reductions in the areas of the spikelets and flag leaf sheath. However, seed yield per tiller would decline.

Although in this experiment there was a close correlation between seed weight and photosynthesising area per seed above the flag leaf node, the interpretation of this relationship is not simple. The apparently obvious conclusion that seed weight is limited by the photosynthetic area per seed is open to question. In cereals, it is generally recognised that limitations on total grain yield per ear may be imposed by the ability of the organs concerned to provide the carbohydrate requirements of the developing grain, the capacity of the grain to fully utilise the photosynthate capable of being produced, the ability of the phloem to translocate photosynthate from the lower organs to the grain, or combinations of these processes. Several recent reviews of these possible limitations are available (Thorne 1966; King, Wardlaw and Evans 1967; Wardlaw 1968; Nealea and Incoll 1968). Provided photosynthetic area is expressed on a per seed basis, there seems no reason why limitations to seed weight could not be similarly described. Statistical relationships alone cannot provide conclusive evidence of the relative importance of these potential limitations. For example, Simpson (1968) observed a correlation of 0.76 between the total grain weight per tiller and an estimate of the total photosynthetic area per tiller above the flag leaf node among 120 wheat varieties and suggested that the size of the source of grain carbohydrate was limiting grain yield. Yet the conclusions of those authors who have studied this relationship at a more basic level have been far from unanimous (e.g. Thorne's 1966 summary of earlier work, and recent experiments by Stoy 1966; Evans and Rawson 1970; Walpole and Morgan 1970). Should this be a major limitation, one would expect that by removing some grain from the ear, the individual weights of the remaining grain would increase; although several authors have observed such increases for wheat (Stoy, 1965; Bingham 1967; Rawson and Evans 1970), others have reported little or no change (Abolina 1959; Buttrose 1962).

Similarly, in P. tuberosa McWilliam and Wardlaw (1965) found no relationship (r = -0.01) between seed weight and percentage seed set when the latter character varied from 11-96%. Labelled carbon did not appear to be redistributed from spikelets containing abortive florets to seeds developing in adjacent spikelets. In addition, as percentage seed set decreased, so also did photosynthesis by the top internode, although photosynthesis by the inflorescence did not; however, glumes subtending abortive florets had apparently lower rates of photosynthesis, and senesced earlier, than glumes subtending developing seeds. Another observation was that, if culms were detached from the plant at anthesis, there was no apparent reduction in photosynthesis by the inflorescence two weeks later, but more of the photosynthate was retained by the glumes. Despite some discrepancies, these results seem to indicate that seeds in adjacent spikelets do not compete for translocated photosynthate, and that seed weight is limited not by the supply of photosynthetic products but by the capacity of the seeds to utilise them.

An alternative interpretation of the present results, and one more in accord with the results of McWilliam and Wardlaw (1965), is that there is no causal relationship between spikelet area and seed weight but that instead, there is some common determinant of the size of both spikelet and seed. Cell size may well be such a factor, as recently suggested by Evans and Dunstone (1970) for wheat. This explanation would agree with many observations in the previous experiments; for example, the small leaves and high rates of apparent photosynthesis of the high N clones during vegetative growth (see experiment 8) could both be due to a lower mesophyll cell size (Wilson and Cooper 1967) in these clones than in the low N clones. Two other observations support this interpretation of the present results. One is the fact that the glumes of P. tuberosa contain very little chlorophyll; that which is present occurs in concentrated bands roughly parallel with the long axis of the glumes, and probably intercepts little of the light reaching the spikelets, so that much of the light may be intercepted by the seeds. The other is the fact that P. tuberosa seeds are green before and long after anthesis, and obviously contain considerable amounts of chlorophyll. Thus, much of the photosynthate required by the developing seeds seems certain to be supplied by the seeds themselves. Significantly, Evans and Rawson (1970) recently demonstrated that, in 3 wheat varieties, photosynthesis by the grain

accounted for 33-42% of the gross ear photosynthesis, and that the ear contributed from 20-33% of the total photosynthate requirement of the grain.

Further support for this interpretation is provided by the results of an unpublished experiment conducted at Canberra in 1965-66. Plants used for this experiment were originally established by Dr. R.N. Oram to examine the inheritance of seed yield and seed retention in P. tuberosa. The experimental population, which consisted of 4 closely-spaced (40 x 40 cm) plants of each of 55 full-sib families in each of 2 replicates, was used by the present author for a genetic analysis of seed weight and associated characters. By methods similar to those in the present experiment, the length and maximum width of florets at anthesis were measured, and a crude estimate of floret area was obtained by multiplying floret length x width. This latter character was found to have a high genetic correlation (r_g = 0.82) with the subsequent weight of seeds in spikelets close to those sampled for floret measurements. Neither floret length (r_g = 0.46) nor floret width (r_g = 0.55) was as closely correlated genetically with seed weight as was the crude area estimate. However, spikelet area was not measured in that experiment.

Although neither of these interpretations of the present results can be accepted conclusively, it is apparent that the correct analysis must be able to account for the peculiar behaviour of clone H 155. The facts that this clone had heavier seeds than would be expected from its spikelet size, and that it also had the largest flag leaves and smallest number of spikelets per head suggest that the developing seeds of this clone benefit ed from an unusually favourable supply of assimilate from the flag leaf, and support the original interpretation. However, whatever the explanation, it is clear that the negative relationship between %N and seed weight can be broken.

GENERAL DISCUSSION AND CONCLUSIONS

As was pointed out on page 3, the response to selection for a character is a function of the phenotypic variability, heritability, selection intensity, generation interval and the amount of genotype x environment interaction. Both genotype x growth stage interactions and repeatability may affect the generation interval, as explained on pages 8-11. It is also important to examine the likely correlated responses which would result (in the absence of preventive measures) from selection for any character.

The results of the experiments which have been described will be briefly reviewed with reference to these genetic concepts. The numbers in parentheses refer to the nine experiments in the thesis.

(a) Phenotypic Variability

Several experiments (1, 2, 3, 4, 7, 9) have shown that there is extensive variability in IVDOM and %N of herbage in the genus Phalaris and within the species P. tuberosa. Some examples could be noted. At the heading stage there was a range of 59-77% IVDOM (61-75% after adjusting values to a common heading date) among Phalaris species and interspecific hybrids grown as undefoliated spaced plants (1). Among eight P. tuberosa strains, IVDOM at the heading stage ranged from 61-68% (1), and among families derived from the most digestible of these strains IVDOM ranged from 59-68% at heading and 28-39% at maturity (2). Individual plants at the heading stage in another population varied from 56% to 76% IVDOM (4).

Herbage %N varied between genotypes, families or strains of P. tuberosa at all growth stages and in all environments examined. In seedlings, %N of sixteen families ranged from 3.5 - 6.0 (7). At the heading stage, the range in %N among eight strains was 1.15 - 1.55 (1), while individual clones ranged from 1.29 - 2.10 %N (9). Within the two experimental selection lines there was extensive variation between plants at the heading stage (3, 4). Among families of the Algerian ecotype CPI 19280, %N of standing dead herbage ranged from 0.38 - 0.66 (2).

From these results it is clear that phenotypic variability of IVDOM and %N among spaced plants is unlikely to be a limiting factor in a breeding program which aims to raise the levels of these characters. However, it must be remembered that such variation may not occur under sward conditions. The limited experimental evidence available

(e.g. a comparison of the herbage quality of the high and low N selection lines under spaced-plant and sward conditions, experiments 4 and 5) suggests that variability of IVDOM may well be maintained in swards, but that variation in %N might be reduced. Lazenby and Rogers (1965) also observed less variation in %N among Lolium perenne genotypes grown as simulated swards than among the same clones grown at wide spacings. On the other hand, as previously noted (page 7), extensive variation for both IVDOM and %N has previously been observed among 55 P. tuberosa full-sib family swards in their second year of growth, at stages approximately corresponding to the heading and mature stages of experiments 2 and 4 (Oram, Clements and McWilliam, unpublished).

