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AN ECOLOGICAL STUDY OF A *CHIONOCHLOA* POPULATION

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SUMMARY

The morphologically variable population of Chionochloa tussocks growing on the summit of Mt Kaiparoro are shown to be hybrids between C. rubra and C. flavescens. This was established by comparing the pattern of variation in three vegetative characters of the Kaiparoro population with that of the parent species. Six sample populations were used for this purpose. Four populations represented different positions along the full range of morphological variation in the Kaiparoro tussocks, and the remaining two typified the parent species as they are found at their respective type localities. Each population consisted of 100 ramets, representing 50 genotypes or tussocks. A transplant experiment involving the four Kaiparoro populations indicated that the morphological variation was mainly under genetical control.

The distribution of the Kaiparoro hybrids correlates with soil oxygen diffusion rates. To measure these a soil oxygen diffusion meter with a platinum electrode was constructed. The Kaiparoro soil oxygen diffusion rates are in the range usually critical for plant growth. Aeration differences between the four sites examined varied over three sampling dates but the relative site levels remained constant. The differences were largest during the wettest seasons.

SECTION A

GENERAL INTRODUCTION

A.1. STUDY AREA AND ENVIRONS

A.1.1. Location and Physiography

The Chionochloa community under study occupies approximately 63 acres on the summit of Mt Kaiparoro (2,660 ft a.s.l.), a large ridge situated about four miles east of the main northern Tararua Range and separated from it by the headwaters of the Mangatainoka River (fig. 1). The crest of this ridge slopes upwards towards the summit at the southern end, rising 200 feet over the length of the tussock grassland clearing. The clearing extends only a short distance down the sides of the ridge. On all but the northern edge the transition zone from grassland through scrubland to forest occurs where there is a marked increase in slope (fig. 2). The area covered by tussock grassland is gently undulating, but small streams dissect the area at irregular intervals, causing considerable local variation in slope.

A.1.2. Vegetation

Forest covers most of Kaiparoro and the surrounding area. A narrow belt of shrubland separates this from the Chionochloa-dominant tussock grassland clearing on the summit. The vegetation of Kaiparoro was described by Druce (1957) but is discussed briefly here for the sake of completeness.

Forest

The surrounding forest is dominated by Nothofagus fusca

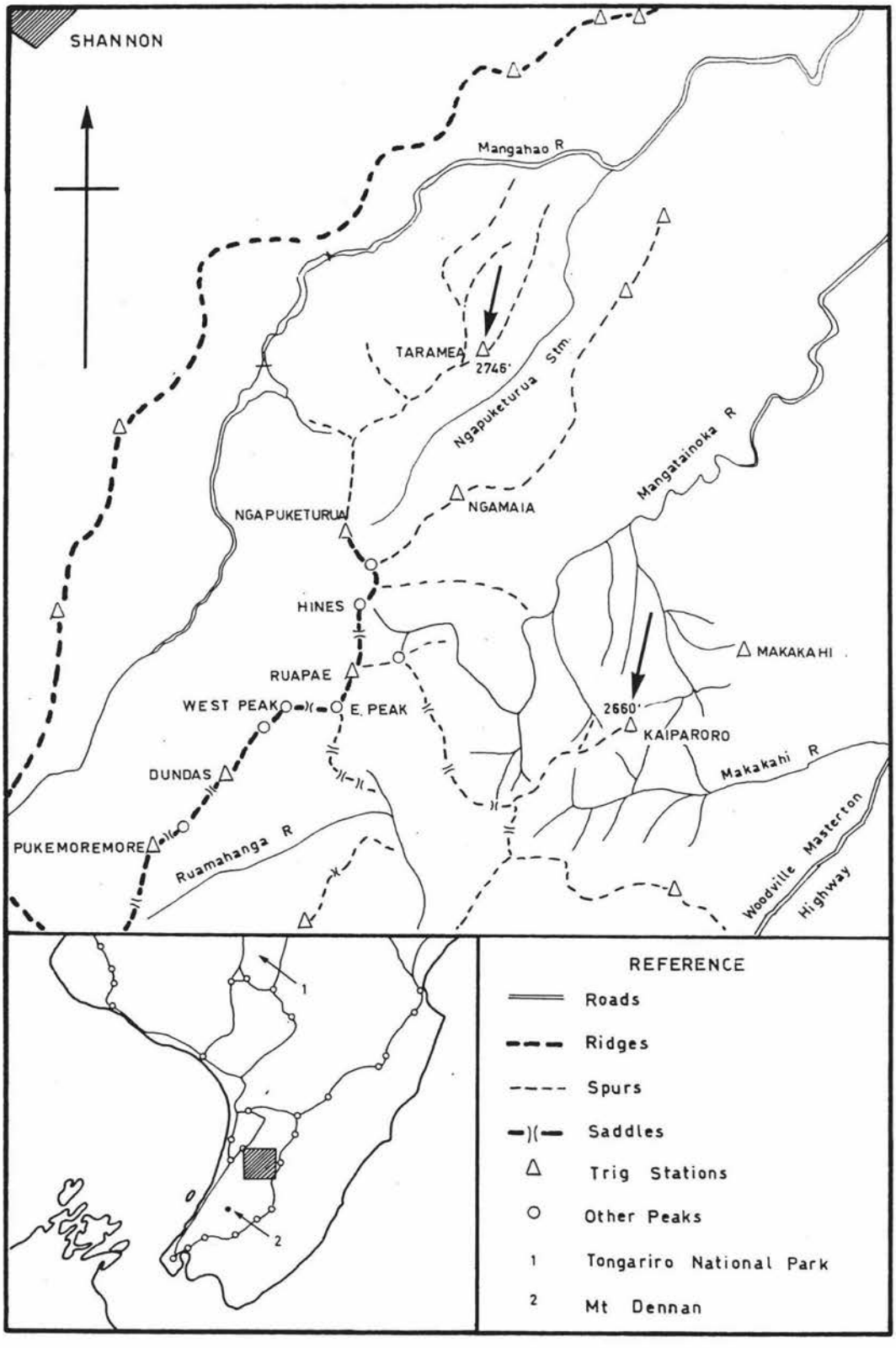


Fig. 1. Locality map.

Fig. 2. Eastern side of the Mt Kaiparoro tussock grassland, looking south.

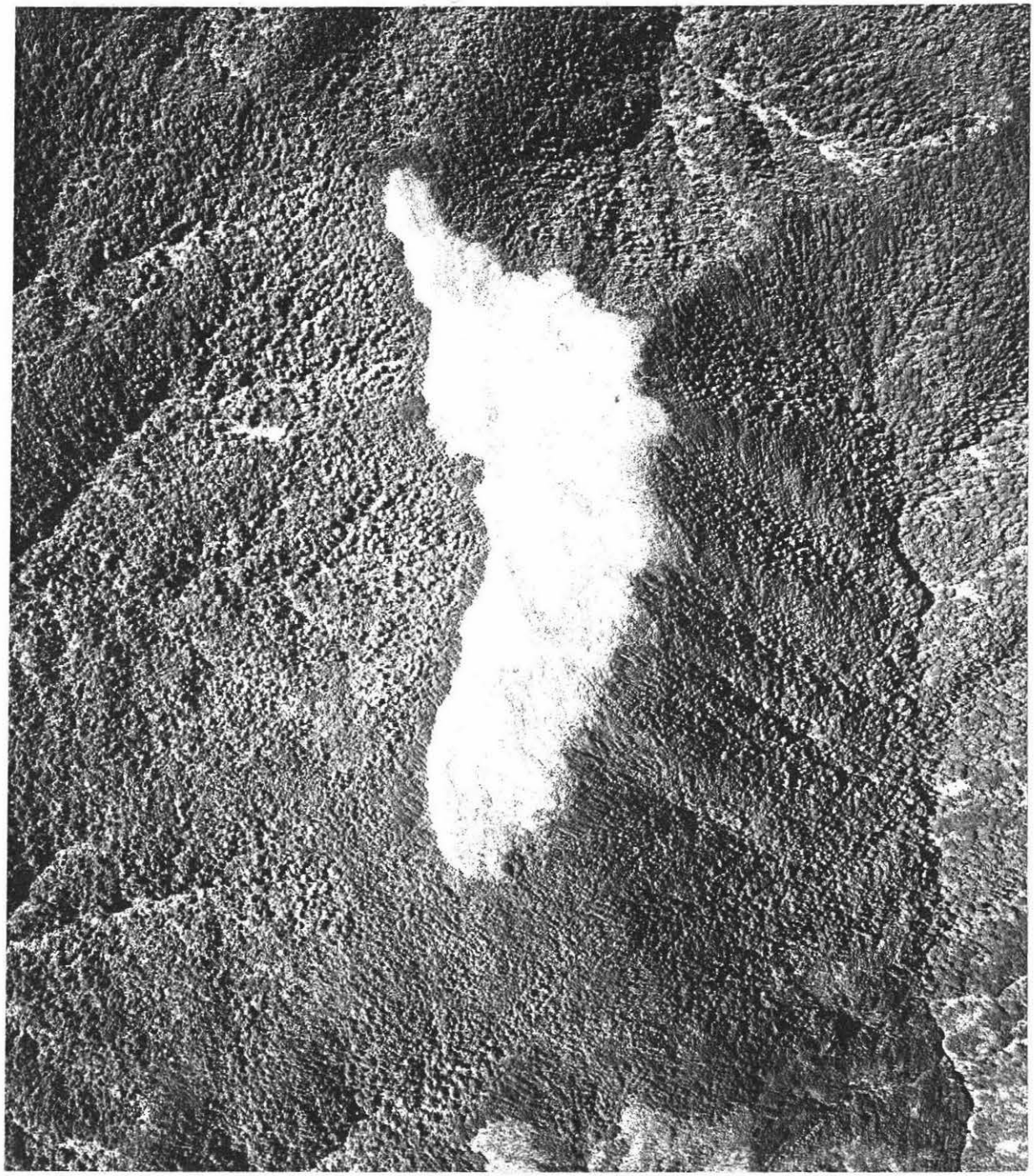


Fig. 3. Aerial photograph of Mt Kaiparoro. The summit area of tussock grassland is shown with its northern end towards the bottom of the page.
Photograph: Copyright N.Z. Aerial Mapping Ltd., no. 7859, taken 4.2.64, approximate scale 1 inch = 800 feet.

(red beech); associated trees are Weinmannia racemosa (kamahi), Myrsine salicina (toro), Phyllocladus alpinus (celery pine), Griselinia littoralis (broadleaf), Podocarpus hallii (Hall's totara), and Carpodetus serratus (putaputaweta). The beech canopy is severely windshorn and furrowed by the prevailing north-west wind. This is clear in the aerial photograph (fig. 3). The forest bears evidence of fire which, on at least one occasion, reached the clearing at the northern end.

Shrubland

Shrubland lies between the forest and the tussock grassland. The greater part of the shrub zone is well defined and narrow, but in areas along the western side, it is rather poorly defined, and merges gradually into the tussock grassland over a distance of up to about 50 metres. The change from scrub to forest however, is nearly always abrupt. In the shrubland the following species may be found: Phyllocladus alpinus (celery pine), Dracophyllum filifolium (turpentine bush), Olearia colensoi (leatherwood), Astelia cockaynei, Coprosma parviflora, C. p. var. dumosa, C. foetidissima (stinking Coprosma), C. colensoi, Cyathodes fasciculata, Neomyrtus pedunculata, Phormium colensoi (mountain flax), Leptospermum scoparium (manuka), and Myrsine divaricata. Druce (1957) stated: "The association of manuka with Olearia colensoi is most unusual; as far as I know it does not occur elsewhere in the North Island." Where the shrub zone is broad, only Dracophyllum, Leptospermum, Phormium, Coprosma parviflora var. dumosa and Astelia extend into the tussock grassland. With the exception of

Astelia, these decrease in importance as the Chionochloa tussocks increase.

Tussock grassland

Druce (1957), commenting on the presence of tussock grassland on Kaiparoro, stated:

> "... the obvious explanation is that poor drainage prevents the forest from invading that area. Very few young beech trees have been seen in the scrub belt adjacent to the forest, i.e., the forest is not advancing at the present time. The vegetation is thus more or less in equilibrium with the environment. As a result of the cool, wet climate the summit is one large seepage area, with the height of the water table and rate of water movement varying from place to place."

Fire does not seem to have been an important ecological factor in maintaining Chionochloa dominance over the summit area. Some of the adjacent forest has been burnt but, according to C. Devenport (pers. comm.), a local farmer, the clearing has not been burnt during the last 40 years. Scattered dead plants, some still standing, suggest that clumps of mature Olearia colensoi have grown in the clearing previously. The reason for their death is not clear, but the possibility of fire cannot be excluded.

Varying numbers of cattle have inhabited the tussock grassland from time to time. Deer are present. Disturbance by these animals may have facilitated the successful establishment of Leptospermum scoparium (manuka) in the area.

There is extreme variability in the morphology of the Chionochloa population and in the distribution of the other species.

Druce (1957) stated his impressions on his first visit to the area: "It appeared to us then that the tussock in the wettest parts was red tussock, but that everywhere else the tussocks were hybrids of various sorts between red tussock and some other tussock, presumably the broad-leaved snow tussock common elsewhere in the Tararuas." He later collected pieces of 68 tussocks and analysing each for 10 characters found nearly every one was different, ranging from those typical of red tussock (C. rubra), at one extreme, to broad-leaved snow tussock (C. flavescens) at the other. The distribution of associated plants was also studied in detail by Druce, who examined 11 sites and found each to be distinct from the remainder.

The following broad relationships exist between vegetation and habitat:

(a) On the well drained slopes where the water table is usually some distance below the surface, the vegetation is 1-1.5 m high and consists mainly of a dense population of Chionochloa tussocks which, though variable, mostly resemble C. flavescens. Scattered plants of Cassinia, Dracophyllum, Phormium, Leptospermum (in some areas), and clumps of Astelia grow between the tussocks. Carpha, Juncus, Carex, and Hierochloe may also be present. (See fig. 4.)

(b) Where the water table is nearer the surface, Cassinia, Dracophyllum, Phormium, Astelia, and Leptospermum are stunted or absent. The Chionochloa tussocks are smaller (up to 80 cm), more widely spaced, and are more like C. rubra. Between the tussocks grow Schoenus, Carex, Juncus, Hierochloe, and Carpha. On the wetter areas

Fig. 4. Chionochloa variants similar to C. flavescens, growing on a well-drained slope at the western side of the Mt Kaiparoro tussock grassland (plot 3).

Fig. 5. Chionochloa variants similar to C. rubra, growing on a level, poorly drained site near the highest point of Mt Kaiparoro (plot 4).

Schoenus may be associated with tussocks about 50 cm high interspersed with a continuous Carpha cover. Carpha dominates in some areas and Oreobolus pectinatus, which is usually present, may be locally dominant.

(c) With the water table close to the surface Gleichenia circinata is usually dominant. Associated with it may be Sphagnum, Schoenus, Carpha, Juncus, Carex, and Hierochloe. Where Chionochloa tussocks are present they are slender and closely resemble C. rubra. In very wet areas Carpha becomes dominant, and both Gleichenia and Schoenus become stunted. Sphagnum dominates in basins and stream beds. (See fig. 5.)

A.2. AIMS OF STUDY

The investigations by Druce (1957), supplemented by a personal appraisal, indicated that the Kaiparoro Chionochloa population possessed a range of morphological characters extending from those typical of C. flavescens (broad-leaved snow tussock) to those typical of C. rubra (red tussock). There appeared also to be a relationship between soil water conditions and the distribution of the Chionochloa variants. Those most like C. rubra occupied the wetter sites and those like C. flavescens, the drier.

The most obvious explanation for the variability in morphology and distribution of the Kaiparoro tussocks is hybridization between C. rubra and C. flavescens. Outside the Kaiparoro area C.

rubra is found growing in wet soils from sea level to about 4,500 ft a.s.l. Although it appears to grow most vigorously in well drained soils it survives, in a stunted condition, in a wetter soil than most of the commonly associated species can tolerate. C. flavescens, on the other hand, is limited to the subalpine zone and usually to the better drained slopes.

Hybridization between two morphologically and ecologically distinct entities such as these species could be expected to give rise to a population of individuals highly variable morphologically and physiologically. Such an occurrence is feasible for species that possess the same chromosome number (Zotov, 1963) and belong to a genus where inter-specific fertility has been demonstrated (Connor, 1963).

On the basis of the available evidence the following working hypothesis was formed:

> That the morphological variation apparent in the Kaiparoro Chionochloa population is the result of hybridization between C. rubra and C. flavescens and that the distribution of the variants is influenced by some parameter of soil water conditions.

The study was designed to test the validity of this hypothesis.

SECTION B

VARIATION IN THE *CHIONOCHLOA* POPULATION

I N T R O D U C T I O N

B.1. SOURCE OF VARIATION

B.1.1. Genotypic vs. Environmental Contributions

Variation among individual plants is under both genetical and environmental control. The genotype exerts an essentially conservative influence on variation. It is transmitted from generation to generation in a broadly predictable, if altered, form that is dependent on the nature and size of the reproductive population system. The environment modifies the gene-initiated processes which govern growth and development. Environmentally induced variation is seldom, if ever, inherited directly but by acting through selection it has an important effect on successive generations. A knowledge of the genetical and environmental contributions to phenotypic variation is essential for an understanding of the phenotypic, phyletic, and ecological relationships in plant populations.

Phenotypic inter-population variation may arise from three sources:

(a) the direct plastic modification of individuals,

(b) genetical divergence as a consequence of selection,

(c) fortuitous genetical divergence.

The taxonomist is mainly interested in (b) and (c), and the gene-

cologist in (b) (which Harberd, 1957, called "genecological differentiation"). In addition both must take (a) into account, as environmental effects are often conspicuous and can never be completely isolated.

B.1.2. Transplant Experiments

Elimination of the effects of direct environmental modification of individuals by cultivating population samples in a uniform environment is commonly attempted. This method may be adequate to permit the kinds of distinction required, given an appropriate experimental design and analysis. In some circumstances, however, it may be unsatisfactory (Heslop-Harrison, 1964). Four important reasons for this are:

(a) Genetically determined differences in the capacity of plants to react adaptively to certain environments may not be expressed in the transplant environment. This can be particularly misleading where physiological responses are being tested.

(b) The test environment, while suppressing some environmentally induced characteristic, may evoke others never exposed in the natural habitats. This exposure of latent genotypic variation is not related to the uniformity of the test environment, but is due to its increasing the penetrance of certain genes.

(c) The genotypes may be carried so far out of their norms that developmental regulation is disrupted, causing increased variation.

(d) Pre-conditioning may affect the subsequent growth and

development of plants. Most of the limitations of the simple transplant techniques are removed when methods of varied-environment or reciprocal transplanting are adopted. The classical example of this approach is the work of Clausen, Keck and Hiesey (1940, 1945, 1948).

B.1.3. Statistical Techniques

Total population variance can be partitioned in such a way that it is possible to estimate the part attributable only to genotypic variation. By determining the relationships between plant variation and environmental variation, estimates can be obtained of the adaptive significance of the genotypic variation. Heslop-Harrison (1964) describes the ideal situation for analysis where a species has encountered a mosaic of habitats while expanding its range, the selective influences of each habitat acting upon the whole available pool of genetical variation. A comparison of between-habitat and within-habitat variances can then be expected to expose, as significant, those differences which are truly adaptive.

B.2. THE SPECIES CONCEPT

The species concept has been the source of much controversy and of some misunderstanding. The three main issues involved were: (a) the species as an integral part of a hierarchal classification system, (b) the nature of species boundaries, and (c) the fixity of species.

(a) Because species are composed of sub-units and are them-

selves the constituents of higher units, it has been concluded that they are mental rather than biological units. To the extent that the integrity of species is maintained by the nature of the breeding system the unit is biologically real, however, and is far from arbitrary.

(b) Many early workers, such as Jordan, Curvier and Linnaeus (Grant, 1963), favoured the separation of species by unbridged gaps. This idea is not generally applicable because the isolation mechanisms preventing interbreeding among species are often incomplete in their operation. The resultant gene exchange causes various degrees of intergrading. It is more realistic to regard species as being separated by relatively distinct gaps or discontinuities.

(c) Although intra-specific variation was long recognised, Darwin was the first to propose its significance as a pre-requisite for natural selection. It is now apparent that species are only relatively stable and that in fact species unable to make adaptive changes often become evolutionary "cul-de-sacs".

The plant classification system has a twofold function of firstly collecting individuals into groups of a convenient size, and secondly of arranging these groups so that the relationships among plants in terms of their past development and present forms, are apparent. These two functions, which both aim at minimising the complications of variation, overlap considerably, but tend to

be incompatible with the genecological or experimental approach where variation and its significance in relation to the environment, are of major importance. The properties of a variational unit suitable for recognition as a named species, according to the 1956 International Code of Botanical Nomenclature, are that its constituent individuals should show an overall resemblance; it should show distinction from other groups of the same kind, and should have some degree of persistence with time. These are essentially the properties of the morphological species of Mayr (1942) and others. Mayr provided a second definition to cover the requirements of those concerned with the dynamics of species populations. Biological species are defined as groups of actually (or potentially) interbreeding natural populations which are reproductively isolated from other such groups.

B.3. HYBRIDIZATION - ITS OCCURRENCE AND EFFECTS ON VARIATION

The term "hybridization" in its broadest sense covers the crossing of any two genetically unlike individuals. Its most commonly accepted meaning, however, is crossing between individuals belonging to species which are normally reproductively isolated. It is used in the latter sense here. Anderson (1949) states that repeated backcrossing of the hybrids to one or both parents occurs most frequently under natural conditions. This results in the gradual infiltration of the germplasm of one species into another and the end result (termed "introgressive hybridization" by Anderson

and Hubricht, 1938) is an increased variability of the participating species.

B.3.1. Ecological Restriction of Hybridization

A connection between hybridization and disturbed habitats has long been apparent. In his excellent monograph on hybridization, Anderson (1949) discussed the powerful control that the environment exerts over hybrids:

> "Hybrids segregate in the second and successive hybrid generations; the habitat ordinarily does not. The flood of hybrid segregants which could result from a species cross is screened out by the nonsegregating habitat in which they would have to live. As a consequence, it is only where man or catastrophic natural forces have 'hybridized the habitat' that any appreciable number of segregates survive."

The key to understanding the action of the environment on hybrid segregates is the realization that habitat requirements are inherited in substantially the same manner as any other character. The requirements of the first generation (F1) hybrids are substantially alike and approximately intermediate between those of the two parents. In succeeding hybrid generations or backcrosses these inherent differences recombine variously, due to segregation and recombination during sexual reproduction. The problem of survival is very different therefore for the first and succeeding hybrid generations. The large number of habitats required for survival of all the diverse hybrids seldom occur. Usually when the habitat has been "hybridized" most of the new habitats are similar to one of the original ones. The hybrids with the best chance of survival are those most closely resembling

one of the parent species. The greater the number of gene differences between the parents, the greater is the number of new habitats necessary for the survival of the segregates. The lack of "recombined habitats" will be the strongest barrier, therefore, where there is the greatest differentiation between two hybridizing entities. The following examples demonstrate this (Anderson, 1949).

No. of gene differences between parents affecting habitat requirements	No. of habitats required
1 pair	3
2 pairs	4
10 pairs	1,024
20 pairs	over 1,000,000

In reality, the plasticity of plants permits the occupation of sub-optimal habitats. The selective force of the environment may, therefore, be less rigorous than indicated by Anderson.

B.3.2. Genetic Basis of Hybridization

The mechanism of heredity determines what genetical material will be produced by hybridization and be available for subsequent modification by the environment. The genetical processes of segregation and recombination determine both the range of the individual character combinations that can occur, and the frequency with which they are likely to occur.

Anderson (1949) introduced the concept of the "recombination

spindle" to illustrate the relationship between the possible and actual recombinations resulting from hybridization. Where the differences between two species are characterized by three variables their relationships can be shown on a cube with each of the three axes representing one variable. If the scales of the axes are such that the two species fall on diagonally opposite corners, the entire range of conceivable recombinations of the three characters are bounded by the walls of the cube, termed by Anderson the "cube of expectations". Hybrids between the two species are not distributed at random in the cube, but lie along a diagonal spindle linking their respective positions. This hypothetical pattern of distribution, which is relatively broad in the centre of the cube and tapers towards the ends, is called the "recombination spindle".

Factors affecting the range and frequency of character combinations

The results of hybridization can be explained in general terms by Mendelian segregation. A simple example where the differences between two hybridizing entities are conditioned by a single factor (with no dominance effects) can be represented as follows:

AA		x		A'A'	parents
		↓			
		AA'			intermediate F1
		↓			
AA		AA'		A'A'	segregated F2
1	:	2	:	1	

With an increasing number of independent genes affecting the same character, the chances of offspring resembling either parent species decrease. (With 10 genes there is only one chance in 1,000,000 that an F2 individual would be the same as one of the parents.) At the same time the chances of producing plants similar to the F1 increases. The number of different genotypes that can produce this intermediate condition increases also. With a very large number of independent genes, the F2 generation would be phenotypically similar to the F1 but highly variable genotypically. These considerations are greatly simplified and disregard the effects of dominance, and of non-random segregation, fertilization, and survival of gametes and zygotes.

Gene linkage modifies segregation and recombination by increasing the proportion of F2 recombinations similar to the parents and decreasing the proportion of segregants similar to the F1. The magnitude of this extremely important effect is apparent in an example given by Anderson (1949). With linkage there may be one chance in 1,000 of obtaining an F2 individual with the same combination of genes as one of the parents, while, without linkage the chances would be 10^{30} for the same number of genes. Linkage may be regarded as a negative force keeping the new recombinations from appearing, or as a strong positive force bringing the hybrid population back to the original types. While it operates in both ways, its positive pull back to the original recombination is stronger. Linkage, therefore, provides a cohesive force within species rather than providing a barrier between species. The cohesive force of linkage is more apparent in the F3 and succeeding generations than in the F2, as the

restriction upon types of recombination is reinforced by the effect on frequencies. These combined influences render unlikely, the possibility that the recombinations of the F3 and subsequent generations could advance very much outside the recombinations of the F2.

An additional influence is the degree of heterozygosity which varies from zero at either end of the recombination spindle to a maximum in the middle. Hybrids resembling original parents tend to reproduce themselves whereas intermediate ones segregate. The combined effects of restriction to the recombination spindle and comparative heterozygosity of forms resembling the F1 would tend to increase, in subsequent generations, the proportion of individuals similar to the original parents. Backcrossing would greatly accelerate this tendency.