(b) Heritability

Much of the observed phenotypic variability was genetic in origin (1, 2, 3, 4, 9). The parameter of main importance is the narrow-sense heritability (see page 8), which is the proportion of the total phenotypic variance attributable to the average effects of genes (or, as it is more generally but ambiguously defined, the ratio of the additive genetic variance to the total phenotypic variance). The heritability of %N at heading for individual plants was low, within the range 0.20 - 0.36 (3, 4). In experiment 2, the reference unit was not a single plant but a family mean value based on three 8-plant rows, and the heritabilities reported in this experiment were consequently higher than would be expected for single plants. In this ecotype, which may reasonably be assumed to be in linkage equilibrium, the heritability of %N at heading was again low (0.27), but that of %N of mature herbage was higher (0.59). Heritabilities of IVDOM at the two stages were 0.55 and 0.78 respectively; these values suggest that, in a breeding program, if the number of possible IVDOM analyses was limited, the optimum number of replicates would be less than three (i.e., that more progress per cycle of selection would be made for a given number of analyses if the replication was decreased and the number of families increased). Alternatively, the high heritabilities of family means suggest that individual selection for IVDOM might be more efficient than family selection. Since the heritability of a single-plant value is not known, the theoretical efficiencies of the two procedures cannot be compared. However, unless the number of analyses was limited, the higher selection intensity that would almost certainly be possible amongst individuals, and the higher phenotypic

standard deviation of individual values would probably outweigh the higher heritability of a family mean. In practice, a decision on the method of selection for either %N or IVDOM would be complicated by the type of selection used for other characters in the breeding program, by the size of the heritability estimates for a given population/environment combination, by the amount of emphasis to be placed on herbage quality as a breeding objective, by the extent to which differences among single plants are maintained in swards, and doubtless by other considerations of a less intellectually stimulating nature (e.g. the availability and adequacy of equipment). In any event, it is clear that in one breeding population the amount of genetic variation for %N was such that four cycles of divergent selection of individual plants were sufficient to produce two experimental populations which, when grown as spaced plants in the field, differed considerably in %N of whole tillers and all tiller components (3, 4), and which differed in %N under sward conditions (5).

(c) Intensity of Selection

This parameter has not been examined in detail in these experiments but some general comments may be made. As has been noted, the selection intensity would be likely to be higher for individual selection than for family selection because of limitations on the number of families which could reasonably be produced. On the other hand, the complexity of the measurements (particularly IVDOM) and the time available for the measurements could impose a limitation; if the possible number of analyses was low, then family selection or progeny testing would increase the value of each individual determination (assuming that herbage from the members of a family was bulked for analysis as in experiment 2). Another factor which would have a bearing on selection intensity would be the general organisation of the breeding program. For example, if the number of IVDOM or %N analyses was restricted, an efficient procedure in a breeding program with several objectives would be a system of independent culling levels; herbage from all individuals or families could be collected, but only that from individuals which had acceptable levels of the remaining characters under selection need be measured for herbage quality. A similar technique could be employed if both %N and IVDOM were to be improved; the material could be screened first for the character most easily measured (probably %N). In practice, the relative emphasis on IVDOM, %N and other characters would markedly influence the intensity with

which each was selected.

(d) Generation Interval

The heritabilities observed for %N (2, 3, 4) and IVDOM (2) suggest that progeny testing would be an inefficient breeding procedure for these characters because of the increased generation interval which would be required. However, progeny testing cannot be completely disregarded. Should it be deemed necessary to select for herbage quality or some other character (e.g. herbage yield) under sward conditions, polycross progeny testing may be a suitable means of providing the quantities of seeds required, (Latter 1964) particularly if the heritability of yield is low or if testing in several environments is required. Nevertheless, progeny testing is unlikely to be efficient for P. tuberosa breeding, provided suitable equipment (McWilliam 1964 b) for controlled hybridisation is available, for two reasons. First, by crossing two genotypes, sufficient seeds are produced for sowing small swards (McWilliam 1964 b; Latter 1964; McWilliam and Latter 1970). Second, it is doubtful if the heritability of any major breeding objective in P. tuberosa is sufficiently low to make progeny testing worthwhile (McWilliam 1963; Latter 1965; Oram 1970 and unpublished; McWilliam and Latter 1970; and experiments 2, 3, 4).

If progeny testing is thus rejected as a breeding method for P. tuberosa (but not necessarily for other species), the remaining selection methods are likely to have the same time requirement for a cycle of selection. However, one possibility may be noted. Should it be acceptable to measure herbage quality on single plants, and should there be no serious genotype x environment interactions for herbage quality, then generation interval could be shorter for single-plant selection than for selection in swards. This is because the generation interval of a single-plant population of reasonable size may be artificially manipulated, as it was in experiment 3. In fact, the generation interval for a species such as P. tuberosa may be shortened to five months using controlled environments. Whether or not selection for a character under such unnatural conditions would be worthwhile can only be accurately predicted if the heritability and phenotypic standard deviation of the character in each environment, the generation interval and selection intensity in each environment, and the genetic correlation between the two measurements are known. However, some conclusions can be drawn about the relative efficiency of selection

for %N of spaced plants in the artificially controlled environment described in experiment 3 and in the natural environment (experiment 4), assuming that improved performance in the natural environment is the criterion for comparison. The necessary theory is described by Falconer (1960, chapter 19). The heritability of %N is approximately the same in each environment (3, 4). Assume that a cycle of selection for %N at heading would take twice as long (i.e. generation interval would be twice as long) in the field as in the controlled environment, and that the intensity of selection in each selection cycle would be similar in each environment. In a given amount of time, the cumulative selection intensity would be twice as high in the controlled environment as in the field. In these circumstances, selection under artificial conditions would only be more efficient if $2 r_g > 1.0$, that is, if $r_g > 0.5$, where r_g is the genetic correlation between measurements of %N made in the two environments.

Two other factors affecting the generation interval, which were described earlier (pages 8-11), should be mentioned. The first is the repeatability (in time) of the character being selected. The present experiments were not designed to provide information on the repeatability of %N and IVDOM since repeated measurements are unlikely to be worthwhile if selection intensity is significantly reduced or generation interval increased as a consequence. However, one could validly ask whether measurements of %N and IVDOM during the first year of growth are likely to indicate the value of a plant in subsequent years; for example, first-year herbage yields may be a poor indication of yield in the second and later years (Biddiscombe et al 1969). The experiments described here provide little information on this point, but some results could be noted. In experiment 5, differences in %N and IVDOM of herbage from swards of the high and low N lines were reasonably consistent at each harvest during the first fifteen months after sowing. In experiment 9, high N and low N clones which had exhibited marked differences in %N during their first year also differed in a subsequent year; however, in the later year the clones were re-established from propagules, so that they could not strictly be called two-year-old plants. In another experiment (Clements 1970) measurements of %N of nine clones at the heading stage in two different years were found to be highly correlated ($r = 0.70$). Thus, although the evidence is inconclusive, it does not seem that first-year measurements of this character are likely to be misleading.

The remaining factor whi ight affect the generation interval is the magnitude of genotype x owth stage interactions for %N and IVDOM (2, 4, 9). If the lity of senescent or dead herbage could be predicted in advance flowering, each cycle of selection could be completed in one year d of two. The genetic analysis in experiment 2 indicated that the ecotype studied, the interaction between families and growth s was such that selection for either character before flowering wou be inefficient despite the reduced generation interval. In exper t 4 it was found that although the high and low N lines differed i at all the growth stages which were examined, the relative differenc tween the lines was greater at heading than at stages immediate efore or after heading. In the same experiment, differences in between the three lines varied from 1.6 digestibility units to 6 digestibility units, depending on the growth stage. For both %N and IVDOM, line x growth stage interactions were very highly significant. On the other hand, among the high and low N clone groups in experiment 9 there was a close correlation ($r = 0.81$) between %N measurements at the heading and ripe seeds stages. Since the ten clones in experiment 9 were the extreme plants in populations which had previously been selected over four generations for high or low %N at heading, this result is perhaps not surprising. Thus, although the genetic relationships between measurements made at different growth stages may be expected to differ in other populations and environments, the present results suggest that unless the measurements are known to be favourably correlated it will be safer to select for herbage quality at the stage at which improvement is required. A more complete discussion of this topic is given in the results section of experiment 2.

(e) Genotype x Environment Interactions

The results of these experiments suggest that genotype x environment interactions for %N and IVDOM in P. tuberosa are small in magnitude and are unlikely to cause problems in a breeding program. The high and low N selection lines which were developed under artificially controlled conditions (3) differed in %N when grown as spaced plants (4) or swards (5) under field conditions. In swards, there were no interactions between lines and defoliation frequencies or levels of applied nitrogen, for either %N or IVDOM (5). When the lines were

grown as seedlings in a glasshouse, they differed in %N when the level of nitrogen in the nutrient solution varied from 2.8 to 280 ppm (6). The IVDOM rankings of the high, low and control N lines were similar under spaced-plant and sward conditions (4, 5). The ranking of P. tuberosa strains for %N and IVDOM of herbage at the heading stage at Palmerston North (1) agreed well with the ranking previously observed at Canberra (Clements, Oram and Scowcroft 1970 and unpublished data).

(f) Genetic Relationships between Herbage Quality Criteria

In these experiments, attention has been concentrated on %N and IVDOM (for reasons outlined on pages 2 and 3), but some other nutritive value criteria were measured in experiment 3. There seems to be no stable genetic relationship between %N and IVDOM in P. tuberosa (1, 2, 3, 4). The relationship may depend on the growth stage (2) and the population (2, 3). In experiment 4 it was found that %N and IVDOM were negatively correlated despite the fact that other variables (leaf:stem ratio, tiller length) had similar effects on each character. In other experiments (2, 4) there was a negative relationship between %N at heading and IVDOM at later stages. In experiment 3, selection for increased %N resulted in a correlated increase in IVDDM during the first two selection cycles, but the correlated response was subsequently reversed (3, 4, 5). These results suggest that, should improvements in both %N and IVDOM be required from a breeding program, selection for both characters would be necessary.

From experiment 3, and from the work of Cooper (1961), it may be concluded that there is a negative genetic correlation between %N and soluble carbohydrate content.

(g) Relationships between Herbage Quality and Other Characters

Every experiment has provided some information on the relationships between either %N or IVDOM and other important agronomic characters. Contrary to most published reports (see page 16) and current overseas opinion, there was no evidence that IVDOM was negatively correlated with herbage yield (2, 4, 5). Among the Phalaris species studied in experiment 1, the annuals were higher in IVDOM than the perennials and matured earlier. In experiment 2 there was a negative genetic correlation (r_g = -0.57) between heading date and IVDOM at heading in a P. tuberosa population. The meaning and importance of these two results are not clear. Even after adjusting values to a

common heading date the annual Phalaris species still had higher IVDOM than the perennial species (1). This suggests that IVDOM may be negatively correlated with perenniality. Although this may impose an upper limit to the improvement of IVDOM (assuming perenniality must be retained), there is a large amount of genetic variation in IVDOM within the perennial species P. tuberosa (1, 2, 4). Also, IVDOM at maturity was not genetically correlated with heading date (2).

The experiments have shown that %N is negatively correlated with seedling vigour (3, 6, 7), tiller weight (2, 3, 4, 5, 9) and herbage yield (5). To the extent that seedling vigour depends on seed weight, the first of these adverse genetic relationships can be broken (9). However, there is a low to moderate negative relationship between %N and net assimilation rate in seedlings (3, 7), which may in some circumstances contribute to the correlation between %N and seedling vigour (3).

The negative genetic relationship between %N and tiller weight would be much more difficult to overcome. As an example of the strength of this association, it may be noted that mean tiller weight at heading among the five high N clones in experiment 9 ranged from 3.2 - 3.8 gm, while low N clone mean values were in the range 7.3 - 8.7 gm. The relationship holds at all growth stages during the reproductive phase (2, 4, 9) under spaced-plant conditions. In swards, the reduced tiller weight of the high N selection line was only partially compensated for by increased tiller density, so that the low N selection line gave consistently higher herbage yields (5). These results suggest that there may be a fundamental negative physiological association between the ability of a genotype to concentrate nitrogen in its tissues and its ability to produce dry matter. Such a suggestion is supported by the results of experiment 7, which showed that seedlings having the ability to take up large amounts of nitrate nitrogen per unit weight of root used their absorbed nitrogen relatively inefficiently in the production of incremental dry weight. Further support comes from biochemical evidence (reviewed by McKee 1962, chapter 2; Sims, Folkes and Bussey 1968; Beevers and Hageman 1969) that nitrate reduction is intimately associated with the photosynthetic production of reducing power, and that nitrate and CO_2 may compete for reducing substances. The implications of this competition are described in the discussion of experiments 6 - 8.

(g) General Comments

These experiments were designed to examine the potential for increasing the nutritive value of P. tuberosa herbage by breeding. The suitability of %N and IVDOM as selection criteria can best be evaluated by considering the estimated gains from selection and the consequent improvement, if any, in animal production; the difficulties of assessing the benefits to grazing animals have already been outlined (pages 12 - 17) and will not be reiterated here. Most aspects of plant breeding methodology are outside the scope of this work. Nevertheless, methodology is important and two aspects will be briefly mentioned.

The first concerns the formation of a suitable base population for a subsequent breeding program. Unfortunately the populations most suitable for a breeding program are not necessarily those most suitable for quantitative genetic analysis, since linkage equilibrium will be temporarily disturbed in a population synthesised from diverse ecotypes. The unstable genetic relationship between %N and IVDOM in the breeding population used to produce the high and low N selection lines (3, 4, 5) is probably an example of this effect. There are therefore good reasons for using naturally occurring ecotypes for genetic analyses, as was done in experiment 2. Nevertheless the formation of a base population is one of the most critical (yet least studied) aspects of a plant breeding program.

Herbage quality is likely in practice to be only one of several breeding objectives for a species such as P. tuberosa. Breeding populations may have previously been established. The present results (3, 4, 5, 6, 7, 9) show that one existing breeding population, derived from thirty Mediterranean ecotypes (McWilliam and Latter 1970), contains extensive genetic variability for %N and IVDOM. Thus, despite the fact that other Phalaris species and other ecotypes of P. tuberosa have relatively high herbage quality (1) it would probably not be worthwhile using these sources to increase the genetic variability for herbage quality in the base population, because of their agronomic deficiencies in characters such as yield and perenniality.

The second methodological aspect which deserves some comment is the sampling of herbage for subsequent nutritive value measurements. This seems certain to remain a controversial topic, for reasons that have been outlined on pages 1, 8-9 and 13-14. It has been assumed in the present work that %N and IVDOM of vegetative herbage are unlikely to be major limitations to the productivity of ruminants grazing

P. tuberosa pastures, and attention has therefore been concentrated on herbage quality during the reproductive phase. As has already been noted (page 13), it may be possible to increase quality during this phase by altering the relative proportions of vegetative and reproductive tillers, or simply by selecting for yield of green herbage during the summer and autumn (Clements, Oram and Scowcroft 1970). There are both advantages (ease of measurement) and disadvantages (probable reduction in seed yield, possible loss of drought resistance) in this approach. There seems little point in performing complicated and expensive chemical or biological assays on mixtures of vegetative and reproductive material, since (as has previously been demonstrated; Clements, Oram and Scowcroft 1970) much of the apparent variation in herbage quality will be due to the relative proportions of tillers at different growth stages, and since grazing animals will in any case select the green herbage (see page 13). For these reasons, and because of the genotype x growth stage interactions described here (2, 4), it is necessary to specify the growth stage at which measurements are to be taken, and to sample only tillers at that stage. In other words, the most sensible approach would be to increase the proportion of high-quality herbage in the pasture and to increase the quality of the low-quality fraction.