B.3.3. Character Association as a Criterion of Hybridity

All multiple-factor characters of an organism are linked with one another so strongly that in species crosses the association persists for an extremely large number of generations. Criteria for the analysis of hybridization under natural conditions are based on this phenomenon. Two criteria were indicated by Anderson (1949):

> "1. The intermediacy of separate characters will be correlated. Hybrids intermediate in one character will tend to be intermediate in others. Hybrids which are most like either parent in any one character will tend to resemble that parent in all other characters.
>
> 2. Variation between individuals will lessen as parental combinations are approached."

Four alternative relationships could be expected to exist among individuals compared in terms of two characters which are not related developmentally:

(a) Separate grouping of individuals indicating the groups were genetically isolated from each other.

(b) Independent variation of characters indicating no relationship.

(c) The characters may be completely correlated suggesting they are affected to the same degree by some common environmental factor.

(d) If the characters tend to be related, the cause could be a less rigorous environmental correlation, or it could be due to introgressive hybridization. Anderson states that if both characters are multifactorial, the only possible explanation is introgression. Character association is a particularly convincing criterion of hybridity if it holds for many characters.

B.4. CHARACTERIZATION OF THE INDIVIDUAL

The success of most taxonomic studies depends to a very large extent on what attributes are selected to characterize individuals, how they are tested, and whether they are relevant to the particular purpose. Davis and Heywood (1963) defined a character as "any attribute (or descriptive phrase) referring to form, structure or behaviour which the taxonomist separates from the whole organism

for a particular purpose such as comparison or interpretation." Ideally all the attributes of an organism should be used but, as this is impracticable, only a few representative characters are selected.

B.4.1. Suitable and Unsuitable Characters

All characters are theoretically of use in classification but some are more suitable than others. In practical taxonomy great reliance has been placed on the use of characters which:

(a) are not subject to wide variation within the samples,

(b) do not have high intrinsic genetic variability,

(c) are not greatly susceptible to environmental modification,

(d) show consistency or, in other words, agree with a natural system of classification which was constructed without using these characters.

Floral characters are widely used as diagnostic features because of their consistency and low plasticity. Vegetative characters are subject to equally valid use however, and should not be regarded as unsuitable. Bailey (1951) discussed the common misconception that internal characters, especially of the vascular system, are more dependable than external characters. He pointed out that internal characters are no more reliable and their use merely adds "more strings to the investigator's bow". As characters may be controlled in different ways genetically, some may be more useful than others for a particular study. McHale and Alston (1964)

successfully used chemical markers in an examination of a Baptisia species complex where morphological characters were unsuitable.

B.4.2. Weighting Characters

The method of interpreting character relationships varies with the object of the investigation. Davis and Heywood (1963) stated that for a general purpose classification each attribute selected should be given equal weight. For the investigation of phyletic relationships, however, it is often useful to weight characters according to their importance. This weighting may be subjective (Anderson's hybrid index, 1936) or objective (discriminant function analysis developed by Fisher, 1936). In the first case more weight is attached to characters which are known to be relatively constant in regions where the parent species occurs by itself. Since the effectiveness of the hybrid index depends largely on correlation due to genetic linkage, greater weight is attached to those characters which are controlled by multiple factors than to those governed by a single gene (Stebbins, 1950). In the second case characters are given weighting coefficients of such values that the ratio of between-population variance to within-population variance is maximised. This has the desired effect of allowing maximum distinction between groups. In both cases care should be taken to avoid including two different characters, such as size and proportions of leaves, which might be governed partly or wholly by the same genetic factors and therefore show developmental correlation.

B.5. VARIATION IN THE GENUS CHIONOCHLOA

Zotov (1963) made the following comments on the Chionochloa genus:

"(1) Chromosome numbers in the genus were investigated by J.W. Calder, Journ. Linn. Soc. London. Botany 51: 1-9, 1937. Five of the species were found to have 2n = 42. They are C. conspicua (Danthonia cunninghamii), C. rigida (D. raoulii), C. rubra (D. raoulii var. rubra), C. flavescens (D. raoulii var. flavescens), and C. teretifolia (D. ovata). The names in parentheses are those used by Calder. The three other species had 2n = 36. These are C. crassiuscula, C. oreophila, C. australis.

"(2) L. Cockayne and H.H. Allan, Annals of Botany 48: 11, 1934, listed a number of hybrids which occur in the subfamily Arundinoideae as here understood. However, it is difficult or impossible now to establish the identity of the species named, since there are no specimens or localities mentioned nor are there any herbarium specimens known on which the list might have been based. Nevertheless, intermediate plants are known to occur in Chionochloa. They are apparently hybrids and apparently fertile. They are, however, never numerous in undisturbed habitats.

"(3) The species of Chionochloa display remarkable similarity in their floral structure. Such differences as do exist do not lend themselves to taxonomic treatment on account of continuous variability within a population and from one population to another. Further studies may well reveal useful characters. So far it has not been found practicable to make a satisfactory separation even between species with 36 chromosomes and those with 42. Again, further cytological studies should help."

Connor (1963) successfully produced nine interspecific hybrids within the Chionochloa genus. Two interspecific hybrids flowered and appeared fertile. No attempt was made to cross C. rubra

and C. flavescens (pers. comm.).

Druce (1957) reported considerable variation in the Kaiparoro Chionochloa population and attributed this to hybridization between C. rubra and some other species, which he presumed was C. flavescens (see A.1.2.).

Greenwood was cited in the Wellington Bot. Soc. Bull. No. 26, (1953) as having found C. rubra in another part of the northern Tararua Range when it was thought to be absent in the North Island south of the Ruahine Range:

> " ——— on a ridge not properly shown on the map, running northwards from just west of Taramia [sic, Taramea] Peak towards the Mangahao. It was associated with the broader leaved variety of snowgrass (Danthonia flavescens), together with intermediates, and was confined to the flatter tops and more exposed areas not covered with leatherwood, altitude 2500-2700 feet."

MATERIALS AND METHODS

B.6. SELECTION OF CHARACTERS AND THEIR MEASUREMENT

B.6.1. Preliminary Measurements

Vegetative characters were used to characterize the *Chionochloa* individuals because:

(a) these characters initially drew attention to the variability of the *Chionochloa* population and were of prime importance in the investigation as defined by the working hypothesis (A.2.),

(b) irregular and brief flowering made floral characters unsuitable,

(c) testing for physiological differentiation was impracticable within the time available.

Leaves of the two putative parent species, *C. rubra* and *C. flavescens*, were taken from their respective type localities (Tongariro National Park; and Mt Dennan, Otaki Forks, Tararua Range) and examined for 20 characters. From these were selected the characters with: low within-species variability, high between-species variability, and with an estimated low degree of environmental modification (see B.4.1.). To meet the last requirement, samples were taken from a large range of phenotypes. Ease of

measurement was also taken into account. The 20 characters examined are given below along with reasons for discarding the unsuitable ones.

x = inconsistent

\- = no difference apparent between species

\+ = difficult to measure

x+ 1. growth form

\+ 2. leaf sheath - length and degree of curling

x+ 3. hairs on sheath - number, length, and distribution

-+ 4. wax on sheath - amount

\- 5. colour of sheath

\- 6. ligule - length, and density of hairs

\- 7. ligule shoulder - shape

8. lateral hairs - distance these extend up the leaf blade expressed as a percentage of blade length

9. asperities - as in (8.)

x+ 10. leaf blade colour

x 11. keel or midrib depth

12. number of ribs/leaf - number of strands of chlorenchyma tissue running longitudinally in the leaf blade

13. flattened leaf width

14. "natural" leaf width - measured on a cross-section in water

15. "natural" groove depth - as in (14.)

\- 16. distribution of chlorenchyma tissue - as seen in cross-

section

- 17. bundle sheath - arrangement of parenchyma cells around the vascular bundles

x+ 18. distribution of sclerenchyma tissue - as seen in cross-section

x+ 19. microhairs - presence and shape

x+ 20. short cells or silica cells - shape

The characters selected are listed in table 1. Preliminary measurements showed a distinct separation of C. rubra and C. flavescens.

Table 1

PRELIMINARY MEASUREMENTS OF THE PUTATIVE PARENT SPECIES FROM THEIR RESPECTIVE TYPE LOCALITIES

	C. rubra		C. flavescens	
	Average	Range	Average	Range
Lateral hairs (%)	3.3	0 - 8.2	7.6	6 - 21
Asperities (%)	100.0	100 - 100	3.7	0 - 23
Number of ribs per leaf	16.5	13 - 19	32.5	30 - 36
Flattened leaf width (mm)	3.7	2.7 - 5.2	10.0	8.0 - 12.2
Degree of leaf inrolling ("Natural" groove depth/ "natural" leaf width)	0.73	0.47 - 1.36	0.89	0.68 - 1.16

B.6.2. Details of Measurement

Only mature leaves gave satisfactory measurements.

Leaf cross-sections

Cross-sections were taken 2%, 20%, and 60% of the distance between ligule and leaf tip. At 2% physical restriction by older leaves had prevented full expression of leaf inrolling characteristics. Tip die-back often prevented measurements being taken at 60%. A distance of 30% up the leaf blade, rather than 20%, was selected as this allowed the required amount of photosynthetic tissue to be left on transplants without interfering with cross-sectioning.

Individual measurements

1. Lateral hairs (%):

These long hairs (about 3 mm) are restricted to the leaf margins, and are epidermal appendages termed "macrohairs" by Metcalfe (1960). The hairs were usually close together and ended abruptly varying distances up the leaf blades. The distance measured was from the ligule to the most distal hair. This was expressed as a percentage of total leaf blade length (fig. 6a).

2. Asperities (%):

The asperities (referred to as "prickles" by Metcalfe, 1960) are also situated along the leaf margin. These were measured in the same way as the lateral hairs (fig. 6b).

The following measurements were taken on a cross-section

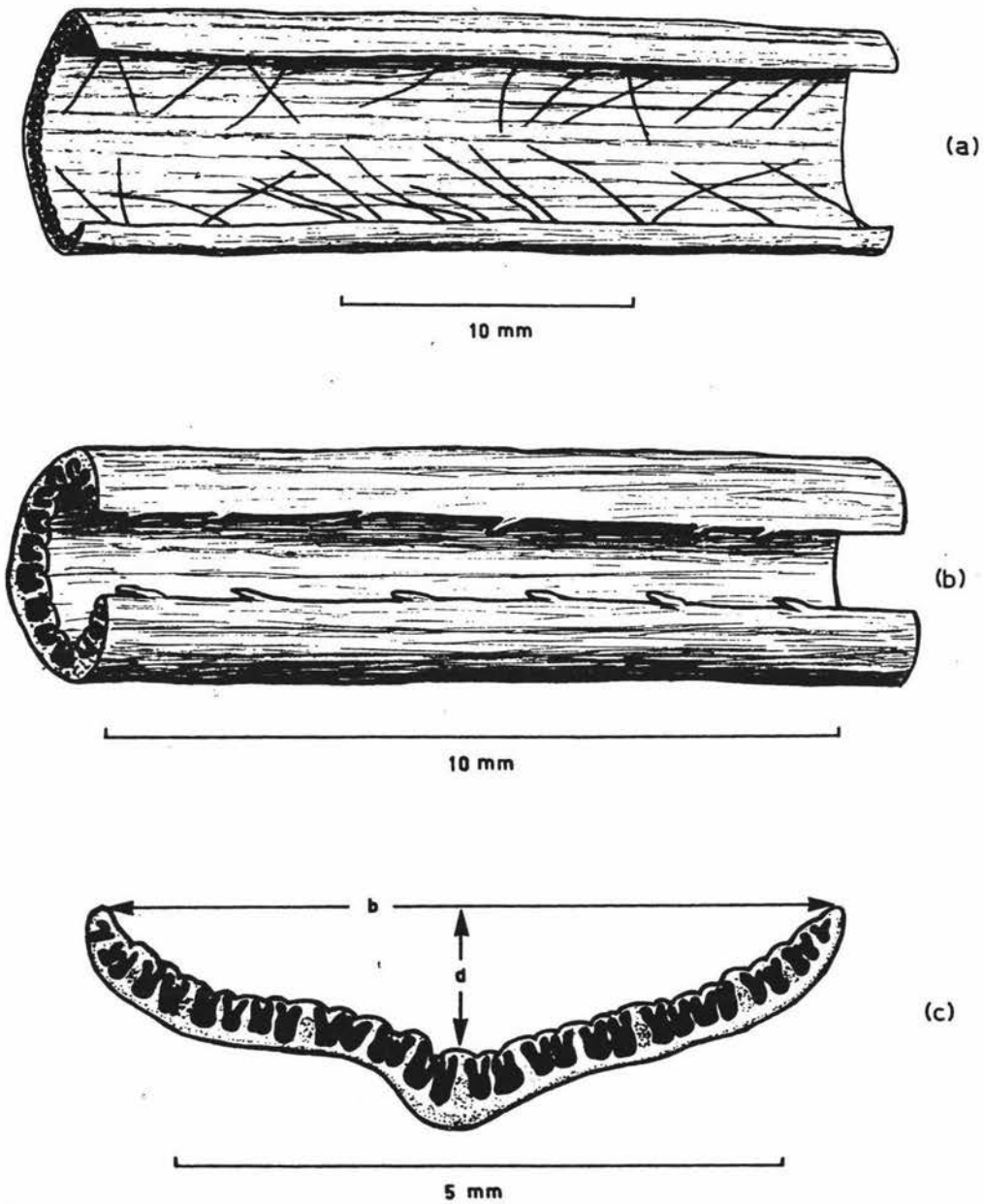


Fig. 6. A portion of two leaf blades showing: (a) lateral hairs, and (b) asperities (distal ends to the left). A cross-section of a leaf blade is shown in (c): b is the "natural" leaf width, and d is the "natural" groove depth. The black areas represent the end view of chlorenchyma strands.

sampled from 30% up the leaf blade and placed immediately in water. This treatment standardized the degree of involution, which is moisture dependent. Measurements were made using a binocular microscope with an optical scale, at 7x magnification (fig. 6c).

3. Number of ribs per leaf:

A count was made of the number of strands of chlorenchyma tissue running longitudinally in the leaf blade.

4. Flattened leaf width:

The width of the leaf section was measured when it was completely unrolled or flattened.

5. "Natural" leaf width:

The distance between the tips of an undisturbed cross-section was measured.

6. "Natural" groove depth:

The inside depth of the groove on an undisturbed cross-section was measured.

B.6.3. Suitability of Selected Characters

Two of the selected characters were discarded after a considerable number of the measurements had been taken.

Asperities

This measurement, which had been highly satisfactory for separation of the parent species, was found to be unsatisfactory for the Kaiparoro plants because of irregular distribution along the leaf

margins. A subjective estimate suggested that some index such as number of asperities per leaf would have been satisfactory in all respects except the practicability of measurement. As a result this character was not used in the final analysis.

Degree of inrolling

Fresh leaf material was available for sectioning only in the case of the glasshouse phenotypes. Unfortunately it was necessary to store the field phenotype leaves in preservative prior to sectioning. This treatment interfered with involution and the leaf inrolling index was of little value for population comparisons.

B.7. SAMPLING TECHNIQUES

B.7.1. Introduction

To test the hypothesis that the Kaiparoro *Chionochloa* population consists of hybrids between *C. rubra* and *C. flavescens* it was necessary to determine the pattern of variation in the Kaiparoro plants and relate this to the putative parent species. Considerable variation appears to exist within *C. flavescens* and possibly also within *C. rubra* throughout their range of distribution. They are therefore difficult to typify and reference to the average broad-leaved or red tussock has a very limited meaning. An attempt was made to characterize the "pure" species by taking samples from their type localities. These may not represent the modal condition

but they do allow reference back to described types. Two further advantages were inherent in use of the type locality populations for comparative purposes:

(a) In the type locality for each species the other species is absent.

(b) The plants growing in the two type localities, by virtue of their geographical placement, represent populations likely to have been among those most recently genetically linked with the Kaiparoro populations.

B.7.2. Placing the Sampling Sites

Kaiparoro

Four sites each with a uniform *Chionochloa* population were chosen to represent the extremes, and two intermediate stages of the full range of morphological variation.

Type localities

One site was chosen at Mt Dennan (*C. flavescens* type locality) and another at Tongariro National Park (*C. rubra* type locality). Transects were placed to sample a range of phenotypes, and in both cases ran from a steep, well drained slope, onto a flat wet area. In this manner it was hoped a range of local within-species variation would be included.

B.7.3. Site Sub-sampling

The number of samples (n) required per plot was calculated using an approximate form of the relationship given by Cochran and Cox (1957, p20). The relationship used was:

$$\text{detectable difference (d) \%} = t\sqrt{2}\ \frac{V}{\sqrt{n}}$$

where V = the coefficient of variation. $t\sqrt{2}$ was taken as approximately equal to 3. The appropriate number of tussocks (n) was calculated for a detectable difference of 10%.

A sample size of 50 tussocks per site was chosen. This allowed for the increased power of multivariate analysis on one hand and transplanting losses on the other. Sampling was conducted in the following manner.

Kaiparoro

On each of the four sites, 20 m x 20 m plots were marked out and the 50 genotype samples from each chosen by a stratified-random system. Each plot was divided into ten 4 m x 10 m rectangles and two genotypes sampled randomly from within each. Systematic-random sampling had two advantages:

(a) Plots could, if required, be analysed for homogeneity and local trends.

(b) Initial location and subsequent relocation of tussocks

was simplified.

The main disadvantage was that collection of a few additional samples to allow for transplanting losses was not possible.

Type localities

A stratified-random system was also used for sampling C. rubra and C. flavescens. A line transect with 50 points about 4 m apart provided the systematic grid. From each of these points a tussock was located at random within a circle about 2 m in diameter. This system had several advantages:

(a) By taking the transect over changing terrain some indication of local variation could be obtained.

(b) The systematic grid made for ease of initial location and subsequent relocation of tussocks and ensured that they were not double sampled.

(c) Random sampling from each grid point eliminated personal bias in selecting tussocks. This was an important factor where large, conspicuously different plants were being examined.

Intensity of sampling

Harberd (1961) found that over-intensive sampling of an Agrostis - Festuca grassland community led to genotype duplication with a resultingly low within-population variance. This gave a spurious indication of significant population differences where within-population variance was used as the error term. There appeared

to be no problem of genotype duplication in the present study. The chances of neighbouring tussocks being sampled was slight and it was not likely, in an intravaginally tillering grass, that many tussocks of the same genotype would occur.

B.7.4. Genotype Sub-sampling

Two ramets were removed from each tussock. Differences between ramets, caused solely by environmental influences, provide an estimate of experimental error.

B.8. TRANSPLANT EXPERIMENT

B.8.1. Experimental Design

The experimental design is shown diagrammatically in fig. 7. A total of 300 tussocks (genotypes) were sampled from six sites. Each tussock was represented by two ramets. As each of the total 600 ramets was collected its leaf blades were removed and stored in preservative for subsequent measurement. The ramets were then transplanted to a common environment in the glasshouse. After about one year, leaves that had grown to maturity in the glasshouse environment were removed and measured. Leaf material was available therefore, to characterize the plants as grown in the field (field phenotypes) and as grown in the common glasshouse environment (glasshouse phenotypes). The original plan of transplanting all

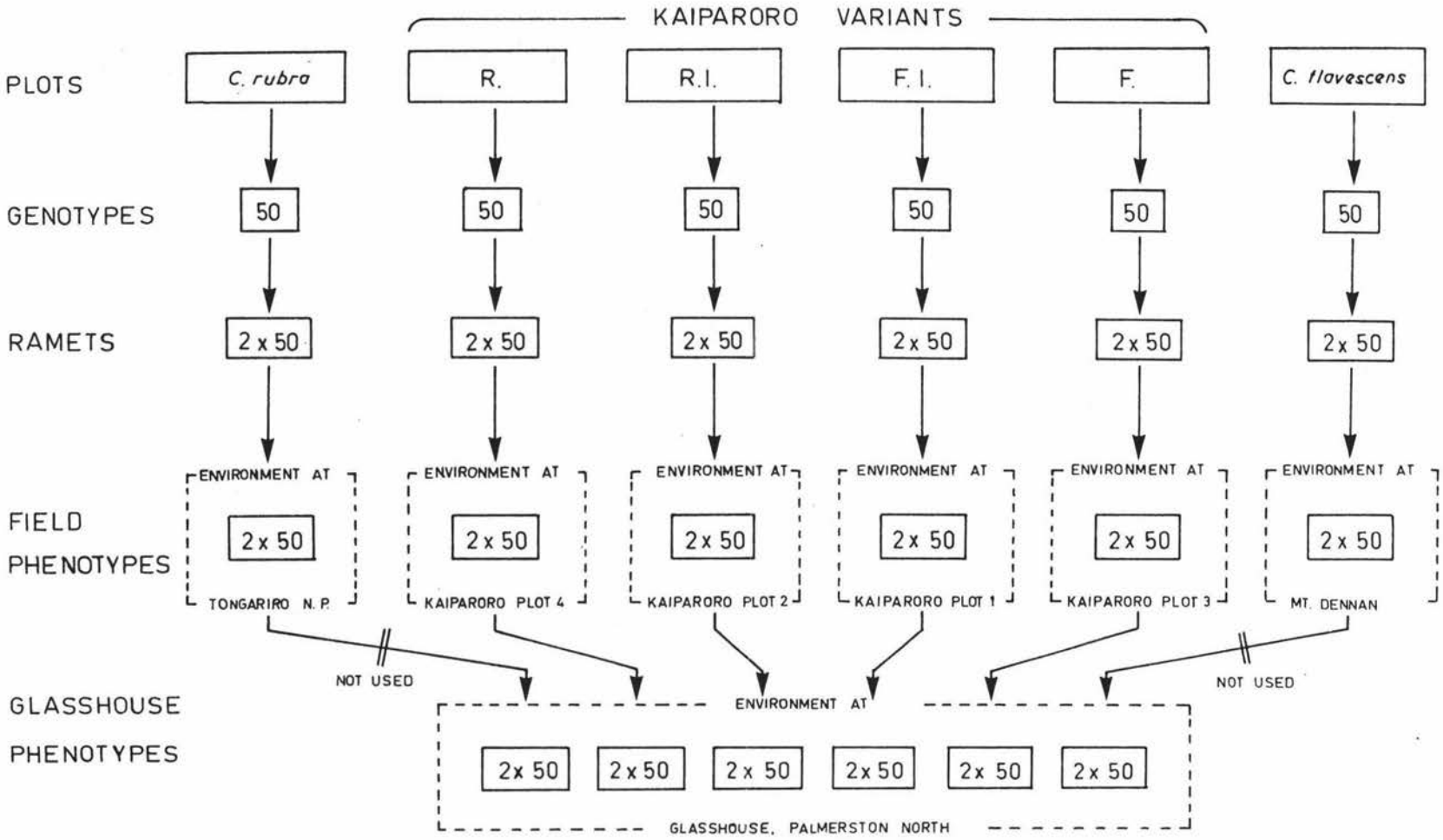


Fig. 7. Experimental design for transplant study.

600 ramets had to be modified when a high proportion of deaths necessitated resampling. Only 400 ramets, those from the Kaiparoro plots, were transplanted. C. rubra and C. flavescens were represented by field phenotype data only.

The 400 Kaiparoro ramets were sampled (second collection) as follows:

Extreme similar to C. rubra	(R.)	from plot 4 on 12.9.64
Extreme similar to C. flavescens	(F.)	from plot 3 on 13.9.64
Intermediate more like C. flavescens than C. rubra	(F.I.)	from plot 1 on 6.11.64
Intermediate more like C. rubra than C. flavescens	(R.I.)	from plot 2 on 7.11.64

Considerable losses in the R. and F. populations required collection of some replacements (9.12.64). Where both of a pair of ramets had died, the replacements were taken from a tussock adjacent to the original. Where only one ramet had died, the replacement came from the original tussock.

B.8.2. Plant Deaths

The first collection of plants was made in June, 1964. Four hundred ramets from Kaiparoro and 100 ramets from Mt Dennan were brought to Palmerston North in moist sphagnum, placed in trays of sand in a propagating pit with bottom heat and automatic mist spray, then

potted out in the glasshouse five weeks later. Fungal colonies, identified as Fusarium sp., were noted at the base of many Kaiparoro plants and, in spite of regular spraying with captan, these plants died. The Mt Dennan plants survived longer and many showed signs of vigorous root growth. Fungal growth appeared later on these plants and fewer died. The evidence suggested that fungal pathogens may have caused the death of the plants. When the second collection of Kaiparoro plants was made, measures were taken to minimise the effects of fungi that may have been present in the field and to reduce the possibility of subsequent infection:

(a) The glasshouse, pots, and tools were treated with formaldehyde.

(b) Ramets were stripped clean of old roots and leaf sheaths.

(c) Each pair of ramets was kept separate from the remainder by wrapping them in newspaper.

(d) Ramets were immersed in fungicide (captan/copper oxychloride) after collection and again before potting.

(e) Ramets were planted directly into separate pots with steam-sterilized soil.

(f) Plants were sprayed with fungicide after placing on the sand bench.

In spite of these precautions, at 8.12.64 losses sustained in populations R. and F. were:

R. 59% death of ramets representing 42% loss of genotypes.

F. 35% death of ramets representing 22% loss of genotypes.

Fungi found in the tissue of dead and unhealthy plants included several belonging to genera noted for their pathogenicity. The majority of plant deaths probably resulted from fungal attack but no test of pathogenicity was made. Short lengths cut from the base (3-4 cm above ground level) of unhealthy and dead tillers were surface-sterilized with 0.1% mercuric chloride, rinsed in sterile distilled water and cut into four sections. These were plated out (two per plate) onto Sabouraud's agar and Czapek Dox agar. The frequency of occurrence of the fungal genera isolated are shown in fig. 8.

The presence of Fusarium sp. in a plant collected directly from the field, and the stringent precautions taken with the transplants, indicated that at least some of these fungi occurred on Kaiparoro. It is conceivable that under the cooler conditions on Kaiparoro the fungi may have been only weakly pathogenic, if at all. The higher temperatures of the glasshouse environment, combined with the physical damage to tillers, may have caused the fungi to become pathogenic.