(h) Conclusions

There is extensive genetic variation for %N and IVDOM in P. tuberosa, and further variation in related species. It should not be difficult to increase the levels of both characters, which are not closely related genetically. The plant breeder wishing to improve either character should clearly define the stage of growth at which maximum improvement is required, and should measure herbage sampled at that stage, but he need not select in more than one environment. A negative relationship between IVDOM and perenniality may impose a limit to increases in IVDOM, but considerable improvement in this character could be achieved without sacrificing perenniality. Increases in %N would be accompanied by reduced tiller weight, and therefore probably by reduced herbage yield, unless selection was concurrently applied to maintain the levels of these characters, in which case the likely improvement in %N would be small. It seems likely that another negative genetic association, that between %N and seedling vigour, could be overcome by maintaining seed weight at a satisfactory level, although seed yield would probably be reduced.

SUMMARY

(1) Thirty-nine strains of 6 annual and 5 perennial *Phalaris* species and 4 categories of interspecific hybrids were grown as spaced plants. Herbage samples, collected as strains reached the heading stage, were examined for Kjeldahl nitrogen content (%N, dry matter basis) and *in vitro* digestibility of organic matter (IVDOM). The strains ranged from 0.72 - 2.54 %N and from 58.5 - 76.7% IVDOM, and there was considerable variation for each character among 8 *P. tuberosa* entries. Heading occurred on average 26 days earlier in annual species than in perennials. After adjusting values to a common heading date it was found that IVDOM was higher in annual species than in perennials (70.5 vs. 66.1), but %N was lower (1.15 vs. 1.70).

(2) Spaced plants of 39 full-sib families from an Algerian ecotype of *P. tuberosa* were analysed for %N and IVDOM at 2 growth stages. At heading, mean IVDOM among the families ranged from 59 - 68%, while IVDOM of standing dead (mature) herbage ranged from 28 - 39%; the heritabilities of family mean IVDOM values were 0.55 and 0.78 respectively at these growth stages. Families did not differ significantly in %N at heading, and the heritability estimate (0.27) was also non-significant. At maturity, %N ranged from 0.38 - 0.66 and the heritability was 0.59. There were highly significant family x stage interactions for both %N and IVDOM, but there was a positive genetic correlation between IVDOM measurements at heading and maturity (r_g = 0.40). At heading, the earliest families were highest in IVDOM. At each stage, %N was negatively correlated with tiller weight. Genetic correlations between %N and IVDOM were usually small and negative.

(3) In a highly variable breeding population of *P. tuberosa*, marked responses were obtained to 3 generations of selection for high and low %N in whole tillers at heading. Total response was similar in each direction, and realised single-plant heritability estimates were 0.25 and 0.20 in the high and low directions respectively. Increaaes in %N were accompanied by reductions in tiller weight and seedling vigour. During the first 2 generations, correlated changes in IVDOM were in the same direction as changes in %N, but in the third generation there were no significant differences in IVDOM between the high and low N lines and a control (randomly selected) line.

(4) High, low and control N selection lines were grown as spaced plants and examined for %N and IVDOM at 5 stages during their first year of growth. Differences in %N between the high and low N lines

were maintained at each growth stage, and in all tiller components (leaf, sheath, stem and head) at the heading stage. Nitrogen concentration in whole tillers at heading was about one third greater in the high N than in the low N line, and there was considerable residual genetic variation for %N in each line. Changes in both the proportions and %N of tiller components contributed to the selection response in each of the selected lines, the latter character being more important. The main difference in tiller composition between the lines was a change in the leaf:stem ratio. The weight of whole tillers and all tiller components was negatively related to %N.

The low N line was marginally higher in IVDOM than the high N line at most growth stages, and markedly superior for IVDOM of mature herbage. Among individual plants at the heading stage there was a small but significant negative correlation between %N and IVDOM. High IVDOM was associated with short, thin stems, low ash content, early maturity and high leaf:stem ratios. Variation in IVDOM between plants at heading was extensive.

(5) High, low and control N selection lines were grown in small swards under four management systems (2 defoliation frequencies and 2 levels of applied nitrogen fertiliser, factorially combined). The frequent defoliation treatments were discontinued after the first year because the Phalaris was rapidly eliminated from these swards. Compared with the high N line, the low N line plots gave higher yields of Phalaris due to a tiller weight advantage. Low N line plots tended to contain a higher proportion of sown grass, but there were no differences between lines in susceptibility to weed infestation. Phalaris herbage from the low N plots was lowest in %N and highest in IVDOM, and gave the greatest nitrogen yield. Phalaris in the high N line plots recovered more of the applied nitrogen fertiliser and gave the greatest yield responses to applied nitrogen, but the low N line had a greater ability to extract non-fertiliser nitrogen from the soil and thus required less fertiliser to provide a given herbage yield. The superior herbage yields of the low N line were therefore most obvious in the absence of applied nitrogen; when nitrogen was not applied, Phalaris yields from the low N plots were one third greater than those from the high N plots during the 1969 and 1970 winter periods.

(6) Seedling vigour of the high and low N lines was examined in 2 glasshouse experiments. Seedlings from the high N line were smaller

than those of the low N line but had similar whole-plant relative growth rates. Differences in seedling weight between the lines were maintained when the level of nitrogen in nutrient solutions applied to the plants ranged from 2.8 to 280 ppm. Seedling dry weights were closely, and positively correlated (r = 0.76) with the weights of seeds from which they originated, and were less strongly correlated with their net assimilation rates.

High N line seedlings were higher in %N of both tops and roots than low N line seedlings, but contained smaller absolute amounts of Kjeldahl nitrogen. They absorbed nitrate nitrogen more efficiently per unit weight of root, but utilised this nitrogen inefficiently to produce incremental dry matter. Low N line seedlings had the highest root:shoot ratios and the lowest leaf area ratios, and allocated proportionately more assimilate to root growth than high N line seedlings.

(7) In seedlings, net assimilation rate was negatively correlated with %N (r= -0.32 and -0.67), but clones which had high %N under field conditions had higher apparent rates of photosynthetic oxygen evolution than low N clones. These apparently conflicting results may have been due to competition between nitrate and CO_2 for photosynthetically produced reducing power.

(8) The relationship between %N and seed weight was examined in ten clones which differed markedly in %N. Seed weight was closely correlated with the photosynthesising area per seed above the flag leaf node (r = 0.85), particularly with that contributed by the spikelets (r = 0.82). In turn, spikelet area was negatively correlated with %N of whole tillers (r = -0.49). Two of the ten clones deviated from an otherwise close negative relationship between %N and seed weight, so that the observed correlations between these characters were non-significant (r = -0.15 to -0.21). From an examination of one of these aberrant clones, with high %N and heavy seeds, it was concluded that photosynthetic area per seed could be maintained (and consequently a satisfactory seed weight might be retained) during selection for %N by concurrently selecting for large flag leaves and a reduced number of spikelets per head.

(9) It was concluded that IVDOM and %N of P. tuberosa herbage could be significantly increased by plant breeders. The breeder should define the growth stage at which improvement is to be maximised and measure the nutritive value of herbage sampled at that stage, but need not select in more than one environment. Although a negative relationship between IVDOM and perenniality may impose an upper limit to the improvement of IVDOM, considerable increases could be obtained

without sacrificing perenniality or other important agronomic characters. Increases in %N would be accompanied by decreases in tiller weight and herbage yield, unless counterselection was applied to maintain the levels of these characters, in which case improvements in %N would be reduced. Another adverse genetic correlation, that between %N and seedling vigour, could probably be overcome.

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APPENDIX I

METHOD FOR DETERMINING KJELDAHL NITROGEN CONTENT OF HERBAGE

The "Kjeldahl technique" is commonly used as a routine analysis for determining crude protein content (%N x 6.25) of herbage. Since there are many modifications of the method from laboratory to laboratory, the technique used for the 3000 analyses that provided the data for this thesis will be described.

MATERIALS

Digestion mixture: To 100 g K_2SO_4 add 1 g selenium powder and 1 litre concentrated H_2SO_4. Heat in a fume cupboard until the solution is clear.

Indicator mixture: 5 volumes of 0.1% ethanolic solution of bromocresol green to 1 volume of 0.1% ethanolic solution of methyl red.

Boric acid indicator mixture: 2% w/v solution of boric acid with water, containing 2% v/v indicator mixture.

Hydrochloric acid: An approximately normal solution is prepared by diluting 89 ml concentrated hydrochloric acid to 1 litre with distilled water. The solution is standardised against Na_2CO_3 previously heated to 270°C, and diluted to exactly N/100.

Sodium hydroxide: Add 500 g NaOH to 2 litres distilled water.

METHOD

The samples to be analysed are dried overnight at 100°C, and each sample, or portion of it, is weighed into a separate, labelled, 100 ml Kjeldahl flask. If the entire sample (up to 1g) is not to be analysed, then either 280 mg or 560 mg is weighed out for analysis. To each flask, 5 ml digestion mixture and a few grains of glass sand are added. The flasks are transferred to electric heating mantles (Kjeldahl digestion racks) in a fume cupboard and the contents carefully boiled, without allowing any acid to evaporate, for $2\frac{1}{2}$ hours. The flasks are then allowed to cool, still in the fume cupboard. A small amount of water is added to the digestates and the flasks are again allowed to cool, after which they are removed from the fume cupboard. The digestates are washed into separate, labelled, 100 ml volumetric flasks and made up to 100 ml with distilled water. Each digestate is now ready for distillation. Provided the flasks are sealed, they can be stored overnight or for several days if necessary.

Distillation is carried out using a Markham still, with the outlet submerged under 5 ml of boric acid indicator mixture in a 100 ml conical flask. From a volumetric flask, 5 ml of digestate is pipetted into the inner chamber of the still, and a quantity (about 10 ml) of sodium hydroxide solution is added. The inlet is sealed and 25 ml of distillate is collected in the boric acid indicator mixture, which is then titrated against the N/100 hydrochloric acid. Before distilling the next digestate, the inner chamber of the still is emptied and flushed with distilled water.

CALCULATION

For 280 mg sample, % N = titre (sample) - titre (blank), where titres are expressed in ml N/100 hydrochloric acid. For samples of other weight, appropriate corrections are made.

NOTES ON THE METHOD

The method can be modified to measure % N of samples of various weights by altering the dilution after acid digestion, the amount of digestate pipetted into the still, and the normality of the hydrochloric acid. However, as a routine procedure the method outlined has been found to be satisfactory and suitable for streamlined operation. The effects of variations in many aspects of the method have been examined, and where necessary procedures have been standardised. The % N estimations are highly repeatable and the recovery of nitrogen from $(NH_4)_2\ SO_4$ and protein standards is not less than 97%, a value which compares favourably with those obtained elsewhere (see Kirk 1950 for appropriate references).

APPENDIX 2

METHOD FOR DETERMINING IN VITRO DIGESTIBILITY OF HERBAGE

This technique, which is a modification of that devised by Tilley and Terry (1963), was developed by K.R. Christian, Division of Plant Industry, CSIRO, Canberra, for Phalaris tuberosa herbage. At Canberra, rumen liquor is obtained from sheep rather than cattle.

MATERIALS (Sufficient for 90 tubes)

Rumen liquor: Rumen contents are removed from a fistulated jersey cow grazing on ryegrass - white clover pastures. The rumen contents are filtered through 8 layers of cheesecloth into a large thermos flask (previously warmed) and the filtered liquor is transported to the laboratory. About 1200 ml of filtered fluid is obtained.

Buffer solution: A stock solution is prepared by dissolving the following chemicals in water and making the volume to 5 litres:-

232.5 g $Na_2HPO_4 \cdot 12\ H_2O$
245.0 g $Na\ HCO_3$
11.5 g NaCl
14.2 g K Cl
1.5 g $Mg\ Cl_2$
1.0 g $Ca\ Cl_2$ (added last)

Some calcium phosphate is precipitated. 800 ml of this solution is diluted (with water) to 4 litres for use.

Pepsin solution: 40 g of 1:2500 pepsin is dissolved in 5 litres of water containing 45 ml concentrated HCl on the day it is to be used. The final solution is 0.1 normal.

Herbage samples: dried and ground to pass a 1 mm sieve.

METHOD

Day 1: Up to 85 herbage samples, including three standards of known in vivo digestibility, are transferred to weighing bottles and dried at 100°C overnight.

Day 2: 0.499 - 0.501 g of each dried sample is weighed into labelled 110 x 40 mm polypropylene centrifuge tubes calibrated at 50 ml. Standards are weighed in duplicate.

Day 3: 4 litres of diluted buffer solution are warmed to 30°C in a 5-litre flask. 1 litre of strained rumen fluid is added (as soon as possible after collection) and is filtered through fine terylene or silk cloth during addition. By means of a stopper fitted with 2 lengths of glass tubing, the mixture is continuously bubbled with carbon dioxide from a cylinder, and 50 ml portions are siphoned into each of the centrifuge tubes. Another outlet fitted with a length of rubber tubing allows the centrifuge tubes to be flushed out with carbon dioxide. The tubes are sealed with rubber stoppers fitted with gas release valves and incubated at 39°C in the dark. Two "blank" tubes, containing buffer plus rumen fluid only (no herbage) are included. The tubes are swirled several hours later.

Day 4: Tubes are swirled once.

Day 5: Tubes are swirled once.

Day 6: After the samples have been incubated for 72 hours, the tubes are unstoppered and centrifuged (M.S.E. "Medium" centrifuge, 8-place angle head, 3000 rpm) for 10 minutes. The supernatants are discarded, and 50 ml pepsin solution, previously warmed to 39°C, is added to each tube. The samples are again incubated at 39°C for 24 hours.

Day 7: Tubes are removed from the incubator and stored in the refrigerator until ready for filtering. About 0.5g pre-ashed filter aid is added to the samples, which are then filtered through glass filter paper (Whatman GF/A, 2.5 cm) in perforated porcelain Gooch crucibles (capacity 35 ml, height 42 mm, diameter 40 mm), washed, and dried at 100°C.

Day 8: Samples drying.

Day 9: Crucibles are weighed to the nearest 0.001 g, ashed for 5 hours at 500°C and reweighed. Loss on ignition represents residue organic matter.

CALCULATION

% *in vitro* digestibility of organic matter (IVDOM) =

$$100 - \left(\frac{\text{mg OM residue (sample)} - \text{mg OM residue (blank)}}{\text{\% OM in sample} \times 0.05} \right)$$

If the calculated *in vitro* digestibilities of the standards deviate from their true (*in vivo*) digestibilities, all values are corrected proportionately.

RUMEN INOCULUM FORTIFICATION EXPERIMENTS

Most of the samples measured were of low *in vitro* digestibility and crude protein content. Wilkins (1966) and others have shown that addition of various "fortifying" supplements to the artificial rumen system may raise the subsequent *in vitro* digestibility of similar poor quality herbage. On two separate occasions the effect of rumen fluid fortification on *in vitro* digestibility measured by the procedure outlined was examined in replicated experiments.

Experiment A1: 4 herbage samples, ranging from 42-72% *in vitro* digestibility, were tested using unfortified rumen fluid and fluid fortified by the addition of 130 mg $(NH_4)_2 SO_4$ per sample. There was no effect due to fortification.