B.8.3. Cultural Details

The successful transplants were collected as the new season's root growth was starting. They were therefore in a condition

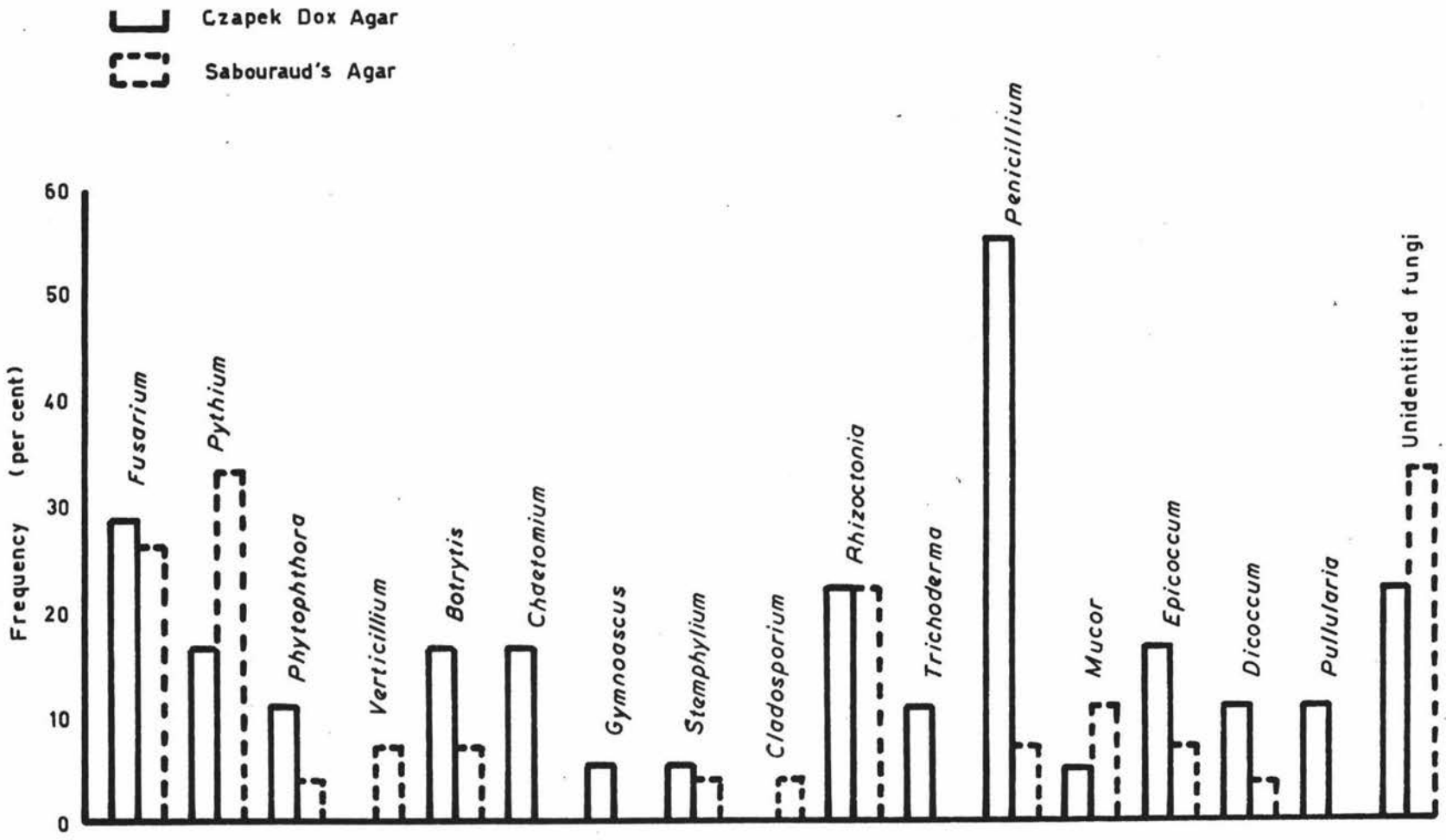


Fig. 8. Occurrence of fungi isolated from dead or unhealthy transplants.

conducive to rapid establishment and mechanical damage to roots was minimised.

Potting mix

This was based on the recommendations of Baker (1957). A mixture of fine sand (50%) and peat (50%) was steam-sterilized and the following fertilizers added:

hoof and horn	2.5 lb/cubic yard
potassium nitrate	4 oz " "
potassium sulphate	4 oz " "
serpentine super phosphate	2.5 lb " "
dolomite lime	7.5 lb " "
lime	2.5 lb " "

Further lime was added to raise the pH of the mix to 6.0.

Sand benches

Pots were watered by a capillary bed of 2 inch deep sand. The supply of water to the pots was from a "Watermac" automatic siphon through a "Cameron" trickle hose to the sand bed. Capillary contact was maintained between the sand and potting mix by wicks of glass wool projecting through the hole in the base of the pots (fig. 9).

During hot weather water was sprayed on the benches and floors to reduce the temperature of the glasshouse. In spite of this, temperatures were at times in excess of 90°F but no adverse

Fig. 9. Chionochloa transplants from four sites on Mt Kaiparoro, after one year's growth in the glasshouse.

plant effects were noticed.

B.9. ANALYSIS OF VARIATIONAL PATTERN

B.9.1. Treatment of Raw Data

Because the measurements of leaf hairs were expressed as percentages, arcsine transformation was necessary. The data transformed according to the table provided by Snedecor (1956) were used in all subsequent calculations.

The ramets were grown on two glasshouse benches. As local environmental variation may have added to experimental variation it was desirable to test for, and remove, any effects. Plants were placed randomly along rows and between benches, with populations R., R.I., F.I., F., and replacements for R. and F. each in separate rows. Individuals of a ramet pair were placed together. This layout allowed testing for environmentally-caused variation along each bench and between benches. In the final analysis there were no along-bench trends, but small between-bench differences were apparent. This was corrected by finding the difference between benches over all populations, and adding it to the lowest bench values. Each of the three variables was corrected in turn. The corrected data were used in all subsequent computations except the hierarchal analysis of variance and the components of variance derived from it for use in Mahalanobis' generalized distance, and canonical axes analysis.

B.9.2. Hierarchal Analysis of Variance

Variance estimates were calculated for the levels of ramets-within-genotypes, genotypes-within-benches, and benches (for the glasshouse populations only). The analysis was conducted on three variables and corresponding cross-products were calculated to provide the dispersion (variance-covariance) matrices required for Mahalanobis' generalized distance and canonical axes comparisons (see App. 1 for computer program). A comparable example for the univariate case is given by Snedecor (1956, p265). Where one ramet of a pair was missing it was allocated the value of the remaining ramet with the loss of one degree of freedom.

B.9.3. Mahalanobis' Generalized Distance (D^2), and Canonical Axes.

The D^2 method of multivariate analysis (Rao, 1952) is used for classifying different groups of individuals according to a significant pattern. Emphasis is placed not on individual variations within a group, but on the group characteristics, or the statistical constants related to the distributions of measurements. Such an approach was suitable in that it constructed a distance index related to the degree of overlap, or conversely, degree of separation of populations. A comparison of all pairs of populations in turn allows representation of the populations in a multidimensional space in such a manner that their mutual relationships are revealed. The information necessary for these comparisons are the dispersion matrices for each population. These are pooled and comparisons made using the

pooled dispersion matrix. Where the dispersion matrices are heterogeneous in nature, pooling is invalid and comparisons cannot be made by the distance technique. This situation applied to the Chionochloa data so the technique was discarded (see B.10.2.).

Seal (1964) preferred the use of canonical axes to the generalized distance approach. He gave two reasons:

(a) Rationalization of the generalized distance technique is heavily dependent on all the variates being measured in the same terms.

(b) Dimensionality of the D^2 comparisons with biometric data will generally be greater than those obtained using canonical axes. The first reason in particular, applies in this study where the following measurements are used: lateral hairs (per cent), number of ribs/leaf (a count), and leaf width (mm). The canonical axes technique would have been used but has the same requirement for homogeneity as the D^2 analysis and was likewise discarded.

B.9.4. Discriminant Function

This statistical technique has the same biological implications as the hybrid index concept (Anderson, 1936), which is dependent on the character association resulting from hybridization. The hybrid index involves the use of multiple measurements to discriminate among groups of individuals. Each individual is characterized by an index composed of the additive, but not necessarily equal, contribution of each variable. The variables may be given

subjective weighting according to their estimated importance, or they may be weighted objectively using a discriminant function analysis.

The general problem can be set up in the form:

$$y = b_1x_1 + b_2x_2 + b_3x_3 + \ldots \qquad \text{(1)}$$

where x_1, x_2, x_3 . . . are the characters measured and b_1, b_2, b_3 . . . are their respective weighting coefficients. Fisher (1936) developed a method for separating two species of Iris. He devised the coefficients of equation (1) such that if an analysis of variance was made of the y values, the ratio of the variance between-species to that within-species would be a maximum. This was achieved by finding the linear function of the measurements which maximised the "t" ratio (the ratio of the mean difference to the standard deviation within-species). In the extension of the method to several species Glenday and Fejer (1956) determined the linear function of the observations which maximised the "F" ratio (the ratio of between-species and within-species variances). This form is adopted here.

The ratio of the between-population variance to the within-population variance in terms of y is (in matrix notation):

$$\frac{b'Vb}{b'Eb}$$

where V and E are respectively the between-population and within-population variance-covariance matrices from the analysis of variance

(see App. 2 for computer program), and b is the column vector of coefficients. The coefficients b which maximise the above ratio are found using the Lagrange multiplier. The solution is found to be:

$$(V - \lambda E)b = 0 \qquad \text{——— (2)}$$

$$b'Eb = 1 \qquad \text{——— (3)}$$

where λ, the maximum value of the ratio, is the highest latent root of $E^{-1}V$ (calculated using a matrix interpretative program on an I.B.M. 1620 computer), the coefficients b being given by equation (2) (see App. 3 for computer program).

A simple iterative procedure for finding the highest latent root of any matrix A is as follows: if $Ac_1 = c_2$, where c_1 is any column vector, then c_{n+1} / c_n converges to the highest latent root of A. No unique solution to the set of equations given by (2) exists, but the ratios of the coefficients are found by putting one value of b equal to unity, deleting one equation from the set, and solving simultaneously for the remaining values of b.

B.9.5. Recombination Spindle

Anderson's technique (Anderson, 1949) for three dimensional representation of the recombination spindle was adapted for comparison of the Kaiparoro variants and the putative parent species (see B.3.2.). Values from the three axes of the cube were used to calculate two composite values for plotting on two rectangular axes. It was possible to realistically represent the position of any individual plant on a

perspective drawing of the cube by plotting its position on these two axes (fig. 10).

The following procedure was adopted to find a and b, the values on the rectangular axes A and B, for any given values of the three leaf variables:

(a) Convert the character values to corresponding values for the sides $X_1 \ldots X_4$, Y_1, Z_1 and Z_2 where the C. rubra mean is represented at E and the C. flavescens mean at C.

(b) Using $X_1 \ldots X_4$ calculate XPER according to the current values of Y_1 and Z_1.

(c) Using Z_1 and Z_2 calculate ZPER according to the current value of Y_1.

(d) Using angles az_1 and az_2 calculate a_1 for the current value of Y_1.

(e) Using $ax_1 \ldots ax_4$ calculate a_2 for the current values of Y_1 and Z_1.

(f) Angle KML = a_2 (alternate angles)

(g) The vertical component of the position of point M in relation to axis B is equal to Y_1 + JK - KL

(h) JK = ZPER sin a_1

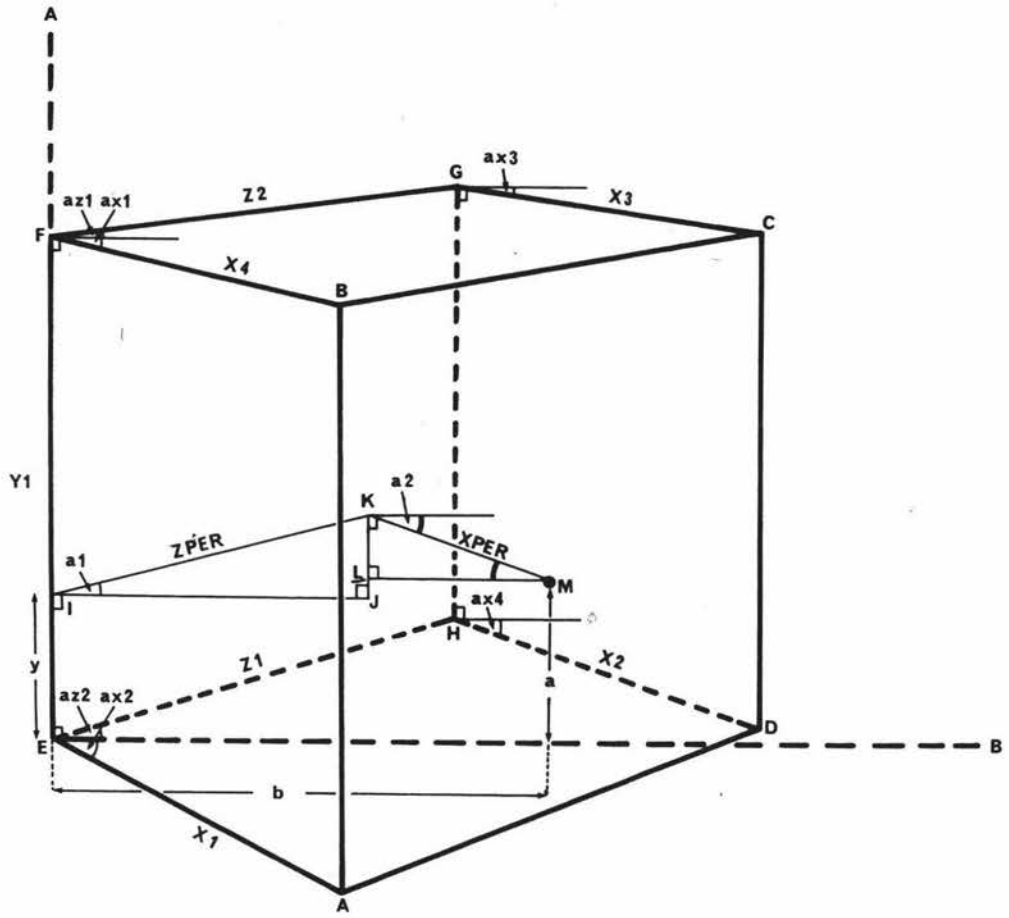


Fig. 10. "Cube of expectations" showing the method for locating individual points on a two-dimensional representation of a three-dimensional graph.

(i) KL = XPER sin a_2

(j) The horizontal component of the position of point M in relation to axis A is equal to IJ + LM

(k) IJ = ZPER cos a_1

(l) LM = XPER cos a_2

Therefore $a = Y_1 + (ZPER \sin a_1) - (XPER \sin a_2)$

and $b = (ZPER \cos a_1) + (XPER \cos a_2)$

Each tussock was characterized by the mean of the ramet pair. The appropriate values of a and b for each set of three variables were calculated using the following Fortran program for the I.B.M. 1620 computer. Comment cards (marked C) indicate the function of each section of the program. Test values were calculated so that program logic and truncation errors could be detected directly. Errors were negligible in all cases selected, for values falling both inside and outside the cube of expectation.

```
C       CALCULATE VALUES FOR 2D REPRESENTATION OF 3D GRAPH
C       PERSPECTIVE ALLOWED FOR
C       LEAD CARD WITH ANGLES X AND Z (IN RADIANS)
C       LEAD CARD WITH VALUE AT ORIGIN AND RANGE FOR X,Y, AND Z AXES
C       LEAD CARD WITH ACTUAL LENGTHS OF ALL SIDES
C       POPULATION LAST CARDS (LP=88 IN COLS. 35 AND 36)
C       FINAL CARD HAS IN ADDITION (LB=99 IN COLS. 37 AND 38)
   34   PRINT 6
    6   FORMAT (//40H REPRESENTATION OF RECOMBINATION SPINDLE)
        PRINT 7
    7   FORMAT (//20H     RECTANGULAR AXES)
        PRINT 8
    8   FORMAT (4X,2H A,8X,2H B,5X,4H NO./)
        N=0
C       READ VALUES FOR ANGLES X AND Z IN RADIANS
        READ 3,AX1,AX2,AX3,AX4,AZ1,AZ2
    3   FORMAT (6F5.4)
C       READ VALUE AT ORIGIN AND RANGE FOR X, Y, AND Z AXES
        READ 1,OVALX,OVALY,OVALZ,DIFX,DIFY,DIFZ
    1   FORMAT (6F7.4)
C       READ ACTUAL LENGTHS OF ALL SIDES OF PERSPECTIVE DRAWING
        READ 10,AE,BF,DH,CG,AD,EH,BC,FG,EF,AB,GH,CD
   10   FORMAT (12F5.2)
C       READ VALUES FOR 3 CHARACTERS, POPULATION LAST CARD VALUE,
C       AND FINAL CARD VALUE
   30   READ 2,AHAIR,RIB,WTH,LP,LC
    2   FORMAT (3F10.4,4X,I2,I2)
        IF(LP-88)31,32,500
C       CONVERT CHARACTER VALUES TO CORRESPONDING VALUES FOR SIDES
C       OF PERSPECTIVE DRAWING WHERE THE TWO SPECIES ARE
C       REPRESENTED AT DIAGONALLY OPPOSITE CORNERS
   31   X1=(WTH-OVALX)*(AE/DIFX)
        X2=(WTH-OVALX)*(DH/DIFX)
        X3=(WTH-OVALX)*(CG/DIFX)
        X4=(WTH-OVALX)*(BF/DIFX)
        Y1=(AHAIR-OVALY)*(EF/DIFY)
        Z1=(RIB-OVALZ)*(EH/DIFZ)
        Z2=(RIB-OVALZ)*(FG/DIFZ)
C       CALC. PERSPECTIVE-ADJUSTED VALUES FOR X AND Z AXES
C       ACCORDING TO THE CURRENT VALUES OF THE REMAINING VARIABLES
        XPER1=X1-((X1-X4)*(Y1/EF))
        XPER2=X2-((X2-X3)*(Y1/EF))
        XPER=XPER1-((XPER1-XPER2)*(Z1/EH))
        ZPER1=Z1-((Z1-Z2)*(Y1/EF))
C       CALC. PERSPECTIVE-ADJUSTED ANGLES IN AN ANALOGOUS MANNER
C       TO ABOVE
        A1=AZ2-((AZ2-AZ1)*(Y1/EF))
        AQ1=AX2-((AX2-AX1)*(Y1/EF))
        AQ2=AX4-((AX4-AX3)*(Y1/EF))
        A2=AQ1-((AQ1-AQ2)*(Z1/EH))
```

```
      SN1=SIN(A1)
      CS1=COS(A1)
      SN2=SIN(A2)
      CS2=COS(A2)
C     CALC. APPROPRIATE VALUES FOR RECTANGULAR AXES A AND B
      A=Y1+(ZPER1*SN1)-(XPER*SN2)
      B=(ZPER1*CS1)+(XPER*CS2)
      N=N+1
      PRINT 9,A,B,N
    9 FORMAT (2F10.4,I4)
      GO TO 30
   32 N=0
      IF(LC-99)30,33,500
   33 PRINT 4
    4 FORMAT (20H PROCESSING COMPLETE)
      PAUSE
      GO TO 34
  500 PRINT 5
    5 FORMAT (14H PROGRAM ERROR)
      PAUSE
      GO TO 34
      END
```

RESULTS AND DISCUSSION

B.10. GENOTYPIC AND PHENOTYPIC VARIATION

B.10.1. Hierarchal Analysis of Variance

Each set of data from the 10 phenotype populations was analysed to obtain estimates of the respective contributions of genotypic and environmental variation to phenotypic variation. Variation at the ramet and genotype levels was calculated for all populations. Bench variation was included for glasshouse phenotype populations (table 2).

(a) Ramets-within-genotypes variation is due to different environmental influences acting on the genetically identical individuals of each ramet pair. Such effects on transplants could include differential pre-treatment (e.g., root damage, ramet size, and fungal infection), and a differential glasshouse environment (e.g., micro-climate and soil mix variation). Field-grown ramets could experience similar effects (e.g., differential micro-climate and nutrition).

(b) Genotypes-within-populations (or benches) variation is due to the genotypic heterogeneity of each population.

(c) Bench variation could be due to environmental or chance differences.

Table 2

CONTRIBUTION OF RAMETS, GENOTYPES, AND BENCHES TO VARIATION IN TEN *CHIONOCHLOA* POPULATIONS FOR THREE LEAF CHARACTERS

POPULATION	SOURCE OF VARIATION	d.f.	CHARACTER	MEAN SQUARE	PARAMETERS ESTIMATED	'F' RATIO	P
1	Benches	1	Hairs	1.3530	$\sigma_R^2 + 2\sigma_G^2 + 84\sigma_B^2$		
			Ribs	6.0110			
			Width	0.2301			
F.I. (Glasshouse Phenotypes)	Genotypes-within-benches	42	Hairs	151.0690	$\sigma_R^2 + 2\sigma_G^2$	5.67	$<.005$
			Ribs	36.7495		8.81	$<.005$
			Width	1.1413		4.47	$<.005$
	Ramets-within-genotypes	32	Hairs	26.5756	σ_R^2		
			Ribs	4.1719			
			Width	0.2552			
2	Benches	1	Hairs	121.7540	$\sigma_R^2 + 2\sigma_G^2 + 80\sigma_B^2$	-	-
			Ribs	77.8090		3.03	.085
			Width	1.5534		2.53	$>.100$
R.I. (Glasshouse Phenotypes)	Genotypes-within-benches	40	Hairs	192.8611	$\sigma_R^2 + 2\sigma_G^2$	17.58	$<.005$
			Ribs	25.6938		4.32	$<.005$
			Width	0.6129		2.56	$<.005$
	Ramets-within-genotypes	35	Hairs	10.9708	σ_R^2		
			Ribs	5.9429			
			Width	0.2393			

Genotypes-within-benches: $d.f. = n_1 + n_2 - 2$
Ramets-within-genotypes: $d.f. = (r - 1)(n_1 + n_2) - m$

Where n_1 = no. of genotypes in bench 1
n_2 = " " " " " " 2
r = " " ramets per genotype
m = " " genotypes with one ramet of the pair missing

Table 2 (continued)

POPULATION	SOURCE OF VARIATION	d.f.	CHARACTER	MEAN SQUARE	PARAMETERS ESTIMATED	'F' RATIO	P
3	Benches	1	Hairs	43.5950	$\sigma_R^2 + 2\sigma_G^2 + 96\sigma_B^2$	-	-
			Ribs	14.8350		-	-
			Width	7.4447		1.39	$>.100$
F. (Glasshouse Phenotypes)	Genotypes-within-benches	48	Hairs	62.2974	$\sigma_R^2 + 2\sigma_G^2$	2.60	$<.005$
			Ribs	19.9401		2.54	$<.005$
			Width	5.3441		6.94	$<.005$
	Ramets-within-genotypes	37	Hairs	23.9539	σ_R^2		
			Ribs	7.8649			
			Width	0.7700			
4	Benches	1	Hairs	28.1255	$\sigma_R^2 + 2\sigma_G^2 + 94\sigma_B^2$	-	-
			Ribs	12.4730		1.48	$>.100$
			Width	1.1857		2.36	$>.100$
R. (Glasshouse Phenotypes)	Genotypes-within-benches	47	Hairs	86.6267	$\sigma_R^2 + 2\sigma_G^2$	2.54	$<.005$
			Ribs	8.4515		3.66	$<.005$
			Width	0.5027		2.69	$<.005$
	Ramets-within-genotypes	42	Hairs	34.1068	σ_R^2		
			Ribs	2.3095			
			Width	0.1868			

Table 2 (continued)

POPULATION	SOURCE OF VARIATION	d.f.	CHARACTER	MEAN SQUARE	PARAMETERS ESTIMATED	'F' RATIO	P
5	Genotypes	49	Hairs	116.3550	$\sigma_R^2 + 2\sigma_G^2$	8.02	$< .005$
			Ribs	22.7829		3.75	$< .005$
F.I.			Width	1.7073		3.89	$< .005$
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	14.5135	σ_R^2		
			Ribs	6.0800			
			Width	0.4392			
6	Genotypes	49	Hairs	70.5852	$\sigma_R^2 + 2\sigma_G^2$	4.01	$< .005$
			Ribs	26.5069		4.62	$< .005$
R.I.			Width	1.1455		2.39	$< .005$
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	17.6086	σ_R^2		
			Ribs	5.7400			
			Width	0.4783			
7	Genotypes	49	Hairs	71.9388	$\sigma_R^2 + 2\sigma_G^2$	5.61	$< .005$
			Ribs	12.5298		5.54	$< .005$
F.			Width	2.0928		3.39	$< .005$
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	12.8123	σ_R^2		
			Ribs	2.2600			
			Width	0.6182			

Table 2 (continued)

POPULATION	SOURCE OF VARIATION	d.f.	CHARACTER	MEAN SQUARE	PARAMETERS ESTIMATED	'F' RATIO	P
8	Genotypes	49	Hairs	34.3986	$\sigma_R^2 + 2\sigma_G^2$	4.58	$<.005$
			Ribs	13.7829		6.21	$<.005$
R.			Width	1.1519		6.16	$<.005$
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	7.5056	σ_R^2		
			Ribs	2.2200			
			Width	0.1869			
9	Genotypes	49	Hairs	16.2321	$\sigma_R^2 + 2\sigma_G^2$	3.01	$<.005$
			Ribs	4.4865		3.25	$<.005$
C. rubra			Width	0.4220		3.84	$<.005$
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	5.3986	σ_R^2		
			Ribs	1.3800			
			Width	0.1098			
10	Genotypes	49	Hairs	65.3234	$\sigma_R^2 + 2\sigma_G^2$	4.14	$<.005$
			Ribs	10.9120		7.42	$<.005$
C. flavescens			Width	2.2942		6.35	$<.005$
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	15.7623	σ_R^2		
			Ribs	1.4700			
			Width	0.3612			

The variance ratio $\frac{\sigma^2_R + 2\sigma^2_G}{\sigma^2_R}$ indicates that the three leaf characters vary significantly from genotype to genotype in all populations ($P < 0.01$). On the other hand the ratio $\frac{\sigma^2_R + 2\sigma^2_G + 2x\sigma^2_B}{\sigma^2_R + 2\sigma^2_G}$ shows no significant difference between benches in the glasshouse populations ($P > 0.05$). In several cases the bench variance was less than the genotype-within-bench variance due to chance bench similarities.

Calculation of the genotype component of variation also provided data for comparisons of populations by Mahalanobis' generalized distance, and canonical axes free from the additional effect of ramet (environmental) or bench variation (table 3). "Environment" here refers to any factor external to the plant that could, by acting before or during the experiment, influence the characters measured.

Figures 11, 12, and 13 summarize much of the information obtained on variation of leaf characters. The mean of each population is shown by a vertical line and the full range of variation by a horizontal line. The rectangles extend for one standard deviation either side of the mean, horizontally for the genotype component of variation and vertically for the environmental component. Both are shown on the same scale so that horizontal elongation of the rectangle indicates that genotypic variation makes a greater contribution to phenotypic variation than does environmental variation. Several facts emerge from this information:

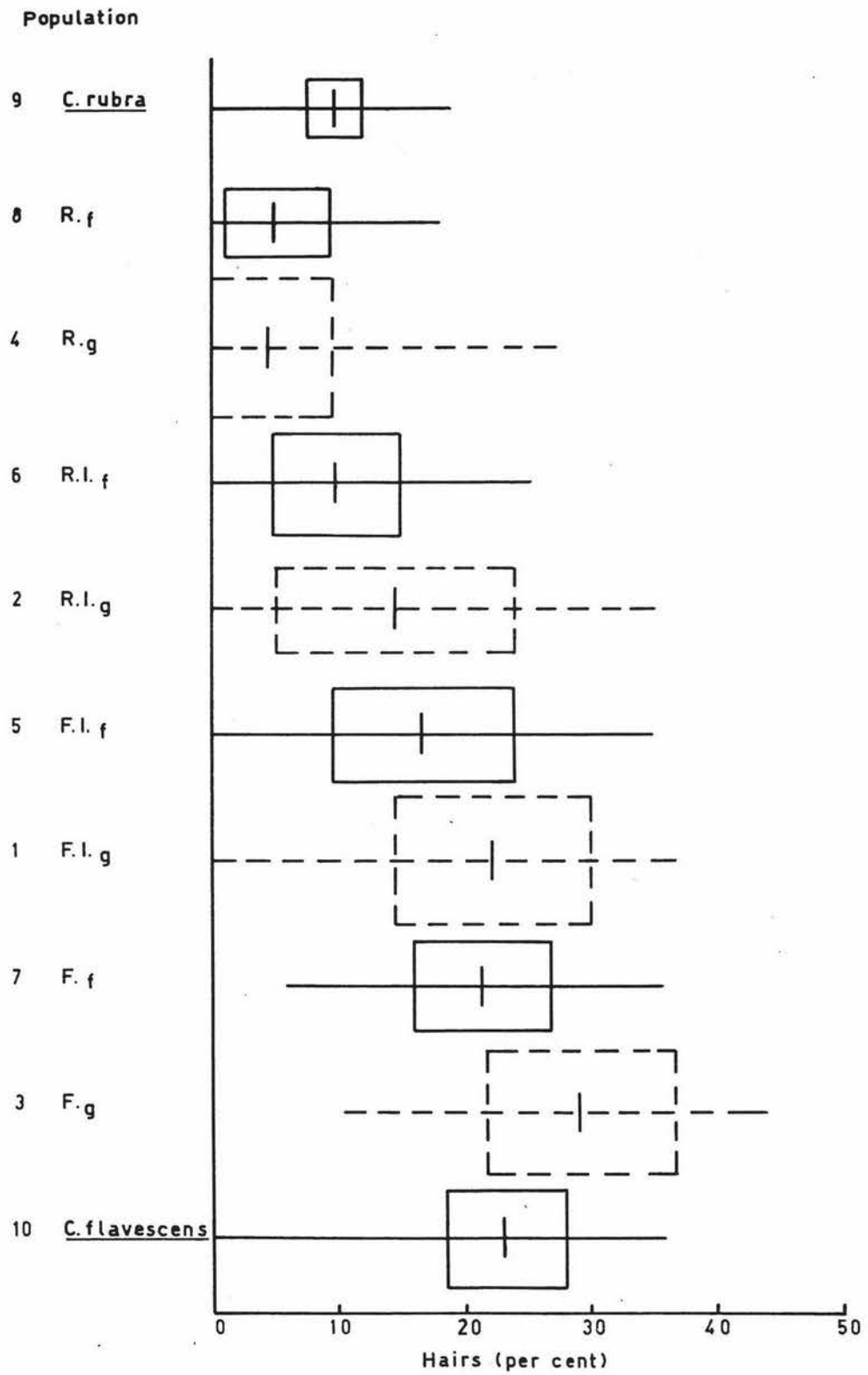


Fig. 11. Comparison of 10 populations, for leaf hairiness. Field phenotypes are represented by continuous lines, and glasshouse phenotypes by broken lines. For explanation of the symbols used, see text (p. 46).

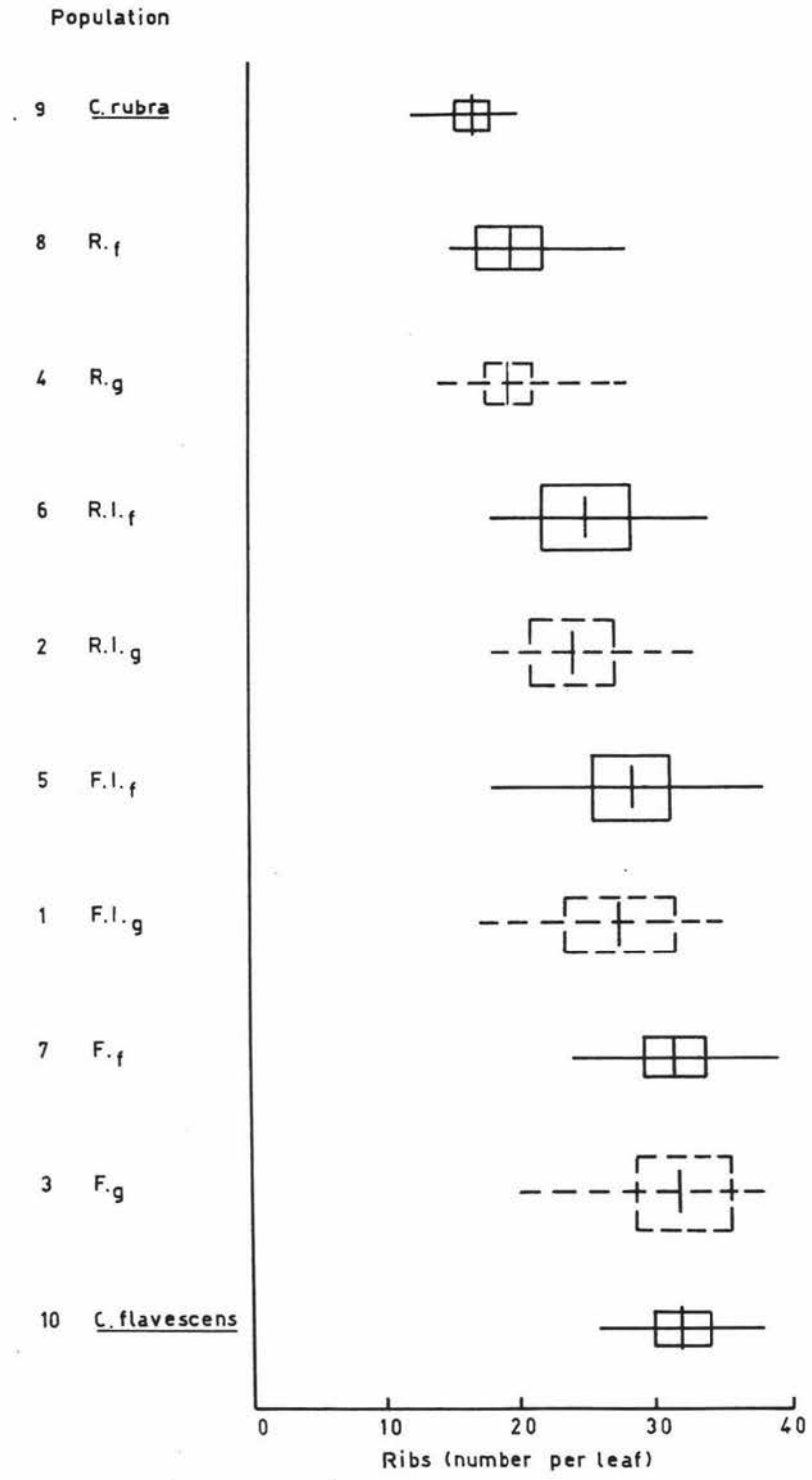


Fig. 12. Comparison of 10 populations, for number of ribs per leaf. Field phenotypes are represented by continuous lines and glasshouse phenotypes by broken lines. For explanation of the symbols used, see text (p. 46).

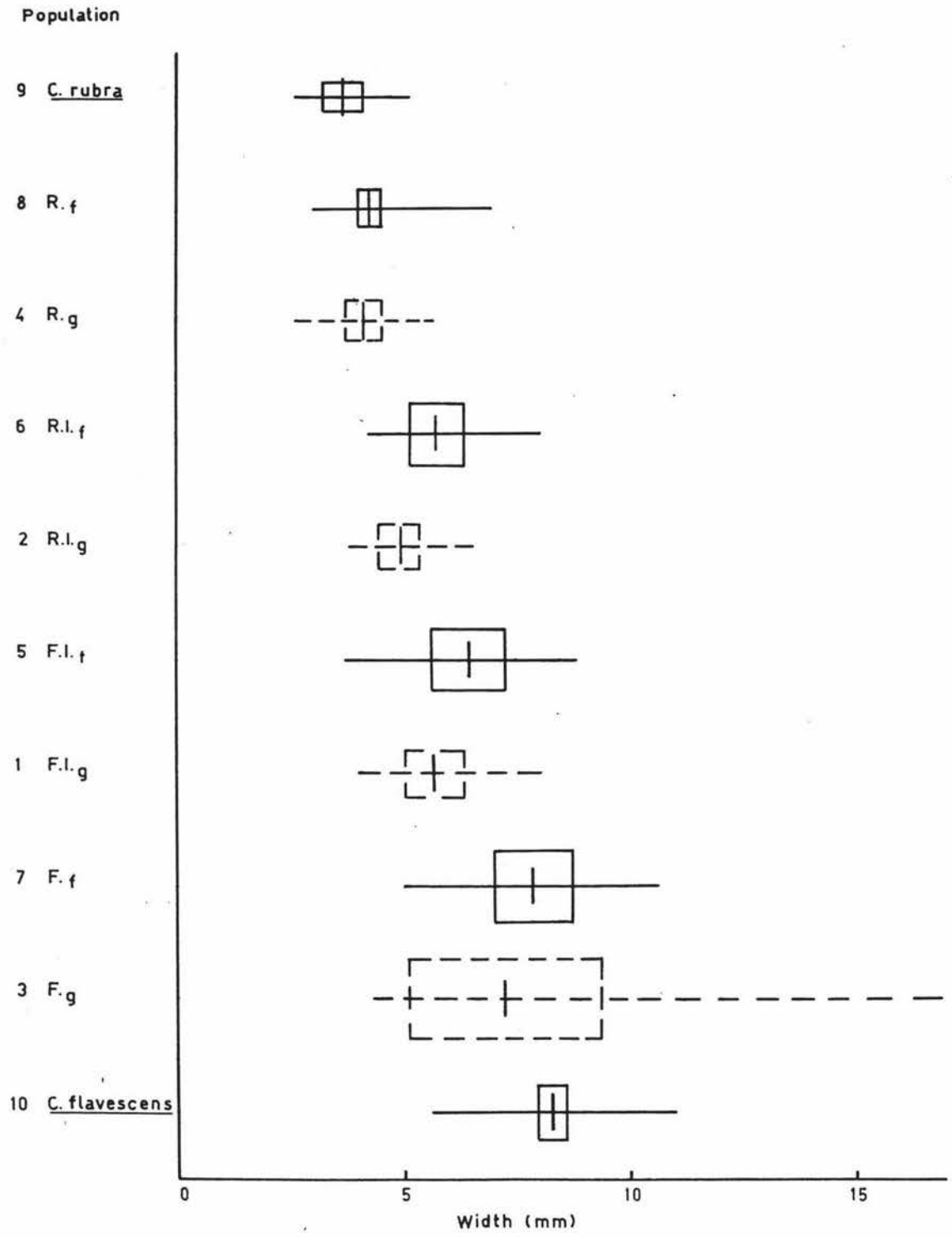


Fig. 13. Comparison of 10 populations, for leaf width. Field phenotypes are represented by continuous lines and glasshouse phenotypes by broken lines. For explanation of the symbols used, see text (p. 46).

Table 3

GENOTYPE COMPONENT OF VARIANCE AND COVARIANCE IN TEN CHIONOCHLOA POPULATIONS FOR THREE LEAF CHARACTERS

POPULATION	SOURCE OF VARIATION	d.f.	CHARACTER	MEAN SQUARES AND CROSS PRODUCTS Hairs	Ribs	Width	PARAMETERS ESTIMATED	GENOTYPE COMPONENT OF VARIATION $S_G^2 = (MS_{gwb} - MS_{rwg})/2$			DETERMINANT
1	Benches	1	Hairs	1.3530	2.8500	0.5580	$\sigma_R^2 + 2\sigma_G^2 + 84\sigma_B^2$				
			Ribs		6.0110	1.1770					
			Width			0.2301					
F.I.											
(Glasshouse Phenotypes)	Genotypes-within-benches	42	Hairs	151.0690	18.0806	2.3313	$\sigma_R^2 + 2\sigma_G^2$	62.2467	8.0325	0.6808	
			Ribs		36.7495	5.4304			16.2888	2.4441	68.0206
			Width			1.1413				0.4431	
	Ramets-within-genotypes	32	Hairs	26.5756	2.0156	0.9696	σ_R^2				
			Ribs		4.1719	0.5422					
			Width			0.2552					
2	Benches	1	Hairs	121.7540	97.3320	13.7526	$\sigma_R^2 + 2\sigma_G^2 + 80\sigma_B^2$				
			Ribs		77.8090	10.9941					
			Width			1.5534					
R.I.											
(Glasshouse Phenotypes)	Genotypes-within-benches	40	Hairs	192.8611	15.6978	1.2306	$\sigma_R^2 + 2\sigma_G^2$	90.9452	7.6909	0.5061	
			Ribs		25.6938	2.6776			9.8755	0.8781	90.9046
			Width			0.6129				0.1868	
	Ramets-within-genotypes	35	Hairs	10.9708	0.3161	0.2185	σ_R^2				
			Ribs		5.9429	0.9214					
			Width			0.2393					

For d.f. used, see Table 2.

Table 3 (continued)

POPULATION	SOURCE OF VARIATION	d.f.	CHARACTER	MEAN SQUARES AND CROSS PRODUCTS Hairs	Ribs	Width	PARAMETERS ESTIMATED	GENOTYPE COMPONENT OF VARIATION $S_G^2 = (MS_{gwb} - MS_{rwg})/2$			DETERMINANT
3	Benches	1	Hairs	43.5950	-25.4310	-18.0160	$\sigma_R^2 + 2\sigma_G^2 + 96\sigma_B^2$				
			Ribs		14.8350	10.5090					
			Width			7.4447					
F. (Glasshouse Phenotypes)	Genotypes-within-benches	48	Hairs	62.2974	5.8878	3.1446	$\sigma_R^2 + 2\sigma_G^2$	19.1718	2.1627	1.3728	
			Ribs		19.9401	4.3217			6.0376	1.3366	216.3462
			Width			5.3441				2.2871	
	Ramets-within-genotypes	37	Hairs	23.9539	1.5624	0.3991	σ_R^2				
			Ribs		7.8649	1.6486					
			Width			0.7700					
4	Benches	1	Hairs	28.1255	18.7306	5.7747	$\sigma_R^2 + 2\sigma_G^2 + 94\sigma_B^2$				
			Ribs		12.4730	3.8458					
			Width			1.1857					
R. (Glasshouse Phenotypes)	Genotypes-within-benches	47	Hairs	86.6267	11.7322	2.8599	$\sigma_R^2 + 2\sigma_G^2$	26.2600	5.3734	1.5059	
			Ribs		8.4515	1.5400			3.0710	0.5718	1.8847
			Width			0.5027				0.1580	
	Ramets-within-genotypes	42	Hairs	34.1068	0.9855	-0.1518	σ_R^2				
			Ribs		2.3095	0.3964					
			Width			0.1868					

Table 3 (continued)

POPULATION	SOURCE OF VARIATION	d.f.	CHARACTER	MEAN SQUARES AND CROSS PRODUCTS Hairs	Ribs	Width	PARAMETERS ESTIMATED	GENOTYPE COMPONENT OF VARIATION $S_G^2 = (MS_g - MS_{rwg})/2$			DETERMINANT
5	Genotypes	49	Hairs	116.3550	11.9929	2.3489	$\sigma_R^2 + 2\sigma_G^2$	50.9208	4.9188	0.9323	
			Ribs		22.7829	4.6286			8.3515	1.7373	109.3053
F.I.			Width			1.7073				0.6341	
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	14.5135	2.1554	0.4843	σ_R^2				
			Ribs		6.0800	1.1540					
			Width			0.4392					
6	Genotypes	49	Hairs	70.5852	4.8355	0.8177	$\sigma_R^2 + 2\sigma_G^2$	26.4883	2.0785	0.3940	
			Ribs		26.5069	4.1605			10.3835	1.5498	27.6184
R.I.			Width			1.1455				0.3336	
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	17.6086	0.6786	0.0297	σ_R^2				
			Ribs		5.7400	1.0610					
			Width			0.4783					
7	Genotypes	49	Hairs	71.9388	3.0961	2.9189	$\sigma_R^2 + 2\sigma_G^2$	29.5633	2.3819	1.9657	
			Ribs		12.5298	3.5980			5.1349	1.5330	32.7810
F.			Width			2.0928				0.7373	
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	12.8123	-1.6677	-1.0124	σ_R^2				
			Ribs		2.2600	0.5320					
			Width			0.6182					

Table 3 (continued)

POPULATION	SOURCE OF VARIATION	d.f.	CHARACTER	MEAN SQUARES AND CROSS PRODUCTS Hairs	Ribs	Width	PARAMETERS ESTIMATED	GENOTYPE COMPONENT OF VARIATION $S_G^2 = (MS_g - MS_{rwg})/2$			DETERMINANT
8	Genotypes	49	Hairs	34.3986	3.8870	1.4709	$\sigma_R^2 + 2\sigma_G^2$	13.4465	1.3922	0.7164	
			Ribs		13.7829	3.4131			5.7815	1.5716	3.5301
R.			Width			1.1519				0.4825	
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	7.5056	0.1027	0.0381	σ_R^2				
			Ribs		2.2200	0.2700					
			Width			0.1869					
9	Genotypes	49	Hairs	16.2321	2.9604	0.6171	$\sigma_R^2 + 2\sigma_G^2$	5.4168	1.5024	0.3044	
			Ribs		4.4865	0.9593			1.5533	0.3727	0.3795
C. rubra			Width			0.4220				0.1561	
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	5.3986	-0.0444	0.0083	σ_R^2				
			Ribs		1.3800	0.2140					
			Width			0.1098					
10	Genotypes	49	Hairs	65.3234	10.5309	6.5953	$\sigma_R^2 + 2\sigma_G^2$	24.7806	5.4930	3.1324	
			Ribs		10.9120	3.7064			4.7210	1.7312	22.8919
C. flavescens			Width			2.2942				0.9665	
(Field Phenotypes)	Ramets-within-genotypes	50	Hairs	15.7623	-0.4550	0.3306	σ_R^2				
			Ribs		1.4700	0.2440					
			Width			0.3612					

(a) The range of characters show considerable population overlap particularly for leaf hairiness.

(b) Genotypic variation is generally greater than environmental variation, but leaf width shows some exceptions.

(c) The four Kaiparoro field populations range between the extremes of C. rubra and C. flavescens except for the R. population which is less hairy than C. rubra.

(d) The standard deviation shows a progressive decrease as the "parental" conditions are approached. This occurs in spite of selection for homogeneity in the Kaiparoro populations and therefore emphasises the high variability of the Kaiparoro Chionochloa.

(e) Genotypic plasticity is most marked for hairs and width, and for both these characters increases towards the C. flavescens end of the scale.

B.10.2. Mahalanobis' Generalized Distance, and Canonical Axes

These techniques require pooling of the dispersion matrices for each population and comparisons are only valid where the matrices are homogeneous. The 10 matrices for the genotype component of variation were tested for homogeneity according to the procedure outlined by Seal (1964). The comparison of the pooled and individual matrices was effected by comparing their determinants according to the following test criterion which is distributed approximately as chi-square with $(h - 1)p(p + 1)/2$ degrees of freedom.

$$-2\left[1-\left\{\sum_{l=1}^{h}\frac{1}{N_l-1}-\frac{1}{N-h}\right\}\frac{2p^2+3p-1}{6(p+1)(h-1)}\right]\ln\left\{\frac{\prod_{l=1}^{h}\hat{\Delta}_l^{(N_l-1)/2}}{\hat{\Delta}^{(N-h)/2}}\right\}$$

where h is the number of populations (h=10)

N_l is the number of sets of observations for the l^{th} population

p is the number of characters making up each set of observations (p=3)

$\hat{\Delta}_l$ is the determinant of the l^{th} dispersion matrix

$\hat{\Delta}$ is the determinant of the pooled dispersion matrix

Comparison of the pooled dispersion matrix (M)

$$M = \begin{matrix} 33.6536 & 3.9899 & 1.1671 \\ 3.9899 & 6.9662 & 1.3691 \\ 1.1671 & 1.3691 & 0.6496 \end{matrix}$$

$$|M| = 454.6700 = \hat{\Delta}$$

and the individual dispersion matrices $M^{(l)}$ (table 3) gave a test value of 693.69, which greatly exceeded the appropriate values of chi-square for 54 degrees of freedom ($67.50_{(p = 0.05)}$ and $76.15_{(p = 0.01)}$). Heterogeneity of the dispersion matrices was demonstrated and, as pooling of the dispersion matrices was therefore invalid, no use could be made of Mahalanobis' generalized distance, and the canonical axes techniques.

B.11. POPULATION RELATIONSHIPS

B.11.1. Discriminant Function

The discriminant analysis was based on maximising the F ratio firstly for the two putative parent species only, and secondly, for all 10 populations. Results of the analysis of variance for three characters are presented in table 4. The highest latent roots giving the maximum ratio of between-population to within-population variance, in all but one case, show a large increase in the ratio. The results of the two analyses are adjusted so that the range of hybrid indices is the same (table 5). The population relationships are shown graphically in fig. 14.

Fig. 14a: The analysis was used to discriminate between C. rubra and C. flavescens and the remaining eight populations placed according to the weighting coefficients thus established. The population distributions show that the Kaiparoro populations at one extreme are inseparable from the C. flavescens population. C. rubra is shown to be slightly different from the Kaiparoro plants representing the other extreme. The remaining Kaiparoro populations lie in intermediate positions. The relationships between the field phenotypes and their respective glasshouse phenotypes indicate that genetical plasticity increases as the C. flavescens extreme is approached.

Fig. 14b gives a similar result.