Experiment A2: 6 samples (including 2 standards), ranging from 20-59% *in vitro* digestibility were tested using fortified and unfortified systems. The two fortification treatments were (a) 10 mg urea per sample; (b) 10 mg urea + 10 mg glucose + 25 mg yeast extract. There was no effect due to urea fortification, but the more complex fortification raised the uncorrected digestibility of all samples by 3% ($P < 0.05$). Since there was no sample x treatment interaction, such an increase had no effect on digestibility values expressed relative to the standards.

It was concluded that fortification of the rumen inoculum was unnecessary, and the system was therefore not fortified.

APPENDIX 3

NUTRIENT SOLUTIONS USED IN GLASSHOUSE AND CABINET EXPERIMENTS

Solutions 1, 2 and 3, which were prepared as described in table A1 contained 2.802 (low N solution), 28.02 (medium) and 280.2 (high) ppm N respectively.

TABLE A1

COMPOSITION OF NUTRIENT SOLUTIONS

Salt	Molar stock solution (g salt per litre water)	Volume of stock solution (ml) per litre of nutrient solution		
		Solution 1	Solution 2	Solution 3
$CaCl_2$	110.99	1.5	1.5	1.5
KH_2PO_4	136.08	1.5	1.5	1.5
$MgSO_4.7H_2O$	246.50	2.2	2.2	2.2
$NaNO_3$	85.00	0.2	2.0	2.0
NaCl	58.46	1.8	—	—
NH_4NO_3	80.05	—	—	9.0
"Minors"	*	1.0	1.0	1.0
"Fe chelate"	**	1.0	1.0	1.0

* Minor elements : 2.86 g H_3BO_3
1.81 g $MnCl_2.4H_2O$
0.22 g $Zn\,SO_4.\,7H_2O$
0.08 g $Cu\,SO_4.\,4H_2O$
0.02 g $H_2\,Mo\,O_4.4H_2O$
} Dissolved in 1 litre water

** Iron chelate: 5.0 g NaOH in 800 ml H_2O
33.2 g E.D.T.A.
24.9 g $Fe\,SO_4.\,7H_2O$

The 3 chemicals are added in the above order and dissolved thoroughly. The solution is aerated overnight and made up to 1 litre.

APPENDIX 4

ORIGIN OF THE PHALARIS STRAINS USED IN EXPERIMENT I

In table A2, the CPI (*= Commonwealth Plant Introduction) number (or name) and country of origin of the 39 *Phalaris* strains used in experiment 1 are given. Further details of the species and hybrids are included in the text (table 2).

TABLE A2
ORIGIN OF *PHALARIS* STRAINS

Strain name or CPI* number	Species	Origin or collection site
19280	*P. tuberosa*	Algeria
19305	"	Morocco
19315	"	"
19351	"	Greece
Seedmaster	"	McWilliam and Schroeder (1965)
General select	"	McWilliam and Latter (1970)
High % N	"	see experiment 3
Low % N	"	see experiment 3
19195	*P. minor*	Libya
19197	"	Egypt
19215	"	Morocco
19224	"	Greece
32269	"	India
32270	"	"
14422	*P. coerulescens*	Portugal
14692	"	Morocco
24457	"	"
15709	"	Italy
Wagga polycross	"	experimental population (Oram)

TABLE A2 (continued)

Strain name or CPI* number	Species	Origin or collection site
14690	P. canariensis	Morocco
18679	"	"
19625	"	Sweden
19241	P. truncata	Algeria
19247	"	"
19251	"	"
14073	P. paradoxa	Italy
19238	"	Greece
14830	P.brachystachys	Israel
15589	"	"
34708	P.californica	California, U.S.A.
——	P.caroliniana	U.S.A.
25776	P.amethystina	U.S.A.
7594 ("Superior")	P.arundinacea	Oregon, U.S.A.
10446	"	Portugal
19145 x 19217	P.brachystachys x minor	Algeria x Morocco (Oram)
"P. davies ii"	P.tuberosa x minor	Hutton (1955)
Australian x 19203	"	Italy x Algeria
P.t.x a. allo	P.tuberosa x arundinacea	McWilliam (1962)
P.t.x c. allo, bulk 1	P.tuberosa x canariensis	Hoen and Oram (unpublished)
P.t.x c. allo, bulk 2	"	" "
(P.t.x c)xt. backcross	"	"

APPENDIX 5

STATISTICAL ANALYSIS OF DATA IN EXPERIMENT 2

The mating system used was essentially the North Carolina "design 1" plan originally described by Comstock and Robinson (1948). There were several modifications. On average, 3 dams were mated to each sire but the number was not constant. The progeny of each cross were not measured individually, but were bulked within each replicate. Consequently, the raw data for analysis were the replicated measurements of family means. The genetic analysis, which takes these modifications into account, is as follows:

TABLE A3

ANALYSIS OF VARIANCE AND COVARIANCE

Source	Degrees of freedom	Expectation of mean squares §
Replicates	r - 1	
Families	f - 1	
Sires	s - 1	$\sigma^2 e + r\sigma^2 d + k\sigma^2 s$
Dams in sires	f - s	$\sigma^2 e + r\sigma^2 d$
Error	(r-1) (f-1)	$\sigma^2 e$
Total	rf - 1	

§ for expectations of mean products, replace σ^2 by cov. in all terms in this column.

r = replicates
f = full-sib families
s = sires
d = dams
e = error

$$k = \frac{1}{s-1}\left[n - \frac{\sum n_i^2}{n}\right],$$

where n = total number of observations (ie, families x replicates), and n_i = no. of dams x replications for the i^{th} sire.

Heritability. The formulae used were developed by Latter (personal communication) and used by McWilliam and Latter (1970) They are

$$h^2 = \frac{\sigma^2 s + \sigma^2 d}{\sigma^2 s + \sigma^2 d + \frac{\sigma^2 e}{r}} = \frac{X}{Y} \quad \ldots\ldots\ldots\ldots (1)$$

$$\text{and var. } h^2 = \frac{V(X)}{Y^2} - \frac{2X \text{ cov. } (X,Y)}{Y^3} + \frac{X^2 V(Y)}{Y^4} \quad \ldots\ldots\ldots(2)$$

The heritability estimated from equation (1) refers to the mean values of families averaged over r replicates, where r can be varied. Standard errors of the estimates are derived from equation (2), since the variance is the square of the standard error. For a test of significance, h^2 can then be compared with its SE.

An alternative test of the significance of h^2 was described by Hayman (personal communication), based on the method of Cochran (1951). Denoting mean square (sires) as m_1, mean square (dams within sires) as m_2, and mean square (error) as m_3 the test is

$$F = \frac{\frac{r}{k} m_1 + \left[1 - \frac{r}{k}\right] m_2}{m_3}$$

This approximate F ratio is compared with F ratios required for significance as usual. The degrees of freedom are for denominator, d.f. = d.f. (error) and for numerator,

$$\text{d.f.} = \frac{\left[\frac{r}{k} m_1 + \left(1 - \frac{r}{k}\right) m_2\right]^2}{\frac{\left[\frac{r}{k} m_1\right]^2}{\text{d.f(sires)}} + \frac{\left[\left(1 - \frac{r}{k}\right) m_2\right]^2}{\text{d.f.(dams in sires)}}}$$

Both tests of significance gave the same results.

Genetic correlations. Following analyses of variance and covariance, the additive genetic correlation between two characters a and b is estimated by

$$r_g = \frac{\text{cov. } s_{ab} + \text{cov. } d_{ab}}{\sqrt{\left[\sigma^2 s_a + \sigma^2 d_a\right]\left[\sigma^2 s_b + \sigma^2 s_b\right]}}$$

An exact test of the significance of this estimate has not yet been published (see Tallis 1959). However, following Robertson (1959) and Falconer (1960) the standard error is approximately calculated as

$$SE(r_g) = \frac{1 - r_g^2}{\sqrt{2}} \sqrt{\frac{SE(h^2_a)\, SE(h^2_b)}{h^2_a\, h^2_b}}$$

An approximate test of significance can then be applied by comparing r_g with its SE. Following McWilliam and Latter (1970), a correlation has been taken as significant if the ratio of r_g to its SE exceeds 2.0. For many correlations the ratio was much higher.