Table 4

MAXIMISING THE RATIO OF BETWEEN-POPULATIONS AND WITHIN-POPULATIONS VARIANCE

SOURCE OF VARIATION	d.f.	CHARACTER	MEAN SQUARES AND CROSS PRODUCTS Hairs	Ribs	Width	'F' RATIO (Diagonal Elements)	MATRIX A = $E^{-1}V$			HIGHEST LATENT ROOT OF A
C. rubra and C. flavescens										
Between-populations = V	1	Hairs	4486.4	5174.2	1545.9	220.04	0.356	0.411	0.123	1550.49
		Ribs	5174.2	5967.6	1782.9	1550.03	1364.389	1573.579	470.138	
		Width	1545.9	1782.9	532.7	784.54	-68.029	-78.461	-23.442	
Within-populations = E	98	Hairs	20.389	3.373	1.803					
		Ribs	3.373	3.850	1.166					
		Width	1.803	1.166	0.679					
Populations 1-10										
Between-populations = V	9	Hairs	3410.8	2076.7	545.1	81.52	58.054	30.356	7.568	226.75
		Ribs	2076.7	1606.4	438.2	181.00	158.322	121.102	29.855	
		Width	545.1	438.2	128.4	154.66	246.212	234.353	82.240	
Within-populations = E	474	Hairs	41.837	4.324	1.208					
		Ribs	4.324	8.875	1.708					
		Width	1.208	1.708	0.830					

Table 5

HYBRID INDICES, CONSTRUCTED FROM THREE LEAF CHARACTERS, FOR TEN *CHIONOCHLOA* POPULATIONS

			Based on all populations			Based on 'parent' populations		
POPULATION	CHARACTER	MEAN	WEIGHTED MEAN	HYBRID INDEX ($\overline{Y}_A$)	ADJUSTED INDEX ($\overline{Y}_A - x$)	WEIGHTED MEAN	HYBRID INDEX ($\overline{Y}_p$)	ADJUSTED INDEX ($\overline{Y}_p - y/z$)
1 F.I. (Glasshouse Phenotypes)	Hairs	22.45	22.45	165.39	67.53	22	104,197	74.23
	Ribs	27.38	100.08			105,251		
	Width	5.62	42.86			-1,076		
2 R.I. (Glasshouse Phenotypes)	Hairs	14.76	14.76	141.15	43.29	15	92,593	53.53
	Ribs	24.33	88.92			93,519		
	Width	4.91	37.47			-941		
3 F. (Glasshouse Phenotypes)	Hairs	29.22	29.22	200.87	103.01	29	121,500	105.09
	Ribs	31.96	116.68			122,851		
	Width	7.20	54.97			-1,380		
4 R. (Glasshouse Phenotypes)	Hairs	4.61	4.61	106.19	8.33	5	73,150	18.85
	Ribs	19.23	70.30			73,930		
	Width	4.10	31.28			-785		
5 F.I. (Field Phenotypes)	Hairs	16.91	16.91	169.62	71.76	17	109,137	83.04
	Ribs	28.42	103.87			109,243		
	Width	6.40	48.84			-123		

Table 5 (continued)

			Based on all populations			Based on 'parent' populations		
POPULATION	CHARACTER	MEAN	WEIGHTED MEAN	HYBRID INDEX ($\overline{Y}_A$)	ADJUSTED INDEX ($\overline{Y}_A - x$)	WEIGHTED MEAN	HYBRID INDEX ($\overline{Y}_P$)	ADJUSTED INDEX ($\overline{Y}_P - y/z$)
6	Hairs	9.97	9.97			10		
R.I.	Ribs	25.04	91.52	114.76	46.90	96,250	95,174	58.14
(Field Phenotypes)	Width	5.67	43.27			-1,086		
7	Hairs	21.47	21.47			21		
F.	Ribs	31.52	115.20	196.17	98.31	121,158	119,685	101.85
(Field Phenotypes)	Width	7.80	59.50			-1,494		
8	Hairs	5.17	5.17			5		
R.	Ribs	19.42	70.98	108.19	10.33	74,648	73,849	20.10
(Field Phenotypes)	Width	4.20	32.04			-804		
9	Hairs	9.83	9.83			10		
C. rubra	Ribs	16.46	60.16	97.86	0.00	63,270	62,580	0.00
(Field Phenotypes)	Width	3.65	27.87			-700		
10	Hairs	23.23	23.23			23		
C. flavescens	Ribs	31.91	116.63	202.95	105.09	122,658	121,097	104.37
(Field Phenotypes)	Width	8.27	63.09			-1,584		

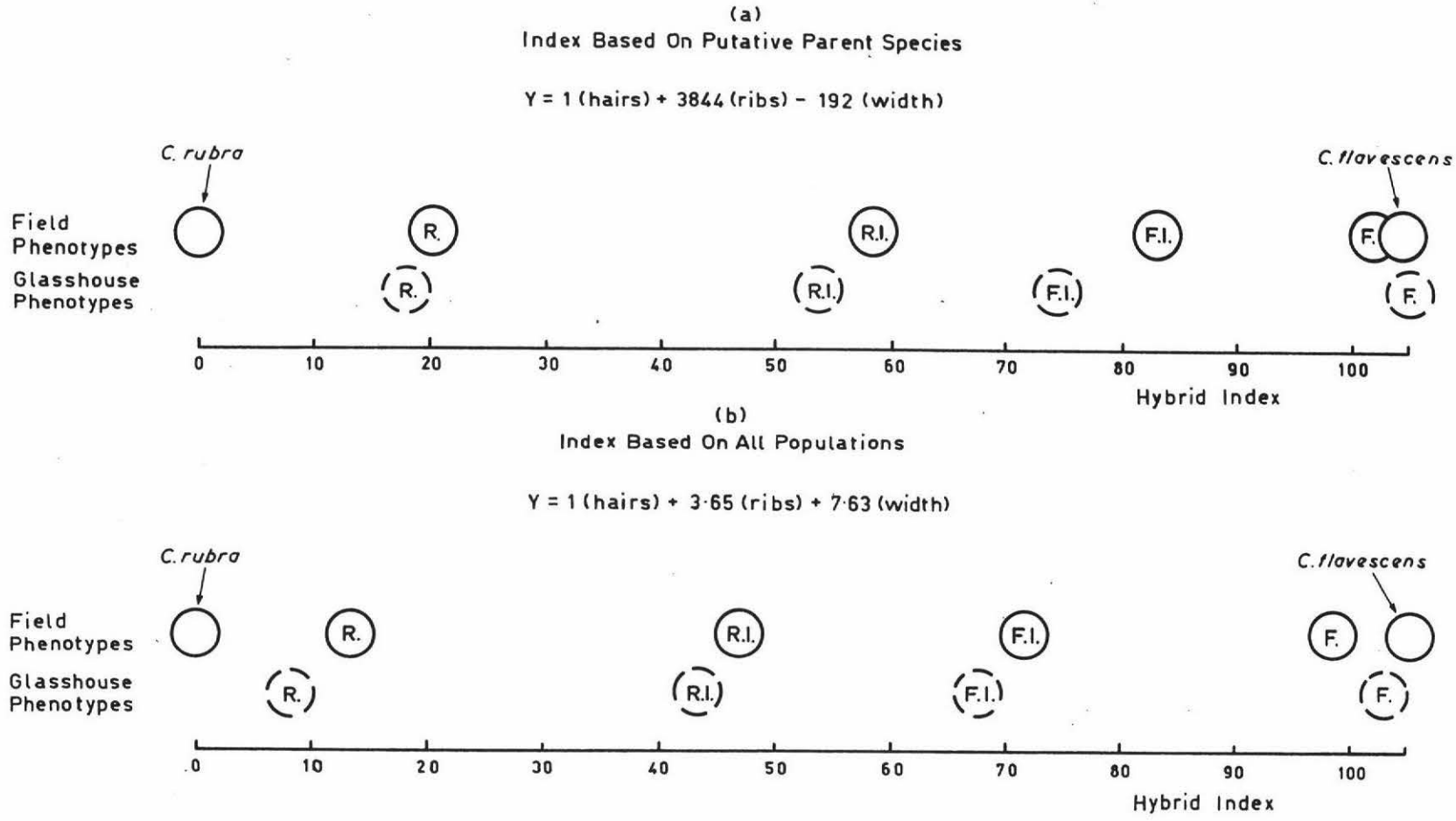


Fig. 14. Ten Chionochloa populations compared using hybrid indices constructed from three leaf variables. For explanation of symbols used, see text (p. 32).

B.11.2. Recombination Spindle

The relationship among population means, and the distribution of individuals about their respective means are shown in fig. 15-20 inclusive.

Population means

The following can be deduced from fig. 15:

(a) The Kaiparoro field phenotypes fall between the conditions characterizing C. rubra and C. flavescens. Some deviation from the straight line joining the two species is evident and is due to the Kaiparoro plants being less hairy than the C. rubra population.

(b) The deviation of the Kaiparoro glasshouse phenotypes from their corresponding field phenotypes shows a progressive increase in genotypic plasticity as the C. flavescens extreme is approached. This is due mainly to an increase in hairiness and a decrease in leaf width.

Individual deviation about the mean

Fig. 16-20 inclusive show the following:

(a) The putative parent species are highly variable for leaf hairiness, C. flavescens in particular. They do not overlap for leaf width and number of ribs per leaf, however.

(b) The populations show a progressive decrease in variability as the "parental" conditions are approached. This supports the hypothesis that the Kaiparoro plants are hybrids (see B.3.3.).

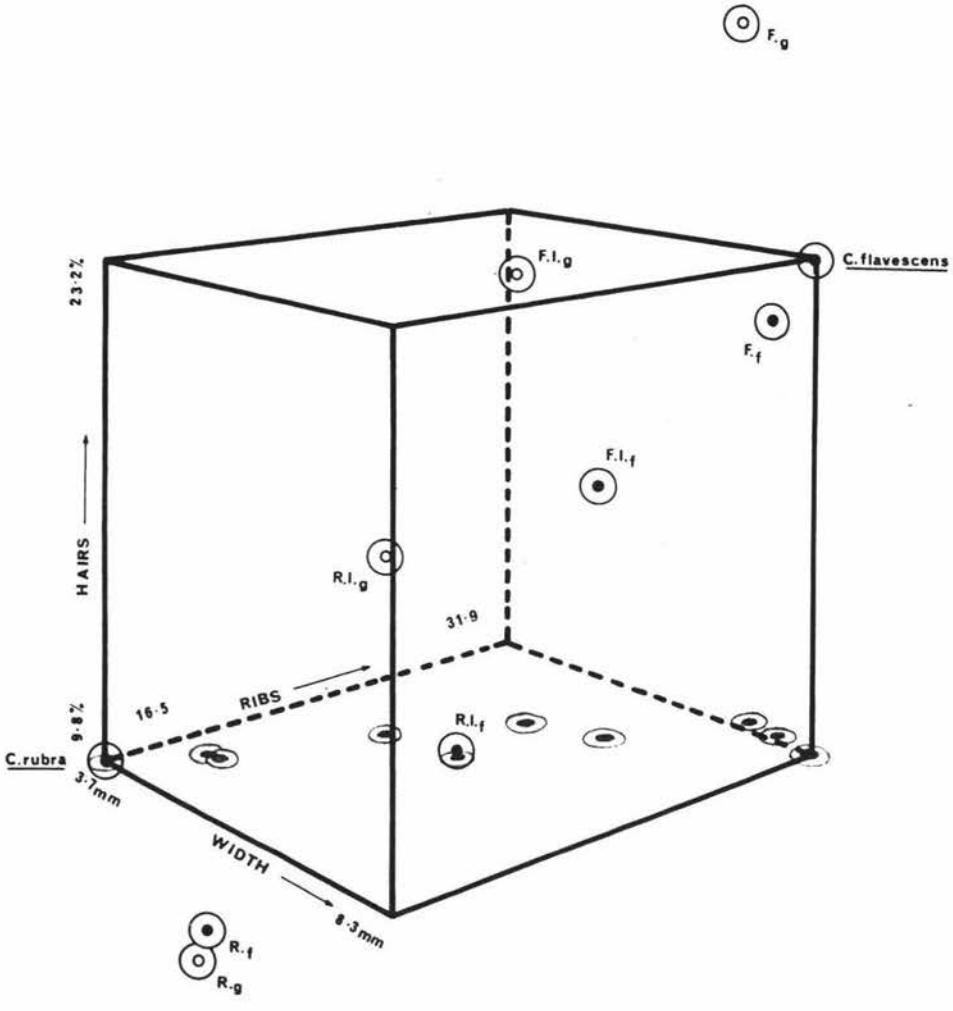


Fig. 15. The means of 10 Chionochloa populations compared for three leaf characters. "Shadows" on the base of the cube are vertical projections from the 10 points. Subscript f = field phenotypes and subscript g = glasshouse phenotypes. For explanation of other symbols used, see text (p. 32).

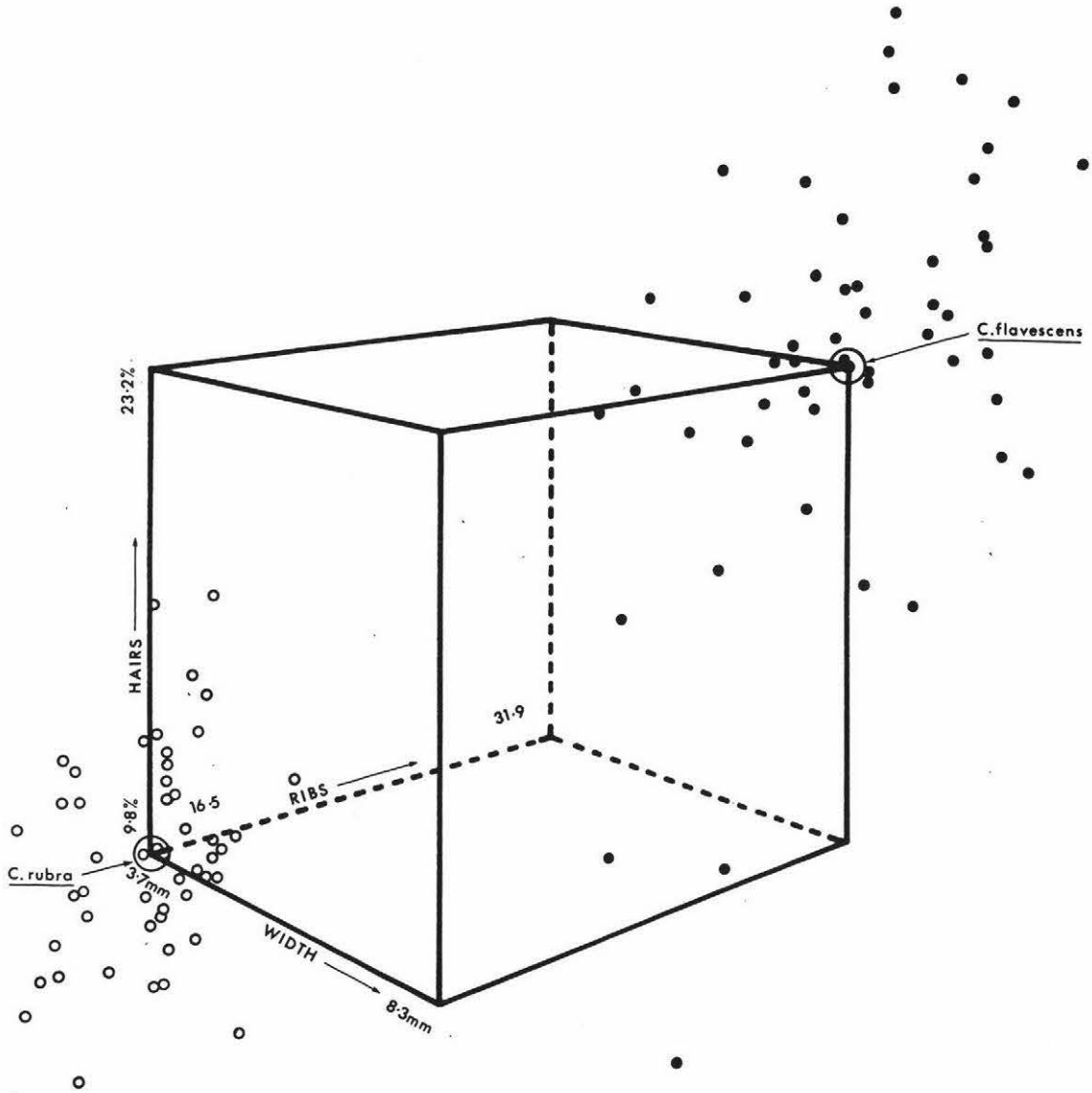


Fig. 16. Distribution of individuals about the means of the C. rubra and C. flavescens populations sampled from their respective type localities.

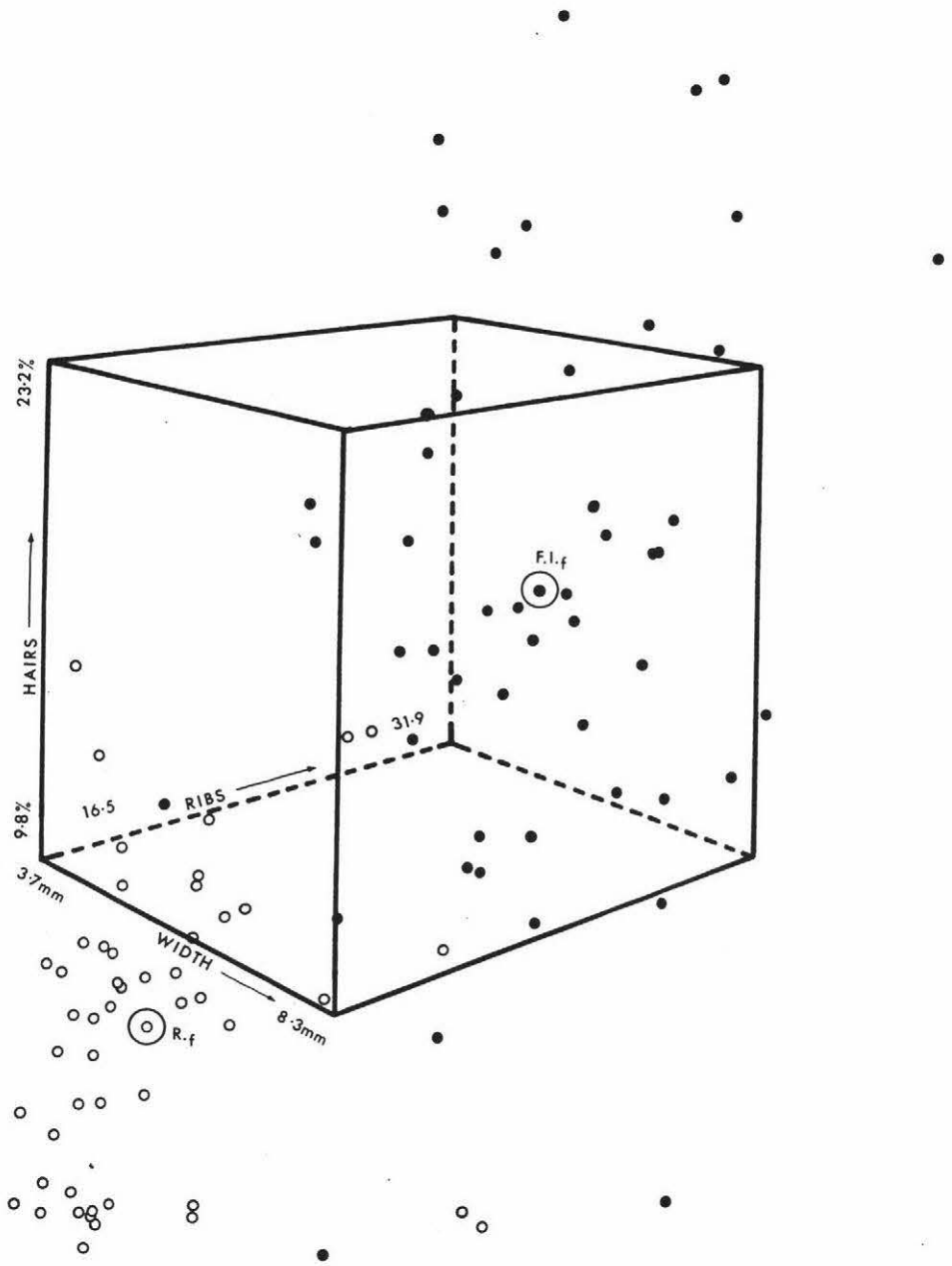


Fig. 17. Distribution of individuals about the means of the Kaiparoro Chionochloa populations R. and F.I. (field phenotypes). For explanation of symbols used, see text (p. 32).

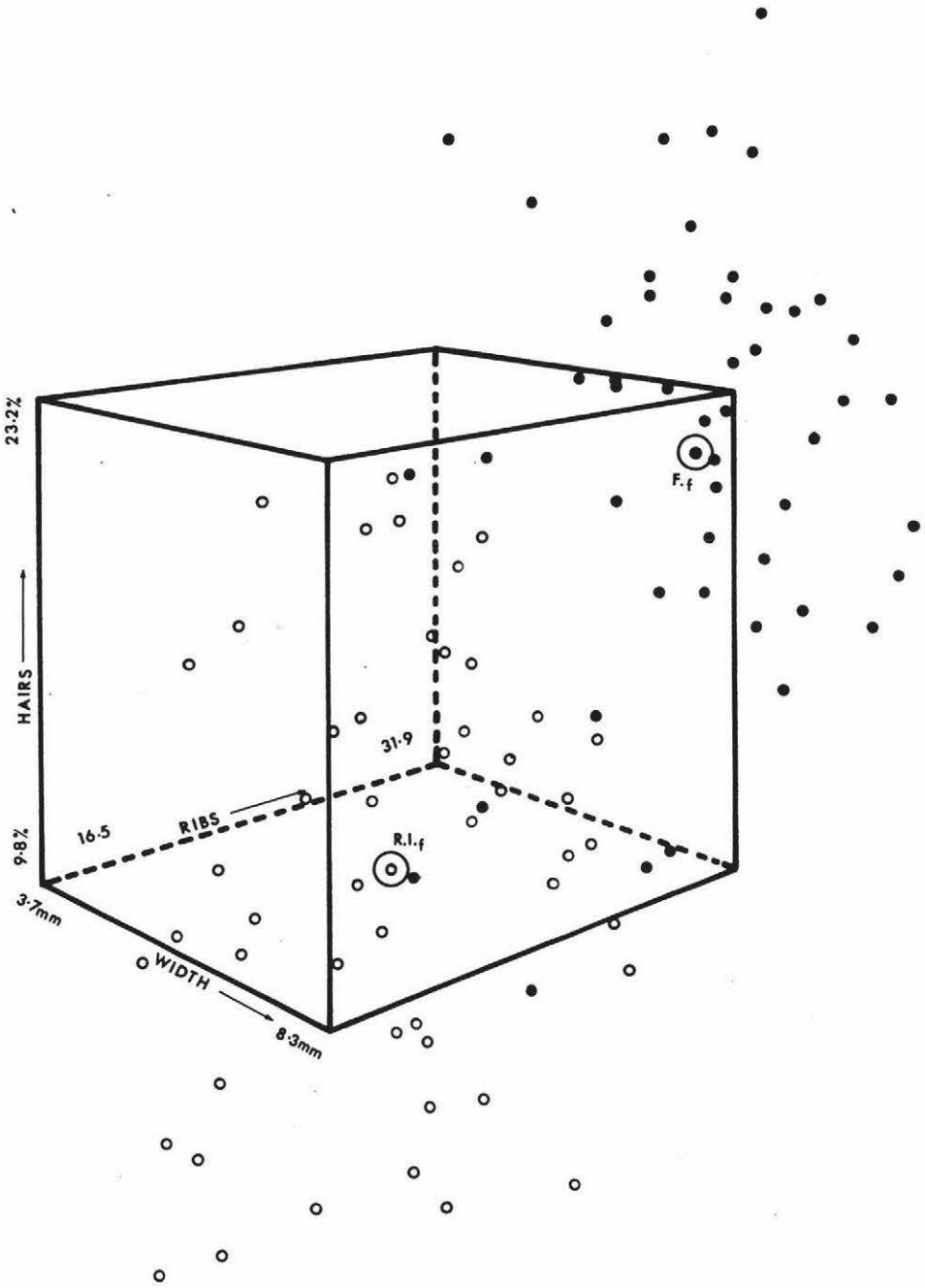


Fig. 18. Distribution of individuals about the means of the Kaiparoro Chionochloa populations R.I. and F. (field phenotypes). For explanation of symbols used, see text (p. 32).

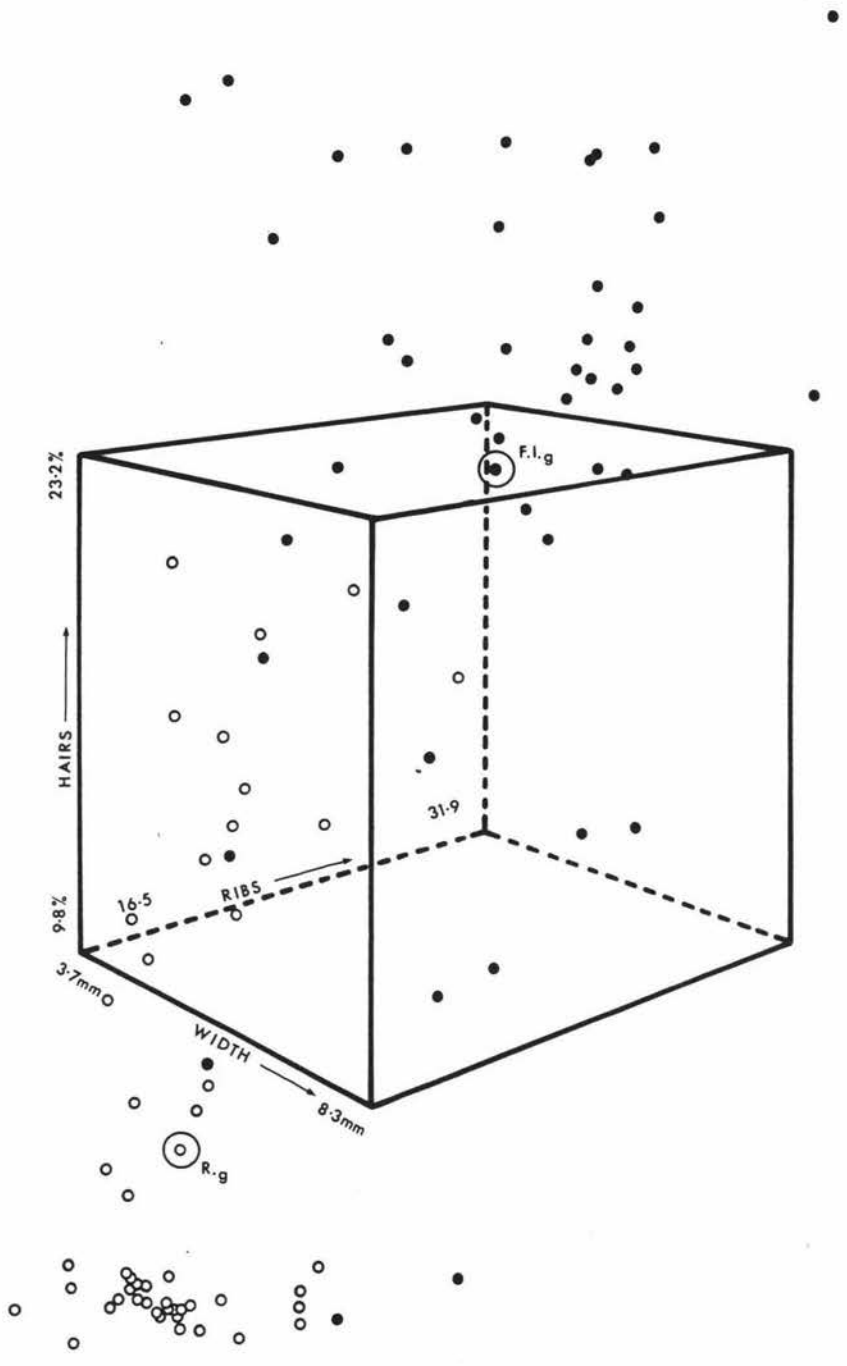


Fig. 19. Distribution of individuals about the means of the Kaiparoro Chionochloa populations R. and F.I. (glasshouse phenotypes). For explanation of symbols used, see text (p. 32).

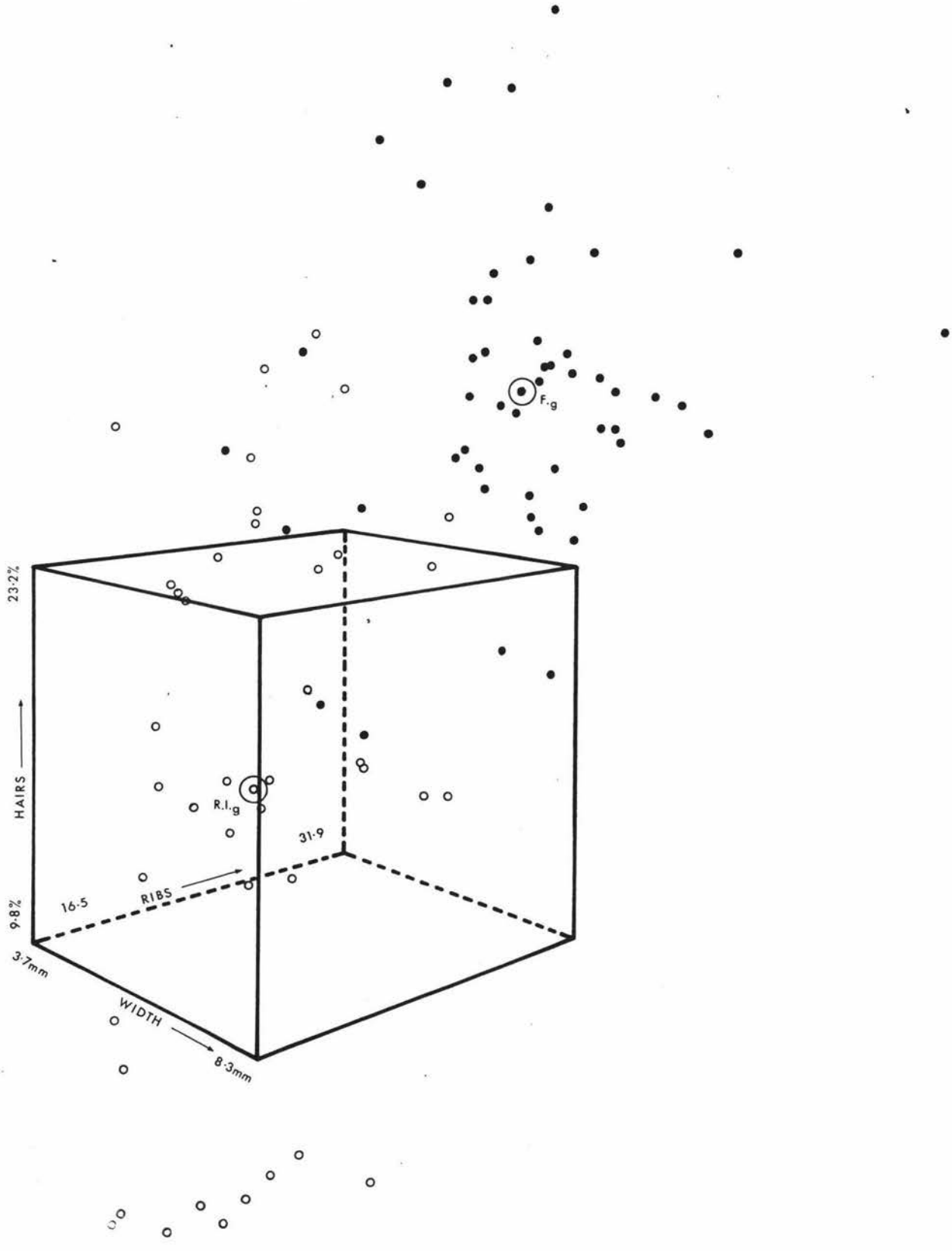


Fig. 20. Distribution of individuals about the means of the Kaiparoro Chionochloa populations R.I. and F. (glasshouse phenotypes). For explanation of symbols used, see text (p. 32).

Table 6

GENETICAL, PHENOTYPIC, AND ENVIRONMENTAL CORRELATIONS AMONG THREE LEAF CHARACTERS FOR TEN CHIONOCHLOA POPULATIONS

POPULATION		PHENOTYPIC CORRELATION*	P*	ENVIRONMENTAL CORRELATION	P*	GENETICAL CORRELATION	P*
1 F.I. (Glasshouse Phenotypes)	r_{HR}	+.243	-	+.191	-	+.252	-
	r_{HW}	+.178	-	+.372	<.05	+.130	-
	r_{RW}	+.839	<.01	+.525	<.01	+.910	.010
2 R.I. (Glasshouse Phenotypes)	r_{HR}	+.223	-	+.039	-	+.257	-
	r_{HW}	+.113	-	+.135	-	+.123	-
	r_{RW}	+.675	<.01	+.773	<.01	+.647	.073
3 F. (Glasshouse Phenotypes)	r_{HR}	+.167	-	+.114	-	+.201	-
	r_{HW}	+.172	-	+.093	-	+.207	-
	r_{RW}	+.419	<.01	+.670	<.01	+.360	-
4 R. (Glasshouse Phenotypes)	r_{HR}	+.434	<.01	+.111	-	+.598	-
	r_{HW}	+.433	<.01	-.060	-	+.739	.063
	r_{RW}	+.747	<.01	+.604	<.01	+.821	-
5 F.I. (Field Phenotypes)	r_{HR}	+.233	-	+.229	-	+.239	-
	r_{HW}	+.167	-	+.192	-	+.164	-
	r_{RW}	+.742	<.01	+.706	<.01	+.755	-
6 R.I. (Field Phenotypes)	r_{HR}	+.112	-	+.067	-	+.125	-
	r_{HW}	+.091	-	+.010	-	+.133	-
	r_{RW}	+.755	<.01	+.640	<.01	+.833	-
7 F. (Field Phenotypes)	r_{HR}	+.103	-	-.310	<.05	+.193	-
	r_{HW}	+.238	-	-.360	<.01	+.421	-
	r_{RW}	+.703	<.01	+.450	<.01	+.788	.056
8 R. (Field Phenotypes)	r_{HR}	+.179	-	+.025	-	+.158	-
	r_{HW}	+.234	-	+.032	-	+.281	-
	r_{RW}	+.857	<.01	+.419	<.01	+.941	<.005
9 C. rubra (Field Phenotypes)	r_{HR}	+.347	<.05	-.016	-	+.518	.089
	r_{HW}	+.236	-	-.011	-	+.331	-
	r_{RW}	+.697	<.01	+.550	<.01	+.757	-
10 C. flavescens (Field Phenotypes)	r_{HR}	+.394	<.01	-.095	-	+.508	-
	r_{HW}	+.539	<.01	+.139	-	+.640	.027
	r_{RW}	+.741	<.01	+.335	<.05	+.810	<.005

* See text.

H = hairs
R = ribs
W = width

(c) Many individuals, and in some cases population means, fall outside the "cube of expectations". This is an understandable deviation from Anderson's hypothetical example (Anderson, 1949), where the hybridizing species were represented by a single point. Where the mean is taken to represent the single point, individuals lying outside the cube can be expected to produce offspring also outside the cube.

(d) The pattern of distribution of individuals about their respective means is similar for all populations. This shows the three characters are related in the same manner for the Kaiparoro populations as they are in the putative parent species populations.

The character association within populations was also examined. Genotypic, environmental, and "phenotypic" correlations were calculated for each population in turn (table 6):

(a) Environmental correlations were calculated from the ramets-within-genotype, mean squares and cross products from the hierarchal analysis of variance (table 3). The probabilities were taken from table 7.6.1. given by Snedecor (1956) for the degrees of freedom shown in table 3.

(b) Because the "phenotypic" correlations were calculated from the genotypes-within-benches (or genotypes) mean squares and cross products, they include more than just the additive effects of the environmental and genotypic components of variation. The genotype component includes a factor of two. Probabilities shown were derived in the same way as in (a).

(c) Genotypic correlations were calculated from the genotype component of variance given in table 3. To avoid the difficulties inherent in determining the appropriate degrees of freedom, the genetical correlation coefficients were tested by difference. The "phenotypic" and environmental correlation coefficients were compared using Snedecor's (1956) z values to decide if they could be regarded as significantly different. Where a difference was shown to exist it was concluded that there must be a significant contribution by a genotypic correlation. The appropriate P values from the "t" test are shown in table 6.

In all cases, only P values at the 10% level or less are shown.

All the significant correlations proved to be positive except in the case of the environmental correlation in population 7. In general the correlation between the number of ribs per leaf and leaf width was the highest. This may be due, to an unknown extent, to developmental correlation.

The correlations between hairs and ribs (r_{HR}) and hairs and width (r_{HW}) are mainly non-significant. The weak correlation shown in the putative parents limits the conclusions that can be drawn from the association of leaf hairs with the other two characters, in the Kaiparoro populations. The use of character association as a criterion of hybridity is dependent on the presence of character association in the parent species.

C O N C L U S I O N S

Distribution of Population Means

The Kaiparoro sample populations range from conditions typical of C. rubra to those typical of C. flavescens. This conclusion is based on observations made in three ways:

(a) by comparing the population means for each of three leaf characters separately (fig. 11-13 inclusive),

(b) by comparing the population means and distribution of individuals about the means for three leaf characters simultaneously (fig. 15-20 inclusive),

(c) by constructing a hybrid index from the additive effects of three weighted leaf characters (fig. 14).

Nature of the Phenotypic Variation

Differences among the Kaiparoro populations are mainly under genetical control. The transplant experiment shows that population differences persist when the plants are grown in the same environment. There is some degree of genetical plasticity, however, and this increases towards the C. flavescens end of the variational scale. The evidence is consistent whether the variational pattern is examined for each character separately (fig. 11-13 inclusive), for the three characters simultaneously (fig. 15), or for a composite hybrid index (fig. 14). Statistical examination of the variation within each

population shows a considerable environmental influence but, in most cases, this is less than the genetical contribution to phenotypic variation.

Reasons for the Existing Pattern of Variation

The existence of a relatively small and variable population of plants ranging in characteristics between two well defined species suggested the hypothesis that they were hybrids between the two species. Detailed examination of the variational pattern of the Kaiparoro plants confirms this hypothesis. The relationships between the Kaiparoro Chionochloa populations and C. rubra and C. flavescens are those expected of hybrids.

The character distribution in the Kaiparoro populations shows that the variation among the individuals lessens as parental combinations are approached. The intermediacy of leaf width and number of ribs per leaf is correlated, although this may be due in part to developmental correlation. These criteria, together with the intermediate distribution of the population means, indicate the occurrence of hybridization.

Conclusions drawn from the detailed analyses of three leaf characters are supported by the subjective, and therefore widely based, estimates formed during the course of the study.

SECTION C

THE SOIL ENVIRONMENT

INTRODUCTION

C.1. CHARACTERIZING THE SOIL ENVIRONMENT

Plants react to all factors of the environment, but often one factor exerts a controlling influence through its excess or deficiency. Visual appraisal suggested that the distribution of the Chionochloa variants on Kaiparoro is influenced by soil water content. The most striking feature of Kaiparoro soils is their wetness. Environmental variables related to water excess are likely, therefore, to exert a major influence on plant growth. One of the most common effects of excess soil water on plant growth is restriction of the supply of oxygen to the plant root zone. Soil aeration was measured as it was considered to be the most likely factor controlling the Chionochloa distribution. Soil water content was also determined for comparative purposes.

C.2. TECHNIQUES FOR MEASURING SOIL AERATION

Wiegand and Lemon (1958) stated that the concentration of oxygen at the root surface increases linearly with the logarithm of soil moisture tension. While this may apply to any one soil, the relationship does not hold under all conditions and it is not possible to estimate soil aeration levels from the moisture content. Poel (1960b) provided an example where relatively high levels of aeration

prevailed in the waterlogged soil of hillside flushes.

Several techniques are available for the direct measurement of soil aeration but they do not have equal significance for plant growth. The partial pressure of oxygen in the soil has been measured either by extracting a sample of the soil atmosphere and testing with an indicator, or less laboriously by using a membrane covered electrode (Willey and Tanner, 1963). These methods estimate a soil oxygen "capacity" characteristic. Cannon and Free (1925), and Hutchins (1926) emphasized that the "rate" of oxygen supply through the soil to the roots is of greater importance to plant growth. Hutchins used an absorber buried in the soil to measure the rate of oxygen diffusion. A similar technique was used by Raney (1949). Russell (1952) stated that evaluation of conditions at the interface between the root and soil system offers the greatest possibility of establishing the influence of soil aeration on plant growth. This interface was thought to be bounded by the root cell walls, and by water films surrounding the roots. As the diffusion coefficient of oxygen in solution is 10,000 times slower than in the gaseous phase, Stolzy and Letey (1964b) maintained the limiting factor for oxygen supply is likely to be diffusion through this moisture film. A technique taking these concepts into account was first successfully adapted for *in situ* soil studies by Lemon and Erickson (1952). Polarographic determinations of oxygen diffusion rates are made by measuring the rate of oxygen diffusion to a small platinum wire electrode inserted in the soil. The values obtained include the effect of the diffusion coefficient of oxygen in water, the concentration of oxygen in solution, the

thickness of the water film around the electrode, the porosity of the soil around the electrode, and the tortuous path encountered by the oxygen molecules as they diffuse to the electrode surface. These factors would also influence the rate of oxygen movement to a root.

C.3. POLAROGRAPHIC TECHNIQUE

C.3.1. Principles of Polarography

When a certain electrical potential is applied between a platinum electrode and an unpolarizable electrode, both inserted into the soil, oxygen is reduced at the platinum electrode surface. The electrical current flowing between the two electrodes is proportional to the rate of oxygen reduction which is in turn related to the rate of oxygen diffusion to the electrode. The oxygen diffusion rate (O.D.R.) can therefore be calculated from the measured electrical current.

The basic principles of this technique, long used for both qualitative and quantitative chemical analysis (Kolthoff and Lingane, 1952), are dependent on the unique properties of the current-voltage (c.v.) curve (fig. 21). As the voltage difference between the electrodes begins to increase, only an exceedingly small current due to ion transfer, the "residual current", flows. When the potential is great enough to dissociate the oxygen molecules the current rises sharply. The current from this reaction, the "diffusion current",

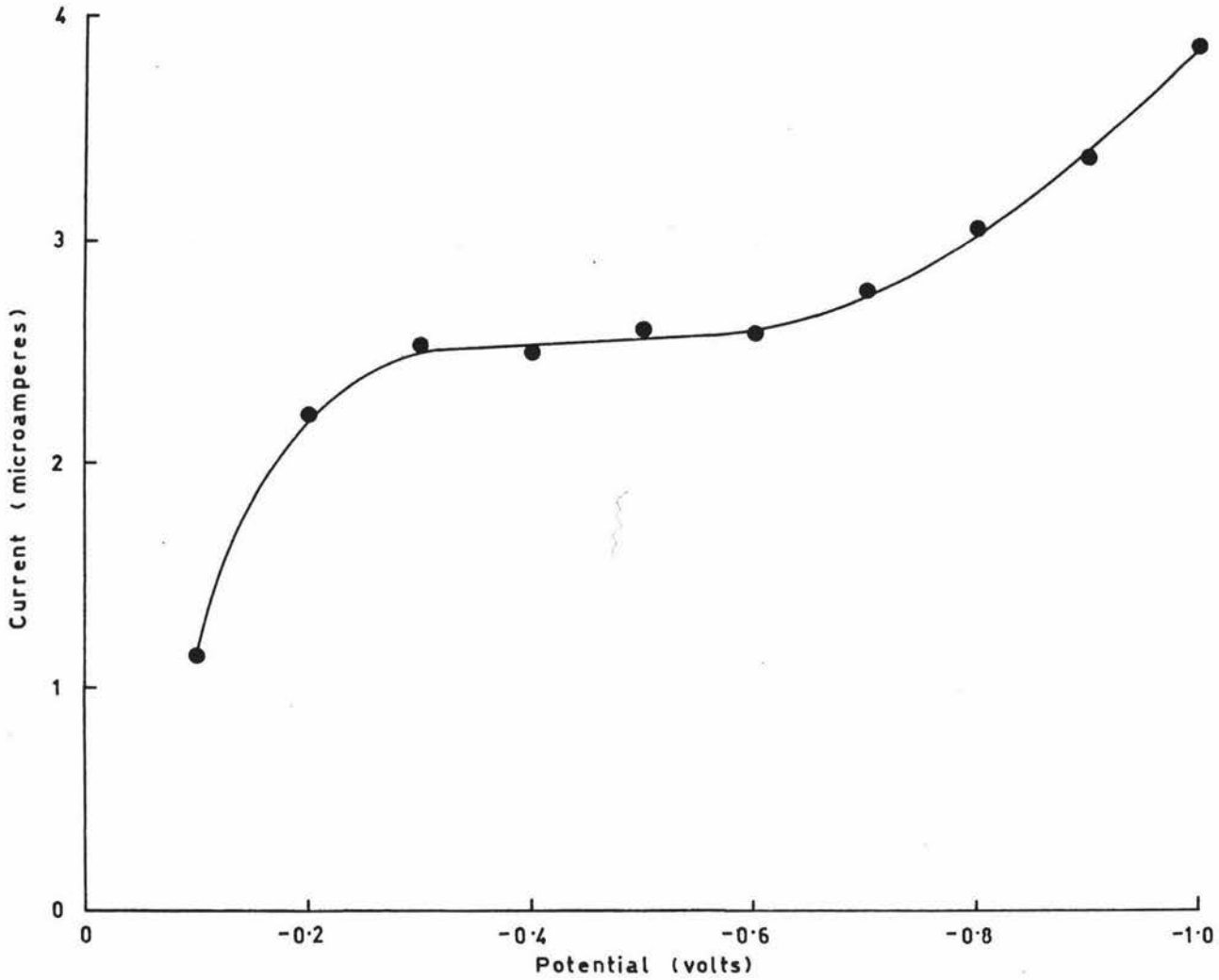


Fig. 21. Typical current-voltage curve with a diffusion-limited "plateau" region between the dissociation curves of oxygen and hydrogen. Each point is the average of four readings determined in a soil-water suspension.

gradually approaches a limiting value which is determined, in a static system, by the rate of oxygen diffusion to the reducing electrode. All electro-reducable and electro-oxidizable substances have characteristic dissociation voltages. Hydrogen dissociates at a greater potential difference than oxygen and accounts for the rise in current beyond the "plateau" region of the c.v. curve. The voltage can be held with least current error in the "plateau" region. Here the current is almost entirely limited by the rate at which oxygen can diffuse to the reducing electrode surface so the calculation of oxygen movement becomes a problem in classical diffusion theory. The presence of "indifferent salts" which can carry a current without participating in the electrode reaction can alter the situation (Kolthoff and Lingane, 1952). This is however, thought to be insignificant in soils. Likewise "residual currents" are too small to require correction. The total current flowing can therefore be considered as the "diffusion current" (Lemon and Erickson, 1955).

The equation relating current flow to oxygen diffusion is derived from Fick's diffusion theory when applied to a hypothetical cylindrical root surrounded by a coaxial film of water (Lemon and Erickson, 1952):

$$i_t = n\ F\ A\ f_{x=0,t} \qquad \text{(1)}$$

where i_t = current in amperes at time (t) in seconds

n = number of electrons used per molecule of oxygen electrolysed (generally believed to equal 4)

F = the Faraday (96,500 coulombs)

A = area of the electrode in square centimeters

$f_{x=0,t}$ = flux at the electrode surface (x distance from the electrode surface is equal to zero), at time (t): or the number of moles of oxygen diffusing to the electrode at time (t)

The O.D.R. is calculated by

$$O.D.R. = \frac{i \times 10^{-6} \times 60 \times 32 \times 10^{8}}{4 \times 96{,}500 \times A} \times 10^{-8} \text{ g cm}^{-2}\text{min}^{-1}$$

The factors 60, and 32×10^{8} are included for the expression of results in minutes and micrograms rather than seconds and moles. The O.D.R. is therefore calculated by dividing the current by the electrode area and multiplying by a constant.

C.3.2. Application of Polarography to Soil Studies

Application of polarographic analysis to *in situ* soil studies has required certain modifications of the technique developed for chemical analysis under controlled conditions. Results and their interpretation vary with the techniques employed, consequently standardization of methods is most important. Letey, Stolzy and co-workers have contributed greatly to this end (Letey and Stolzy, 1964; Birkle *et al.*, 1964; Stolzy and Letey, 1964a).

Electrodes

The indicator or polarizable electrode consists of a small platinum wire mounted on the end of an insulated probe.

The area of the exposed reducing surface must be accurately determined and remain constant.

Electrode length was discussed by Poel (1960a) who found that with short wires the current density was sufficiently high to render end-effects significant. Lengths over 2 mm were considered to be satisfactory but the most commonly used lengths are 4 and 6 mm.

Electrode radius affects results in such a way that its influence cannot be easily removed. Letey and Stolzy (1964) examined a model system where the effects of inserting electrodes of varying radii in a hypothetical cylinder of water were calculated. Eighteen, 22, and 25 gauge wire (0.102 cm, 0.064 cm, and 0.046 cm diameter respectively) gave increasing O.D.R. values and differences were greater in wetter conditions. Comparable calculations by Kristensen and Lemon (1964) confirmed this. They compared critical diffusion path length with root radius and found the finer the roots the better they were adapted to lower aeration conditions.

Radius effects have further implications in relation to soil moisture content. Theoretical considerations suggest that O.D.R. is expected to increase as soil moisture decreases. Measurements taken as a function of soil moisture content showed this to be true only to a point, after which the O.D.R. decreased with further decrease in the

moisture content (Lemon and Erickson, 1955; Lemon and Kristensen, 1960). Birkle et al. (1964) compared 22 and 25 gauge electrodes and found the effect was more pronounced with the larger 22 gauge. It was concluded that the decrease in O.D.R. with decreasing soil water content was an artifact caused by disruption of the moisture films surrounding the electrode. This decreased the effective reducing area. Results indicated that hydroxyl ion build-up was not responsible.

Electrode strength is important for in situ measurements. The choice of wire involves a compromise between a large diameter for rigidity and small diameter for better simulation of the plant root. When large diameter wire is used (or is incorporated as part of a rugged tip such as that used by Wiersum, 1960), the existing solid-liquid-gas geometry is altered so that the measurement does not properly characterize the oxygen diffusion to a root growing in that environment. Although 25 gauge wire is most satisfactory in this respect, many workers have resorted to the use of 22 gauge wire for field experiments.

The non-polarizable reference electrodes most commonly used are calomel or silver-silver chloride, connected to the soil by a saturated potassium chloride bridge through a porous plug or cup. The electrode must be large enough to prevent polarization. Letey and Stolzy (1964) overcame the problem by rolling silver foil (total surface area 2,000 cm^2) inside a potassium chloride filled container.

Relative positioning of the platinum and reference electrodes in the soil is not critical. Birkle et al. (1964) found no distance

effect up to 2.5 metres.

Operating voltage

Lemon and Erickson (1955) and Poel (1960a) determined c.v. curves for soil-water suspensions and concluded that the voltage could be fixed with least current error on the flat portion of the curve at -0.8 volts. Birkle et al. (1964), however, found that in unsaturated soil a straight line relationship occurred up to -0.8 volts. Tackett and Pearson (1964) also found that the classical c.v. curve occurred only in saturated soil samples. Failure to observe a plateau for unsaturated soil measurements throws doubt on the current being diffusion controlled. Preliminary investigations by Birkle et al. (1964) indicate that the current is diffusion controlled under conditions of low O.D.R. but not under high O.D.R. These authors stated that any potential between -0.55 and -0.75 volts could be used, but because -0.75 volts is close to the level of departure from linearity, a safer value of -0.65 volts was recommended. They provide an approximate method for conversion of results taken at different voltages.

Establishing steady state

Completion of the electrode circuit results in a high initial current which decays exponentially as the oxygen in the immediate vicinity of the electrode is reduced. After three to five minutes a relatively steady diffusion gradient is established and it is the current flow under these conditions that should be measured. Experi-

mental results indicate that steady state conditions are reached more rapidly in wet soils than in dry soils (Lemon and Erickson, 1955; Birkle et al., 1964).

Temperature

O.D.R. measurement depends on such temperature-controlled factors as the solubility of oxygen, the diffusion coefficient, and the rate of reaction. Letey et al. (1962a) estimated an O.D.R. increase of 1.8% per degree centigrade rise in soil temperature, for a given oxygen concentration in the soil. Ingram (1964) found a 4.9% increase in current per degree rise in temperature, expressed as a percentage of the value at 25°C. He recommended correction of all values to such a standard temperature. For most purposes, however, it is desirable to include the temperature effect in O.D.R. values.

Sampling

Heterogeneity of the root environment, in both space and time, necessitates careful choice of sampling techniques.

Space sampling problems are intensified by the small volume of soil affected by the platinum electrode. Soil variation along a gradient requires suitable placement of the electrode. Stolzy et al. (1961) found O.D.R. usually decreased with increasing soil depth. As no single measurement can characterize the whole soil profile it is often necessary to compromise by measuring in the zone of greatest root density. Random variation due to micro-edaphic irregularities requires increased sampling. Letey and Stolzy (1964) built an oxygen

diffusion meter capable of using up to 10 electrodes simultaneously. This overcomes many of the problems related to sample number but interpretation of profile gradients in relation to root distribution and plant response is difficult.

Sampling in time requires knowledge of plant oxygen demand, soil oxygen supply, and their inter-relationships. Oxygen deficits of extremely short duration may have significant effects on plant growth and development. Erickson and Van Doren (1960) found a 24 hour deficit, just prior to flowering in peas, reduced yields by up to one third. Tomato plants were stunted by early periods of low oxygen availability, especially on bright days. Technical problems may prevent the use of continuous recording to detect the most critical periods of soil oxygen supply. Birkle et al. (1964) reported that electrode "poisoning" caused lowered O.D.R. values after electrodes had been in the soil for two weeks. It is not clear from their explanation, however, if the effect was due to the changing properties of the platinum surface or the geometry of the soil-water system surrounding the stationary electrode.

C.3.3. Validity of the Polarographic Technique

Theoretical considerations

Several workers have examined the validity of the cylindrical shell model used for the application of Fick's law of radial diffusion. The root, or analogous platinum electrode, is assumed for the purposes of the model, to be uniformly cylindrical with a coaxial cylinder of

water immediately outside it and with the soil atmosphere beyond. Covey and Lemon (1962) studied the situation in the transient state existing before a steady diffusion gradient had established. They found the model was not applicable to individual measurements but the characterization of a system by a large number of measurements did approach agreement with the model. This supported the conclusion that the phase geometry of the gas-liquid-solid system around an electrode or plant root is a complex configuration unique to the particular electrode or root position. Information from experiments on the oxygen requirement of roots was applied by Wiegand and Lemon (1958) in a theoretical approach used to determine critical oxygen concentration at the root surface. This work lends considerable support to empirically determined values of O.D.R. critical to root growth.

Lemon and Erickson (1955) and Birkle et al. (1964) measured current flow as a function of oxygen concentration in a soil suspension. A linear relationship indicated that no substance other than oxygen was reduced.

Correlation of O.D.R. and plant response

Both field measurements and results obtained under controlled conditions support the validity of O.D.R. as a measure of the soil aeration requirements of plants. Use of a wide variety of experimental techniques, plant materials, and criteria for assessing plant growth and development has, however, limited the conclusions that can be drawn from the literature.