APPENDIX 6

GENETIC ANALYSIS OF DATA FROM EXPERIMENT 4

The genetic analysis of this experiment was complicated by unequal observations within subclasses. There were unequal numbers of progeny per dam within each replicate and (for the high N line) unequal numbers of dams per replicate; and there were unequal numbers of plants, disregarding dams, within each replicate.

Two alternative statistical genetic analyses of the % N data were examined, based on two different models. In the first analysis, replicates were ignored and the data were assumed to be from a completely randomised experiment. The underlying model (designated model A) was

$$Y_{jk} = \mu + d_j + E_{jk}$$, so that the observed %N of the kth offspring of the jth dam consisted of the population mean μ, plus an effect due to the jth dam, plus an individual error E_{jk}.

The more detailed analysis (model B) included two additional parameters. The observed value of an individual consisted of the above components, plus an effect due to the ith replicate and an effect due to interaction between the ith replicate and the jth dam, thus:

$$Y_{ijk} = \mu + r_i + d_j + (rd)_{ij} + E_{ijk}$$

The calculations of degrees of freedom, sums of squares and mean squares in each model are straightforward (see, eg, Snedecor and Cochran (1967) for model A and Bancroft (1968) for model B). The expected mean squares in model A are also straightforward, but those in model B are complicated. Weir (personal communication) has provided the following method of estimating the expected components of the observed mean squares in model B.

Let $X_1 = \sum_i \sum_j \frac{n_{ij}^2}{n_{i.}}$, $X_2 = \sum_i \frac{n_{i.}^2}{n_{..}}$, $X_3 = \sum_j \frac{n_{.j}^2}{n_{..}}$,

$X_4 = \sum_i \frac{\sum_j n_{ij}^2}{n_{i.}}$, and $X_5 = \sum_j \frac{\sum_i n_{ij}^2}{n_{.j}}$,

where i and j are the subscripts in model B (i = 1, 2....r and j = 1, 2....d) and n is the number of plants in the subgroup specified by i and j (the notation is that of Bancroft 1968).

Then the mean squares due to the sources referred to in table A4 as replicates (R), dams (D), replicates by dams (RXD) and error (E) have the following expectations:

$$\text{EMS (R)} = \sigma^2 e + \frac{1}{r-1}\left[(X_4 - X_1)\,\sigma^2 rd + (N-X_2)\,\sigma^2 r + (X_4-X_3)\sigma^2 d \right]$$

$$\text{EMS (D)} = \sigma^2 e + \frac{1}{d-1}\left[(X_5-X_1)\,\sigma^2 rd + (X_5-X_2)\sigma^2 r + (N-X_3)\,\sigma^2 d \right]$$

$$\text{EMS (RXD)} = \sigma^2 e + \frac{1}{(r-1)(d-1)-Z}\left[(N-X_4-X_5+X_1)\,\sigma^2 rd + (X_2-X_5)\sigma^2 r + (X_3-X_4)\,\sigma^2 d \right]$$

$$\text{EMS (E)} = \sigma^2 e \quad ,$$

where N is the total number of plants measured, Z is the number of missing dam x replicate combinations and $\sigma^2 d$, $\sigma^2 r$, $\sigma^2 rd$ and $\sigma^2 e$ refer to the variance due to dams, replicates, replicates x dams and error respectively.

Using these equations the nine constants (K_2, K_3 ..... K_{10}) in table A4 are estimated, and these in turn allow the four variances to be estimated.

The expected mean squares for each model are presented in table A4, together with the degrees of freedom and observed mean squares of %N for each of the high N and low N selection lines. Constant K_1 (model A) is estimated as in appendix 5.

Heritibility estimates were calculated as follows, for model A

$$h^2 = \frac{\left\{\frac{4}{1+F}\right\}\sigma^2 d}{\sigma^2 d + \sigma^2 e} \quad , \text{ and for model B}$$

$$h^2 = \frac{\left\{\frac{4}{1+F}\right\}\sigma^2 d}{\sigma^2 d + \sigma^2 r + \sigma^2 rd + \sigma^2 e}$$

where F is the average coefficient of inbreeding of individuals within a selection line.

The approximate standard errors of the estimates in both cases were calculated as

$$SE = \left[\frac{4}{I-F}\right] \sqrt{\frac{2\left[1+(K_1-1)\right]^2 (1-t)^2}{K_1(K_1-1)(d-1)}}$$

(adapted from Falconer 1960) ,

where d is the number of dams and t is the intraclass correlation. The comparison of h^2 with its SE furnishes an approximate test of the significance of the heritability estimate.

Genetic correlations were calculated using model A (for reasons explained in the text) after analyses of variance and covariance, from the formula

$$r_g = \frac{\text{cov. } d_{ab}}{\sqrt{\sigma^2 d_a \, \sigma^2 d_b}} ,$$

where subscripts denote two variables a and b. Standard errors of genetic correlations were calculated as in appendix 5.

TABLE A4

ANALYSIS OF VARIANCE OF % N (WHOLE TILLERS) OF INDIVIDUAL SPACED PLANTS WITHIN THE HIGH N AND LOW N SELECTION LINES IN THE S_4 GENERATION

Source	Degrees of freedom		Mean squares §		Expected mean squares
	High N line	Low N line	High N line	Low N line	
Model A ($Y_{jk} = \mu + d_j + \epsilon_{jk}$)					
Between dams	7	8	0.125986*	0.104650**	$\sigma^2 e + k_1 \sigma^2 d$
Within dams	153	184	0.056598	0.031776	$\sigma^2 e$
Total	160	192			
Model B ($Y_{ijk} = \mu + r_i + d_j + (rd)_{ij} + \epsilon_{ijk}$)					
Between replicates	9	9	0.163044	0.069522	$\sigma^2 e + k_2 \sigma^2 r + k_3 \sigma^2 d + k_4 \sigma^2 rd$
Between dams	7	8	0.125986	0.104650	$\sigma^2 e + k_5 \sigma^2 r + k_6 \sigma^2 d + k_7 \sigma^2 rd$
Replicates x dams	49	72	0.044163	0.023631	$\sigma^2 e + k_8 \sigma^2 r + k_9 \sigma^2 d + k_{10} \sigma^2 rd$
Error	95	104	0.052927	0.034172	$\sigma^2 e$
Total	160	193			

* $P < 0.05$, ** $P < 0.01$

§ There is no valid F test for any of the above model B mean squares.

TABLE A5

VALUES OF CONSTANTS IN EXPECTED MEAN SQUARES IN TABLE A4

Constant	High N Line	Low N Line
K_1	19.2902	21.4313
K_2	2.8126	2.2683
K_3	16.0828	19.2942
K_4	0.2426	0.1267
K_5	2.2990	2.2602
K_6	0.3824	0.1255
K_7	19.2902	21.4314
K_8	2.3827	2.1143
K_9	-0.0546	-0.0139
K_{10}	-0.0445	-0.0158

APPENDIX 7

CALCULATION OF LEAF AREA VIA LENGTH x BREADTH REGRESSION

The close correlation between leaf area and length x maximum breadth (LxB) of grass leaves was used to estimate leaf area in several experiments. A total of 192 leaves were measured for LxB and for "true" leaf area to provide data for predictive equations. "True" leaf area was obtained by cutting out and weighing leaf outlines traced on uniform paper. The weights obtained were converted to leaf area using standards of known area.

Since it was thought that the predictive equations could vary for different categories of leaves, leaves were classified in various ways and the regressions were calculated and compared. These comparisons are summarised in table A6.