Root growth is one plant function that has a critical O.D.R. Published results are summarized in fig. 22.

Top growth response to soil oxygen levels is most apparent in controlled studies with different degrees of soil aeration. Because of differences in O.D.R. in various parts of the root zone it is difficult to assess a single value as critical. Results indicate important reproducable effects however (fig. 22). Letey et al. (1965) pointed out that Williams (1964) used an 18 gauge electrode which in wet soils would give values 35% lower than for a 25 gauge electrode. Also in very dry soils, readings may be anomalously low due to incomplete wetting of the electrode surface. These factors were thought to contribute to the comparatively low figures given by Williamson. Production of dry matter and changes in height of plants are little affected by O.D.R. values greater than 40×10^{-8} g $cm^{-2} min^{-1}$.

Seedling emergence rate in wheat was reduced when the O.D.R. was less than 75×10^{-8} to 100×10^{-8} g $cm^{-2} min^{-1}$ (measured at -0.8 volts with an 18 gauge electrode). Total emergence was little affected however (Hanks and Thorpe, 1956). Erickson and Van Doren (1960) found O.D.R. up to similar levels reduced the percentage seedling emergence in sugar beet, potatoes, and peas.

Mineral accumulation in top growth is affected by soil aeration, varying with different ions and plants. Oxygen diffusion rates of less than 30×10^{-8} to 40×10^{-8} g $cm^{-2} min^{-1}$ reduced the concentration of the more important macronutrients and increased that of several other minerals (Stolzy and Letey, 1964b).

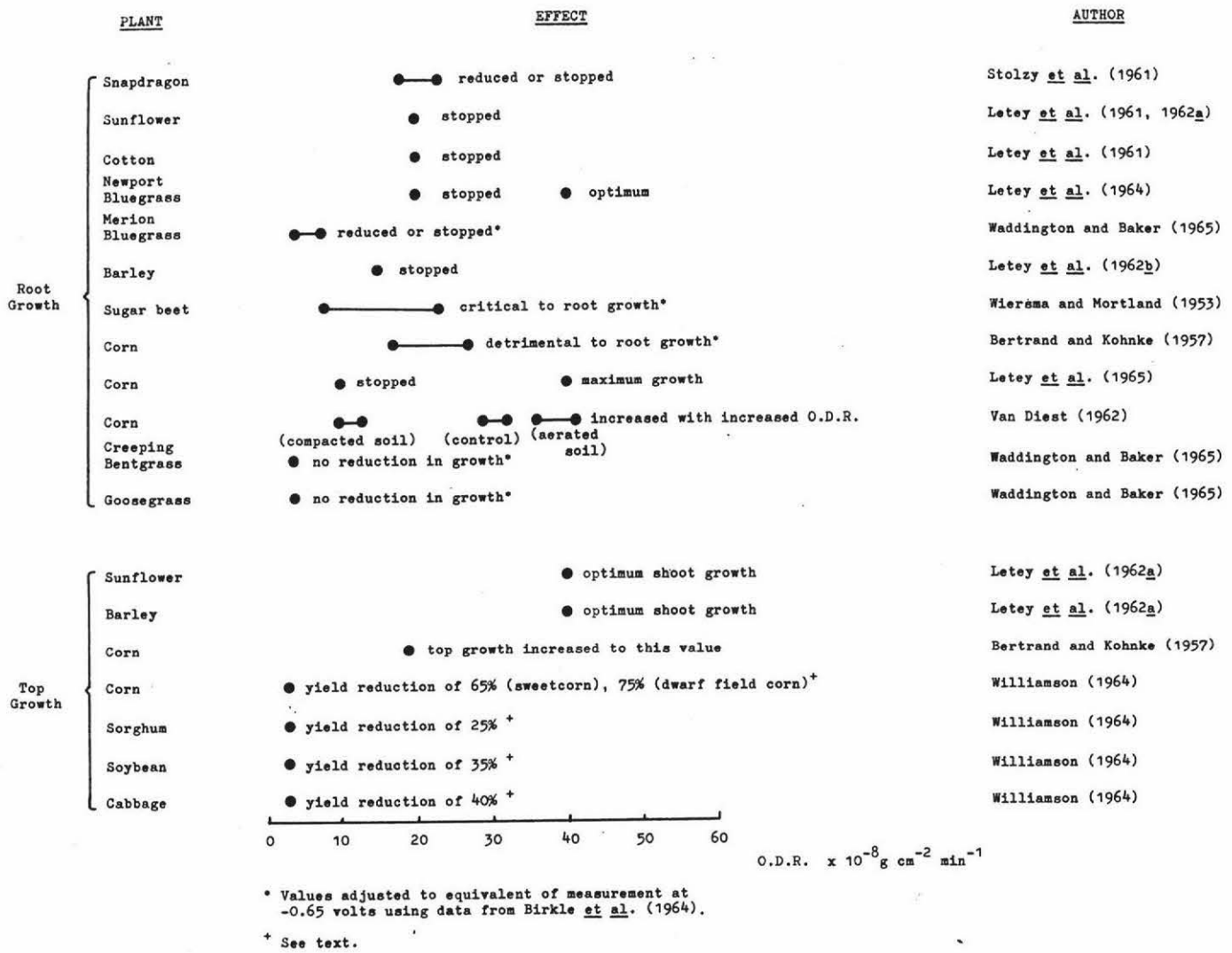


Fig. 22. Diagrammatic summary of published results relating root and top growth to soil oxygen diffusion rates.

Ethanol build-up in tomato xylem exhudate occurred when the soil O.D.R. fell below 38 x 10^{-8} (Fulton and Erickson, 1964). A small reduction in oxygen supply below this value was associated with a large increase in ethanol. The effect was thought to be detrimental to plant growth because of ethanol toxicity, and the reduced supply of energy available under anaerobic respiration.

Field correlation of O.D.R. with plant response has substantiated theoretical and experimental findings. Erickson and Van Doren (1960) varied oxygen availability in the field by varying tillage, compaction, and irrigation. Tomatoes showed response up to an O.D.R. of 40 x 10^{-8} g $cm^{-2}min^{-1}$ comparable with 44 x 10^{-8} to 52 x 10^{-8} g cm^{-2} min^{-1} obtained in glasshouse studies (measurements at -0.8 volts with a 25 gauge electrode). Poel measured oxygen diffusion rates in plant communities of some non-cultivated areas of the British Isles. In one area (Poel, 1960a) oxygen diffusion rates ranging from 25 x 10^{-8} to 5 x 10^{-8} g $cm^{-2}min^{-1}$ were measured through a range of plant communities: Pteridium sp. / Pteridium sp., Calluna sp. / Juncus acutiflorus, Carex flacca, Holcus lanatus / Juncus acutiflorus / Juncus conglomeratus (waterlogged). In a study of 13 plant communities in a hill grazing area (Poel, 1960b) an O.D.R. of 17 x 10^{-8} g $cm^{-2}min^{-1}$ was constantly associated with the occurrence of Molinia and Nardus. Poel (1961) studied the transition from Pteridium aquilinum requiring good drainage, to Juncus acutiflorus growing in wetter areas. Oxygen diffusion rates of 17 x 10^{-8} g $cm^{-2}min^{-1}$ and above were found in soils with Pteridium while Juncus grew in soils with O.D.R. less than 14 x 10^{-8} g $cm^{-2}min^{-1}$.

These results indicate that under wet conditions where diffusion rates are less than 20×10^{-8} g $cm^{-2} min^{-1}$ very narrow ranges of O.D.R. can determine the type of plant communities present.

MATERIALS AND METHODS

C.4. POLAROGRAPHY

C.4.1. Equipment

Oxygen diffusion meter

This meter maintains a given potential difference between a polarizable electrode and a non-polarizable electrode and measures the resulting current. A meter was constructed by the author using a circuit similar to that described by Ingram (1964) (fig. 23). The potential difference between the electrodes is determined using a null-point balancing system. This avoids the error inherent in the use of a sensitive voltmeter giving absolute readings. Where there is no "plateau" on the c.v. curve, accurate voltage settings are particularly important because a small shift in voltage can give a relatively large current error. The meter and electrodes are shown in fig. 24.

Electrodes

A strong, yet slender, electrode was constructed from a 21 cm length of 0.48 cm diameter brass rod which had platinum wire soldered in one end and an electrical socket on the other. The brass rod was insulated by sheathing it in P.V.C. tubing and sealing both ends with "Araldite" epoxy resin. A brass tube (0.95 cm diameter)

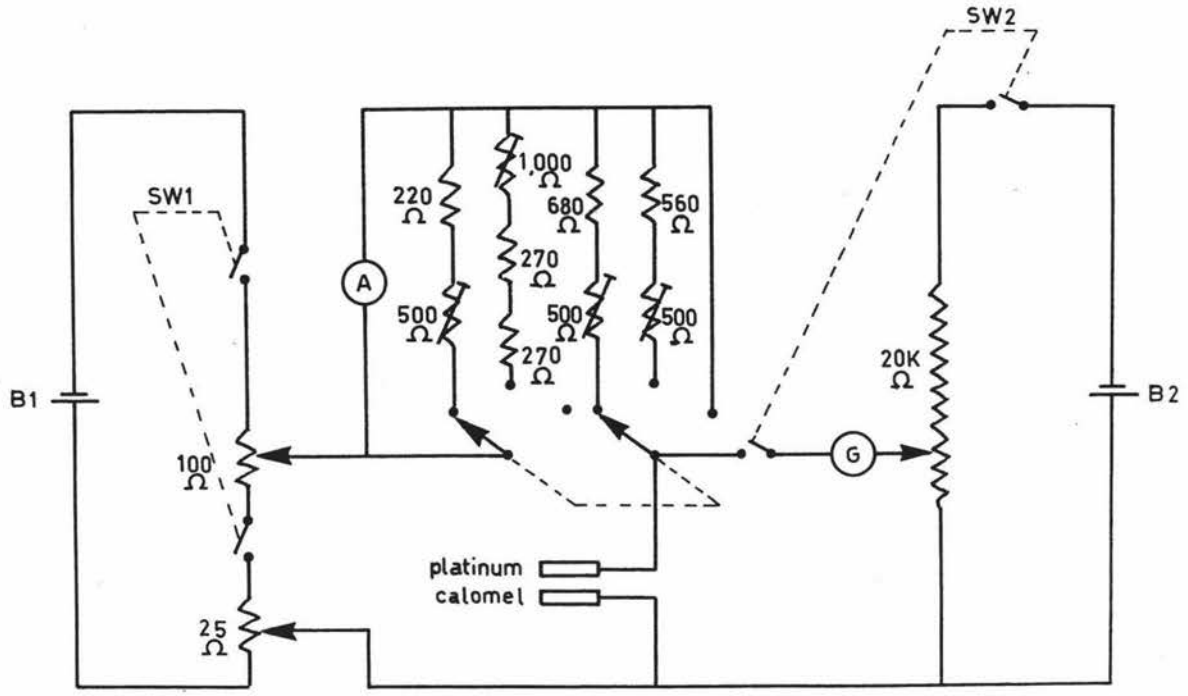


Fig. 23. Circuit diagram of oxygen diffusion meter. Operating voltage for the electrode circuit is provided by the dry cell B1 (Eveready no. 6, 1.5 volt) and regulated by 100Ω and 25Ω wire-wound variable resistors giving coarse and fine adjustments respectively. Current flow is measured by a 0-25 microammeter (1,250Ω) and a balanced-resistance shunt system which is controlled by a two pole - three position rotary switch to give x1, x2, and x4 ranges. A double pole - double throw switch controls the main circuit (SW1). The protective shunts shorting both meters when this switch is in the "off" position are omitted from the diagram for clarity. Electrode potential is determined using a null-point balancing system under the control of a key switch (SW2). A Weston standard cell (B2) provides a known voltage (1.0183 volts at 20°C) which is divided by an accurate 20KΩ wire-wound resistor. The electrode circuit is balanced against the known potential difference of the standard cell circuit using a 30-0-30 microammeter (G) to find the null-point.

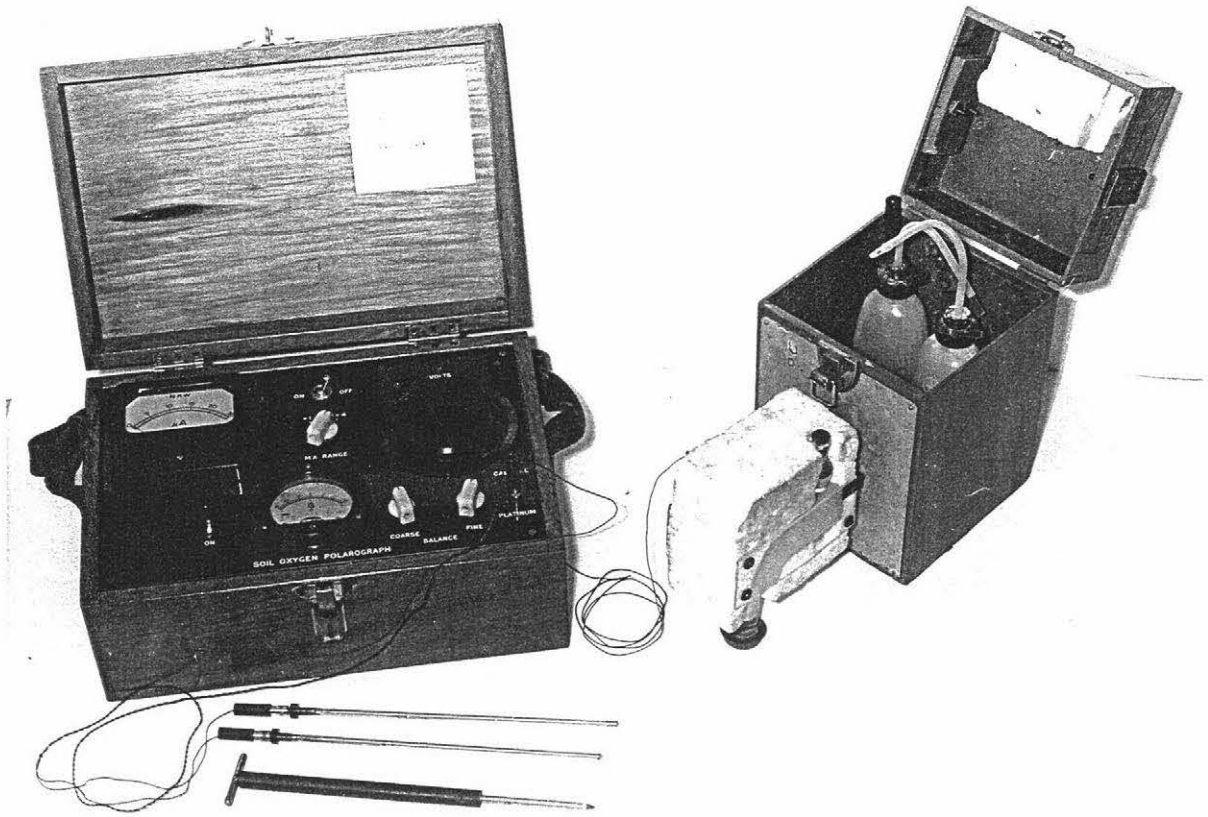
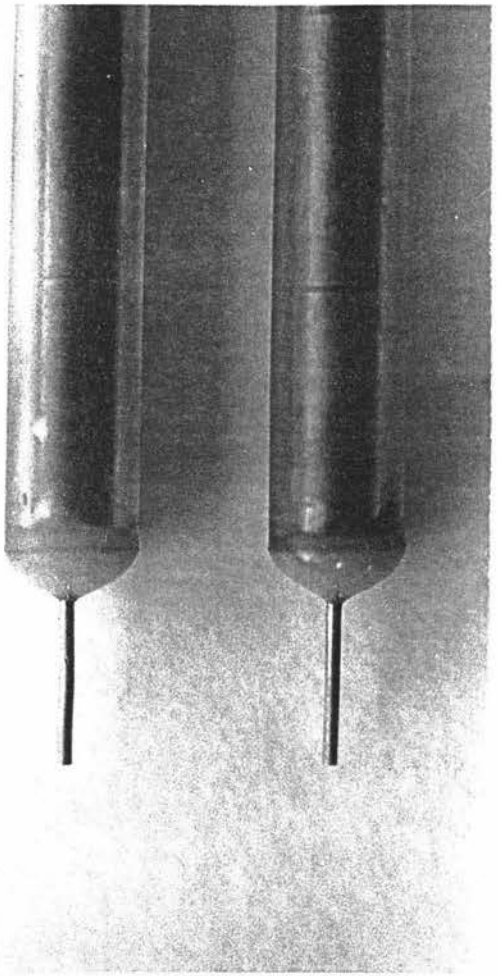


Fig. 24. Equipment used for measuring oxygen diffusion rates. Visible on the meter are the microammeter (left rear) and range switch (centre) used for measuring the diffusion current. Input voltage is varied by means of coarse and fine adjusting knobs (right front) and the null-point balance against the standard cell is determined using the galvanometer (centre front). The standard-circuit voltage is controlled by an accurate resistor which is pre-set according to a calibrated scale (right rear). A toggle switch (centre rear) controls the main circuit and a key switch (front left) the standard circuit. The calomel cell is mounted in a protective block of polystyrene and can be attached to the side of the carrying case by a pot magnet. The two platinum electrodes and the probe can be seen at the front.

(a)



(b)

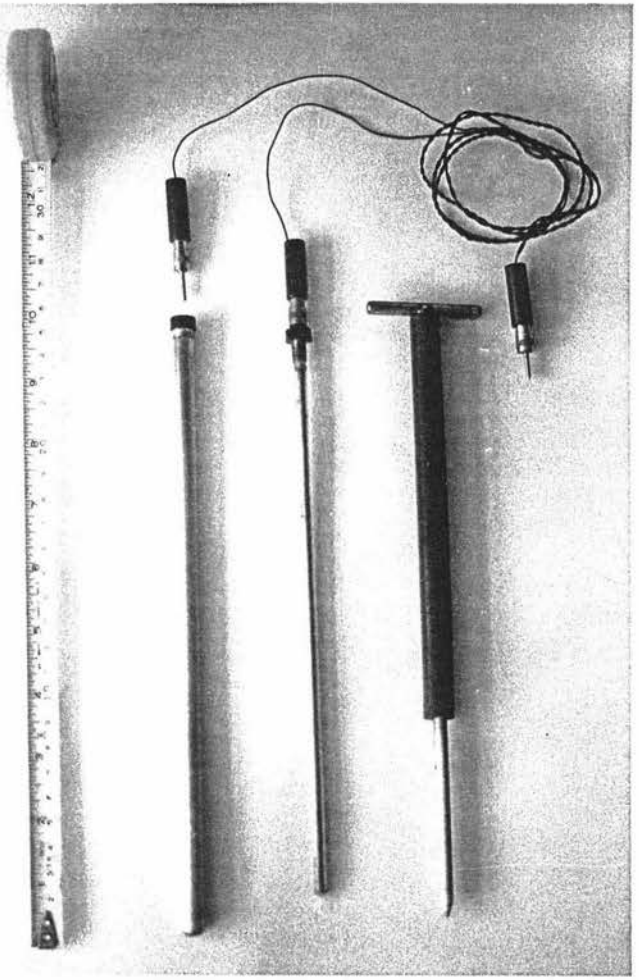


Fig. 25. (a) Close-up view of platinum electrodes. Note the well-defined junction between the "Araldite" seal and the platinum wire. This ensures that the area of exposed platinum wire can be readily determined and remains constant throughout use. (Length of wire is 6 cm.) (b) Two electrodes, one screwed in its carrying case, can be seen together with the plugs which make electrical contact at the top. The probe has a spacing tube to ensure penetration to the required depth.

sealed at one end and threaded at the other provided a protective case. The electrode wire was exposed for 6 mm of its length (fig. 25). Standardization on 25 gauge wire (0.045 cm diam.), or 22 gauge wire (0.064 cm diam.) which is more robust but has less suitable characteristics, was recommended by Stolzy and Letey (1964). Twenty-three gauge wire (0.057 cm diam.) was used, however, as it was the only size available. Some authors have referred to gauge size without quoting the diameter and this can lead to confusion. The gauges quoted above are based on the "Brown and Sharpe" standard.

A large calomel electrode was mounted in a polystyrene block for protection, and could be secured to the side of the carrying case with a pot-magnet during use.

C.4.2. Procedure

A dummy probe was inserted to a depth of 8 cm in the soil, then replaced by the platinum electrode which was inserted so that the exposed wire penetrated into relatively undisturbed soil. A modification was adopted to enable two electrodes, wired in parallel, to give simultaneous readings. The average of the additive readings then gave a sample less affected by micro-edaphic variability. Contact between the potassium chloride bridge of the calomel electrode and the soil was established by positioning the porous block firmly against the soil surface. Where the soil surface was dry the contact area was moistened with distilled water. The current flow was recorded five minutes after establishing the potential difference of -0.65 volts between the electrodes.

Ten positions on each of the four Kaiparoro plots were located by a systematic-random sampling technique similar to that used for the plant sampling. O.D.R. measurements were taken on three occasions from these marked positions.

11.5.65 Using one platinum electrode two readings were taken at each of five locations in each plot (40 readings representing 40 electrode positions).

7.9.65 and 14.2.66 Using two platinum electrodes coupled in parallel one reading was taken from each of 10 locations in each plot (40 readings, each one representing a total of two electrode positions).

Measurement of current-voltage curves

Current was measured as a function of voltage for tap water, soil-water suspensions, and soil *in situ*. The suspensions were made by first sieving the soil then mixing it thoroughly with tap water. The mixtures used were 25%, 50%, and 75% soil (by volume) and also field capacity. Where necessary, glass baffles were used to minimise movement of the beaker contents. Electrodes were inserted to 5 cm depth. Current flow was read after one minute and an interval of one minute allowed to elapse before the meter was switched on for the next reading. After one run of readings for potential differences from -0.1 to -1.0 volts, the suspension was restirred and the next run taken changing the voltage in the opposite direction. *In situ* measurements were taken in a similar manner but the electrodes were reinserted for each run.

In all cases four runs were taken using two electrodes wired in parallel.

C.5. SOIL WATER CONTENT

Soil water content was determined by calculating the loss of water from samples dried in an oven at 105°C. Initially, cores of a known volume were collected so that moisture content could be expressed as a percentage by volume, as well as per cent by weight. Using the relationship $\frac{\text{volume of sample}}{\text{oven dry weight of sample}}$ soil volume densities (cc/g) were calculated. The moisture content of samples collected subsequently without regard to their volume could then be expressed as per cent by volume using the relationship:

$$\text{sample volume (cc)} = \text{oven dry weight (g)} \times \text{volume density (cc/g)}$$

Soil samples from approximately 4-9 cm depth were collected on four occasions (3.4.65, 11.5.65, 7.9.65, 14.2.66) from each of the locations in each plot used for oxygen diffusion determinations.

RESULTS AND DISCUSSION

C.6. SOIL OXYGEN DIFFUSION RATES

C.6.1. Current-voltage Curves

The classical c.v. curve was found only in saturated soil-water suspensions or in water alone. This agrees with work reported by Birkle *et al*. (1964) and Tackett and Pearson (1964). Fig. 26*a* shows the curves obtained for tap water and three soil-water suspensions. These curves have the familiar flat "plateau" region. In some curves a small peak is apparent at approximately -0.3 volts. No explanation for this can be offered. Readings taken in unsaturated soil gave a curvilinear relationship with no "plateau" region (fig. 26*b*). Each point indicated on the c.v. curves is the average of four determinations. Each determination is in turn the average of the current flowing through two platinum electrodes wired in parallel.

C.6.2. Time to Establish Steady State

The change in current with time was recorded for *in situ* measurements taken at four different sites (fig. 27). Equilibrium was reached more rapidly in the wet soils but in all cases the rate of change was very small from four to five minutes after application of electrode potential.

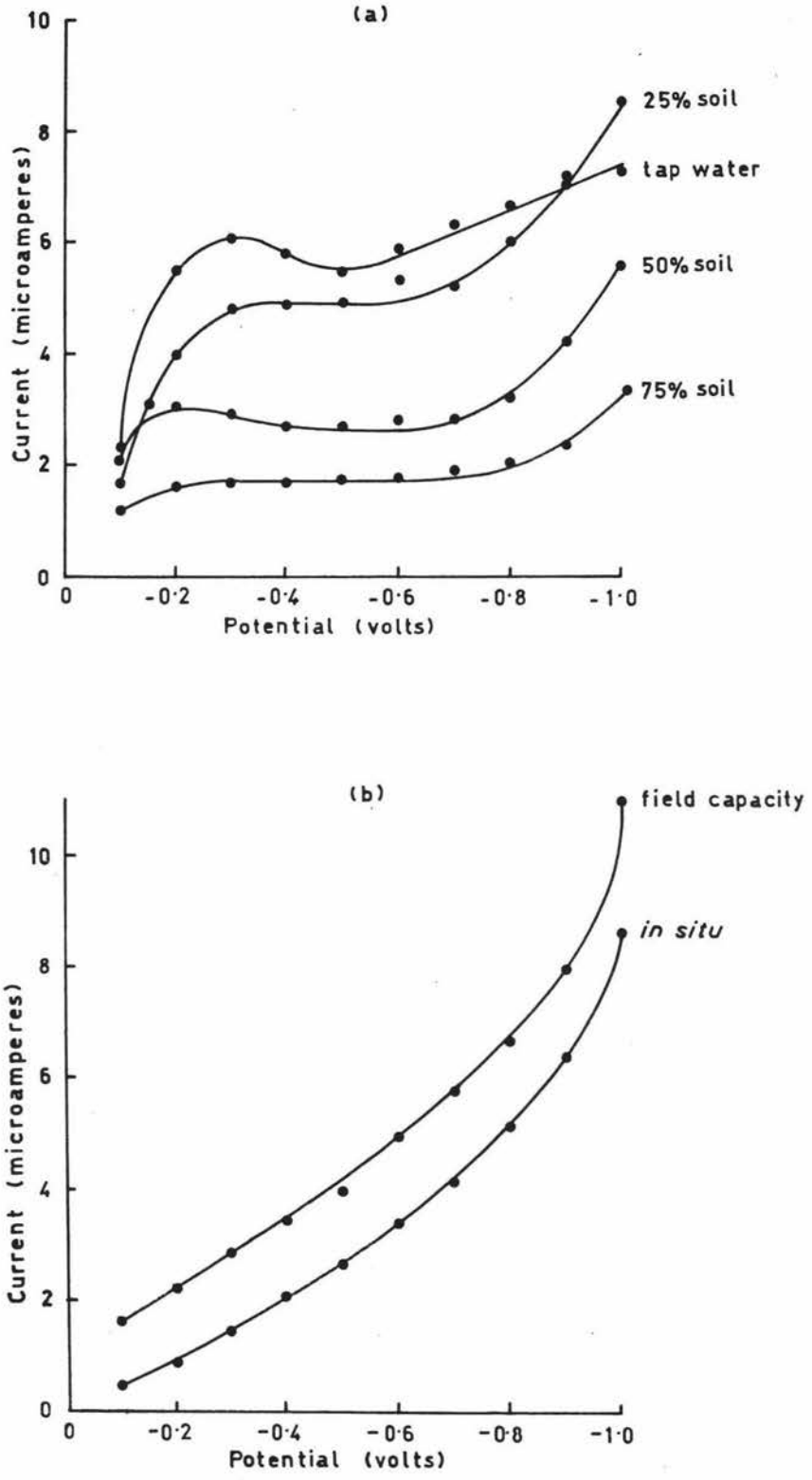


Fig. 26. Current-voltage curves showing: (a) the classical form, with a diffusion-limited "plateau", and (b) the curvilinear relationship obtained for in situ determinations.