TABLE A6

COMPARISON OF REGRESSIONS OF LEAF AREA ON L x B FOR DIFFERENT CATEGORIES OF LEAVES

Comparison	F ratio and significance of variation	
	due to average regression	between individual regressions.
Leaves from seedlings vs. mature plants	8066.2***	0.0472 (NS)
Seedling leaf number 1,2,3,4,5	7845.7***	0.0092 (NS)
Completely vs incompletely expanded leaves	53609.0***	0.0228 (NS)
High, low and control %N selection lines	53792.5***	0.1171 (NS)
Leaf types within experiments †	7598.3***	0.0056 (NS)
Overall regression: leaf area = (0.802 ± 0.003) L x B - 0.002, (r =0.998)		

† the 192 leaves were sampled from 3 different experiments.

*** P<0.001 , NS = not significant.

In the experiments, the shorter regression A = 0.8 (L x B) was used to calculate leaf area since the "error" resulting from this simplification was negligible.

The data from which the equation was derived are plotted in fig A1.

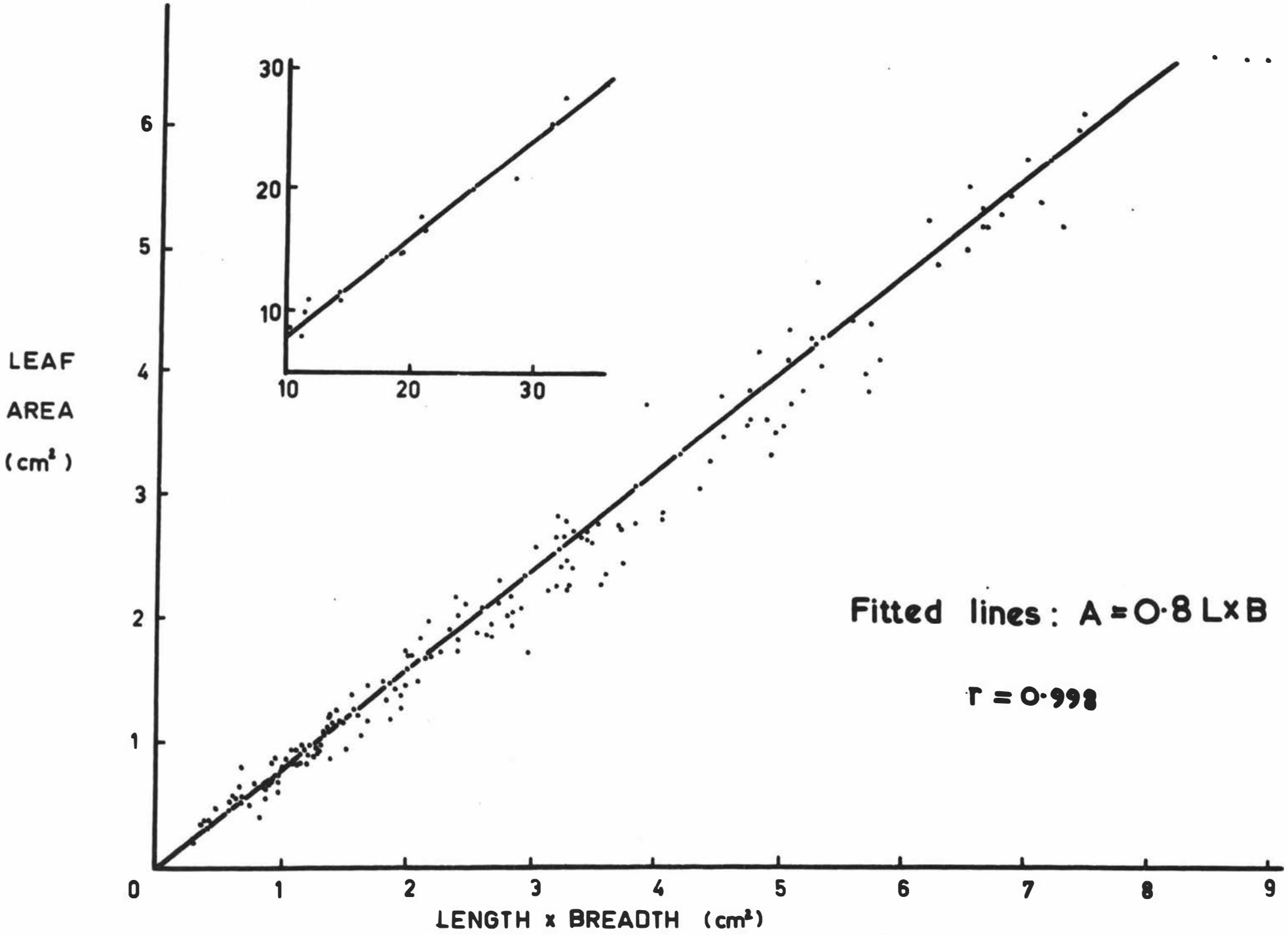

FIG. A1.– Regression of leaf area on length x breadth measurements, leaves <10cm² in area. Inset: leaves 10–30 cm².

APPENDIX 8

TABLE A7

MONTHLY RAINFALL (INCHES) DURING THE FIELD EXPERIMENTS, 1968-70*

Month	1968	1969	1970	
January	2.30	3.18	0.08	
February	2.02	2.76	0.35	
March	0.91	1.09	3.27	
April	4.54	2.68	0.97	
May	5.05	3.92	3.86	
June	6.02	2.64	4.71	
July	2.93	1.54	4.51	
August	2.51	2.51	2.77	
September	2.64	1.29	6.50	
October	5.33	1.49		
November	1.92	1.31		
December	5.92	3.65		
Total	42.09	28.06		

* Records from Grasslands Division, D.S.I.R., approximately 1 mile from the site of experiments.

Breeding for Improved Nutritive Value of *Phalaris tuberosa* Herbage.

Ph.D. Thesis, Massey University, 1970.

R.J. Clements.

ABSTRACT

The perennial pasture grass *Phalaris tuberosa* is noted for its drought resistance, productivity, and persistence under grazing, but its usefulness is limited by its low herbage quality during summer and early autumn. The experiments reported in this thesis assessed the potential for increasing the *in vitro* digestibility of organic matter (IVDOM) and the Kjeldahl nitrogen content (%N, dry weight basis) of *P. tuberosa* herbage by breeding.

Extensive genetic variation for both characters was found between *Phalaris* species and hybrids, between *P. tuberosa* ecotypes, between full-sib families derived from an Algerian ecotype, and between individual plants from a broadly-based breeding population. Since IVDOM of annual species was higher than that of perennials, the only interspecific hybrids significantly superior to *P. tuberosa* in IVDOM were those having an annual species as one parent (*P. tuberosa* x *minor*; *P. tuberosa* x *canariensis*; and annual x annual crosses). None of the hybrids examined were superior to *P. tuberosa* for %N. Since the potentially useful hybrids all showed reduced perenniality, it was concluded that interspecific hybridisation is not a suitable source of herbage quality variation for a *P. tuberosa* breeding programme.

The heritability of IVDOM in an Algerian ecotype of *P. tuberosa* was found to be high and, despite significant genotype x growth stage interactions for this character, artificial selection should produce a single population with increased IVDOM at all growth stages. Apart from

a consistent association with early maturity, increased IVDOM was not adversely correlated with yield or any other important agronomic character. There was no stable genetic correlation between IVDOM and %N.

The heritability of %N was lower than that of IVDOM, but the amount of genetic variation for this character was considerable, as indicated by significant responses to divergent selection for %N of individual plants in a broadly-based breeding population. Differences in %N between these divergent selection lines were maintained at all growth stages, in spaced plants and swards, and under several experimental treatments (e.g. levels of nitrogen). Such differences were due in part to changes in the relative proportions of tiller components (especially leaf : stem ratio), and in part to changes in %N of these components.

The suitability of %N as a selection criterion is reduced by negative genetic correlations with tiller weight (and hence yield) and seedling vigour. Little increase in %N would be possible without sacrificing yield. The adverse relationship with seedling vigour is primarily due to an underlying negative correlation between %N and seed weight, and it appears that this could be overcome during selection for %N by concurrently selecting for large flag leaves and a reduced number of spikelets per head.