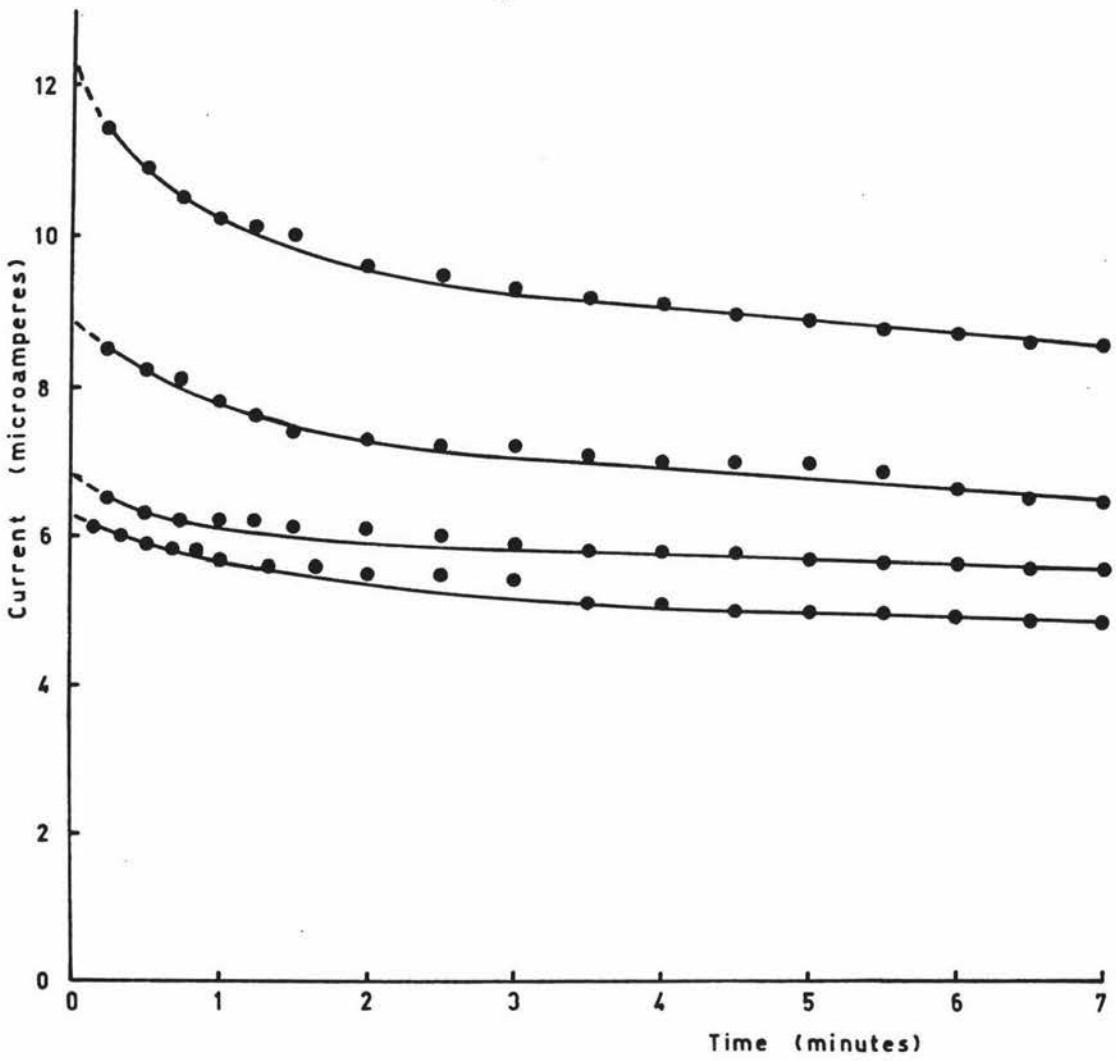


Fig. 27. Current as a function of the time elapsed from application of electrode potential. Determinations were made in situ at four different sites on a lawn.

C.6.3. Oxygen Diffusion Rates in Kaiparoro Soils

The components of variance from the hierarchal analysis of variance shown at the top of table 7 indicate the importance of micro-edaphic variation. Readings were taken at five localities within each of the four Kaiparoro plots (main-samples). At each locality two measurements were taken (sub-samples). The respective components of variance are:

plots	S^2	= (243.84 - 27.32)/10	= 21.65
main-sample	S^2	= (27.32 - 12.12)/2	= 7.60
sub-sample	S^2	= 12.12	

Variation between readings taken at the same location contributed to over half the total variation in readings among locations. In spite of this, greater precision in subsequent determinations was obtained by halving the number of sub-samples and doubling the number of main-samples. The reason is evident from the relationship:

$$\text{S.E. of mean} = \sqrt{\frac{S_S^2 + (s)S_M^2}{s\ m}}$$

If (s m), the total number of samples per plot, is fixed, the expression under the square root sign is least when $s = 1$. Only if S_M^2 had been zero, that is, if there had been no main-sample component of variance, would it have been possible to increase precision to any level by increased sub-sampling.

The F ratios (table 7) indicate significant plot differences

Table 7

SOIL OXYGEN DIFFUSION RATES MEASURED AT FOUR KAIPARORO PLOTS ON THREE OCCASIONS

SAMPLING DATE	SOURCE OF VARIATION	d.f.	MEAN SQUARE	PARAMETERS ESTIMATED	'F' RATIO	P	PLOT	MEAN, DIFFERENCE	DETECTABLE DIFFERENCE
11.5.65	Plots	3	243.84	$\sigma_S^2 + 2\sigma_M^2 + 10\sigma_P^2$	8.93	$<.005$			
	Main samples-within-plots	16	27.32	$\sigma_S^2 + 2\sigma_M^2$	2.25	.047	3 2 1 4	14.8 8.6 (6.2 •) 8.0 (0.6 NS) 5.2 (5.2 •)	$4.7_{(.05)}$ $6.4_{(.01)}$
	Sub-samples-within-main samples	20	12.12	σ_S^2					
7.9.65	Between-plots	3	36.36		12.31	$<.005$	3 2 1 4	6.9 4.9 (2.0 •) 4.1 (0.8 NS) 2.3 (1.8 •)	$1.6_{(.05)}$ $2.1_{(.01)}$
	Within-plots	36	2.95						
14.2.66	Between-plots	3	30.22		2.22	$>.100$	1 2 3 4	16.6 15.5 18.1 14.0	
	Within-plots	36	13.61						

O.D.R. values expressed as x 10^{-8} g cm^{-2} min^{-1}

NS $P > .05$
• $P \leq .05$
•• $P \leq .01$

on 11.5.65 and 7.9.65 ($P < 0.01$). The individual plot means and their differences are indicated together with the differences required for significance at the 5% and 1% levels of probability. Under the high oxygen diffusion rates prevailing at 14.2.66 plot differences were not significant according to the F ratio and no "t" test could be made on the plot means.

C.7. WATER CONTENT AND ITS RELATION TO O.D.R. IN KAIPARORO SOILS

Soil water content was measured on four occasions. On three of these days the samples were collected at the same time and from the same place as the O.D.R. measurements. On all but one sampling day (11.5.65), significant differences were demonstrated among plots (table 8). On this occasion cores were taken with a 1 inch diameter sampler, and compression of the plot 4 soil, which is high in organic matter, gave anomalously low water content results. At the time of sampling, plot 4 was noticeably wetter than the remaining plots, being the only one with water lying at the ground surface. Plot means for the four sampling dates are shown graphically in fig. 28. The comparable plot means for O.D.R. are also shown. An overall inverse relationship between the two variables is apparent. The change in distribution of plot means over time is not parallel however. This emphasises the ineffectiveness of attempting to estimate soil aeration from soil water content under varying circumstances. The relationship between soil water and O.D.R. is relatively constant for any one site

however (fig. 29). Plot numbers are shown in circles centred on their respective means.

Table 8

SOIL WATER CONTENT MEASURED AT FOUR KAIPARORO PLOTS ON FOUR OCCASIONS

SAMPLING DATE	SOURCE OF VARIATION	d.f.	MEAN SQUARE	'F' RATIO	P	PLOT	MEAN, DIFFERENCE	DETECTABLE DIFFERENCE
3.4.65	Between-populations	3	462.30	34.75	< .005	4	77.1	9.7 **	$3.31_{(.05)}$
						2	67.4	4.6 **	$4.43_{(.01)}$
	Within-populations	36	13.31			3	62.8	0.3 NS	
						1	62.5		
11.5.65	Between-populations	3	92.27	1.08	> .100	1	61.3		
						2	70.9		
	Within-populations	16	99.78			3	62.3		
						4	65.7+		
7.9.65	Between-populations	3	679.00	3.90	.028	4	86.3	5.8 NS	$11.98_{(.05)}$
						2	80.5	10.3 NS	$16.05_{(.01)}$
	Within-populations	36	174.17			3	70.2	0.9 NS	
						1	69.3		
14.2.66	Between-populations	3	297.39	7.05	< .005	4	66.9	8.0 **	$5.9_{(.05)}$
						2	58.9	2.5 NS	$7.9_{(.01)}$
	Within-populations	36	42.17			1	55.4	0.2 NS	
						3	55.2		

+ Low value due to compression of soil causing loss of water during collection of samples.

NS P > .05
* P ≤ .05
** P ≤ .01

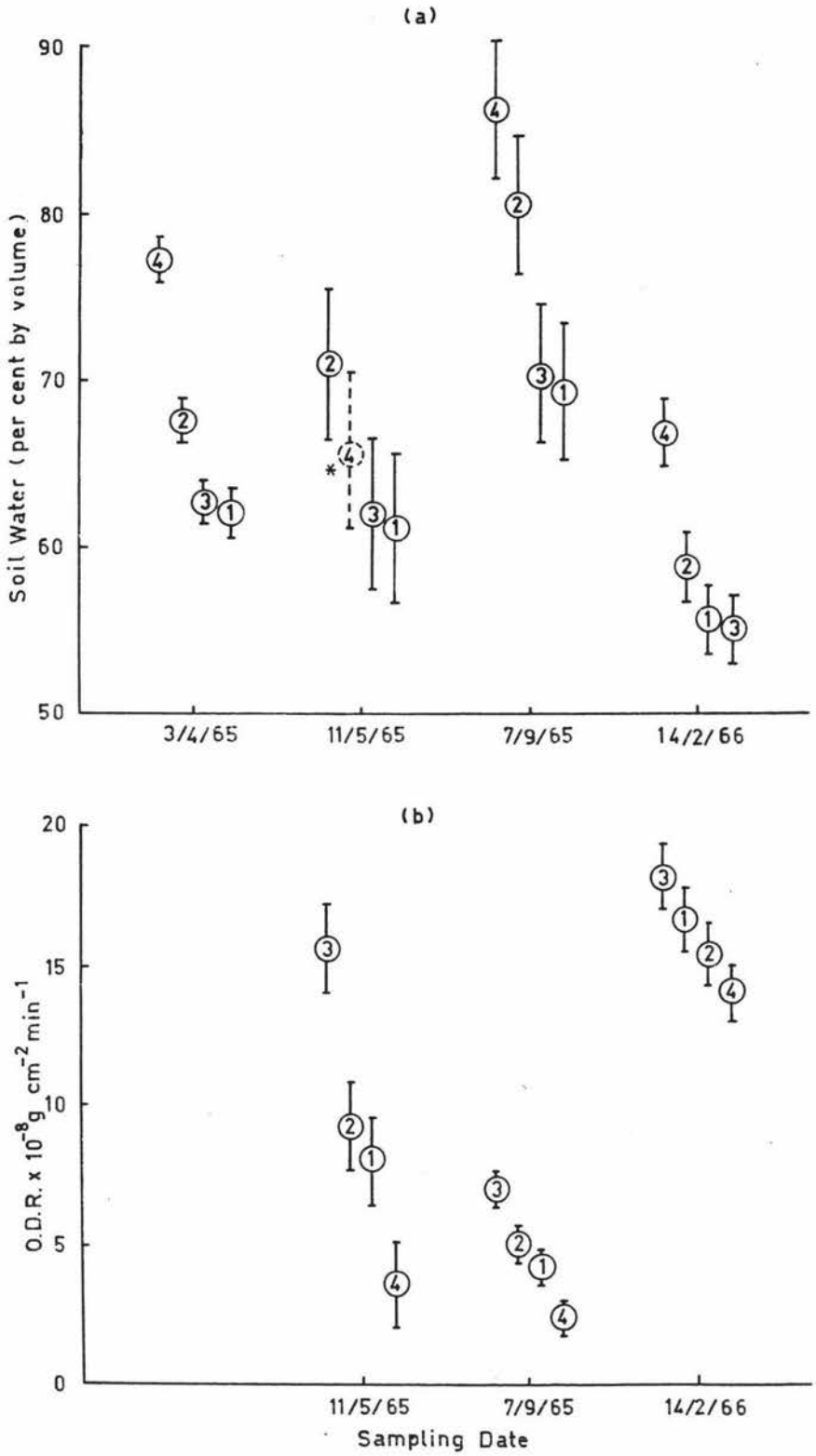


Fig. 28. (a) The mean soil water content of four Kaiparoro plots, sampled on four occasions. (b) The mean soil oxygen diffusion rates of four Kaiparoro plots sampled on three occasions. The vertical bars indicate ± one standard error.
* This result is anomalously low. For explanation, see text (p. 75).

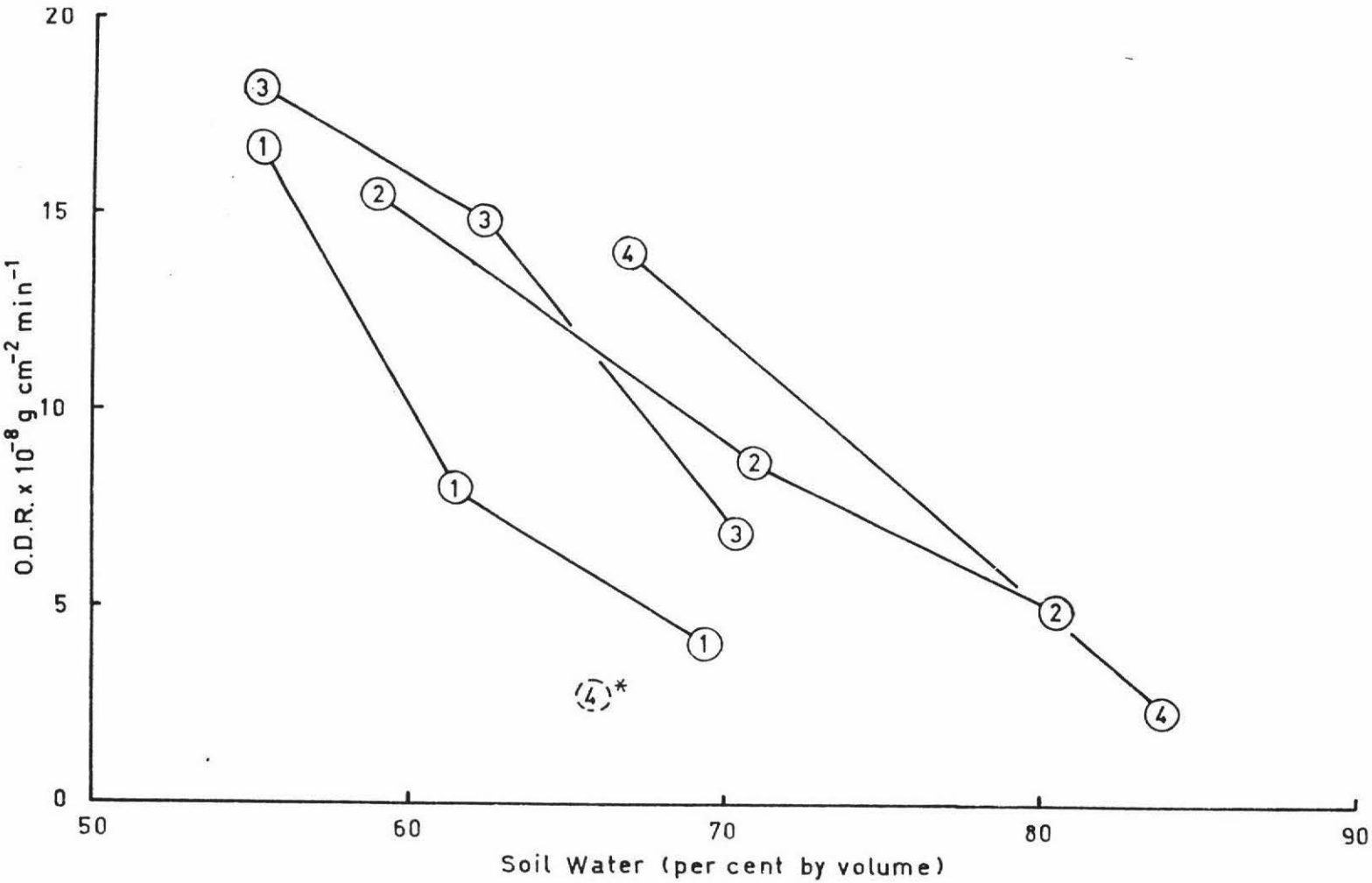


Fig. 29. Soil oxygen diffusion rate as a function of soil water content. The means are shown for four Kaiparoro plots sampled on three occasions.
* This value shows an anomalously low soil water content. For explanation, see text (p. 75).

CONCLUSIONS

The oxygen diffusion rates in the Kaiparoro soils are in the range critical for plant growth. Plot differences vary in magnitude over time but the relative plot levels remain constant. During the wettest seasons these differences appear to be large enough to be biologically, as well as statistically, significant.

SECTION D

PLANT-ENVIRONMENT RELATIONSHIPS

INTRODUCTION

D.1. PLANT - ENVIRONMENT RELATIONSHIPS

Large environmental differences between sites exert such a rigorous control over the distribution of vegetation that direct causal relationships between plants and their environment are easily discerned. Smaller environmental differences, however, permit the operation of chance factors which may cause the differential establishment and growth of any of a number of suitable plants.

To the extent that vegetational differences are determined by environmental differences, the two can be expected to correlate. However, correlation does not necessarily imply direct causal relationships as the correlated variables may themselves be determined by some other common factor. To establish if plant - environment correlations result from a direct causal relationship they must be examined in the light of biological information such as the conditions necessary for optimum plant growth at various stages of the life cycle, and the nature and extent of competition from other plants. The history of environmental change should also be considered as the distribution of vegetation at any one time may have been determined by environmental conditions no longer prevalent.

RESULTS AND DISCUSSION

D.2. RELATIONSHIPS BETWEEN SOIL O.D.R. AND THE DISTRIBUTION OF THE KAIPARORO CHIONOCHLOA VARIANTS

A positive correlation exists between soil O.D.R. and hybrid index showing that the higher the soil aeration levels the more closely the Chionochloa plants growing in these soils resemble C. flavescens (fig. 30a). The correlation is high for plots 2, 3, and 4 on all three sampling days. Plot 1, however, deviates from this straight line relationship. On no occasion did its oxygen diffusion rates differ significantly from those of plot 2. Lack of an absolute correlation does not exclude the possibility that a causal relationship exists. Several alternative explanations are feasible.

(a) The Chionochloa variants may tolerate a range of soil aeration conditions.

(b) The pattern of soil aeration on Kaiparoro may have affected the initial establishment of the tussocks and have since altered with little or no effect on the mature plants. These would probably persist for a long time because Chionochloa tussocks are thought to be long-lived and little seedling establishment occurs in undisturbed populations (Mark, 1965). Harper and Sagar (1953) described a similar case where the distribution of three Ranunculus species in a permanent grassland was influenced by soil drainage which

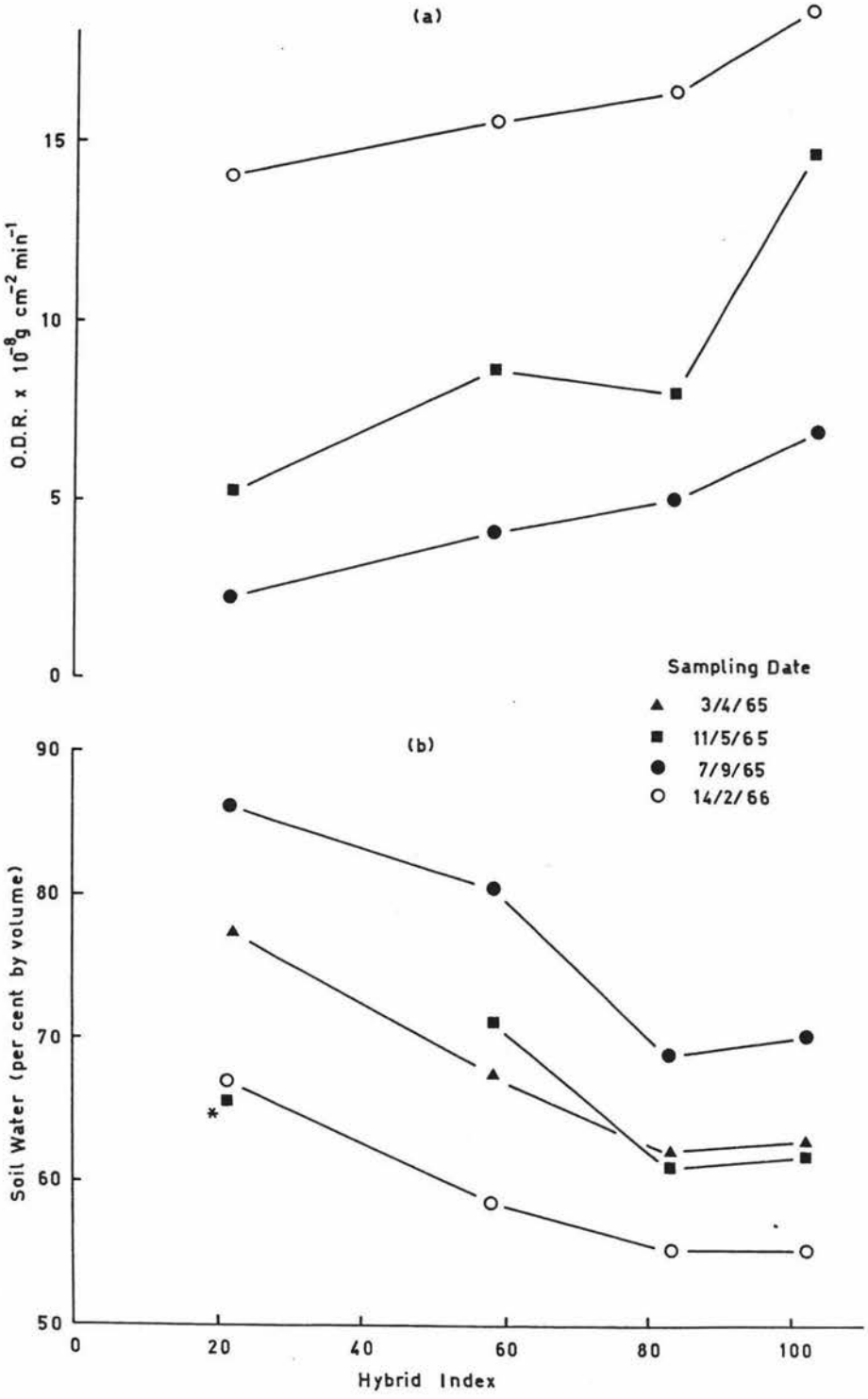


Fig. 30. (a) Relationship between hybrid index and soil oxygen diffusion rate at each of four Kaiparoro plots sampled on three occasions. (b) Relationship between hybrid index and soil water content at each of four Kaiparoro plots, sampled on four occasions.

* This result is anomalously low. For explanation, see text (p. 75).

affected seedling establishment but not the growth of the mature plants.

(c) Plants can be affected by very brief periods of soil oxygen deficiency, especially at critical stages of the life cycle (see C.3.2.). It is possible that O.D.R. sampling did not coincide with the most critical period.

(d) The hybrid index, which is based on three morphological characters, may not typify exactly the variants' physiological relationships.

The relationship demonstrated between soil oxygen diffusion rates and leaf characters is in accordance with the proposition that the Kaiparoro variants are hybrids between C. rubra and C. flavescens. C. rubra appears to tolerate wetter, and therefore more poorly aerated, soils than C. flavescens. As physiological and morphological traits are inherited in approximately the same manner, it is expected that the Kaiparoro variants most closely resembling C. rubra would occupy the sites with the lowest oxygen diffusion rates.

A less consistent relationship exists between the hybrid index and soil water content measured on four occasions (fig. 30b). This is expected if soil O.D.R. does govern the distribution of the Kaiparoro Chionochloa variants because the correlation between soil water content and O.D.R. is not absolute (see C.7.).

CONCLUSIONS

It is likely that the correlation demonstrated between the hybrid indices of the Kaiparoro Chionochloa variants and soil oxygen diffusion rates arises from a direct causal relationship. Experimentation under controlled conditions would be required for the evidence to be conclusive, however.

SECTION E

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

Results confirm the first premise of the working hypothesis:

> "That the morphological variation apparent in the Kaiparoro *Chionochloa* population is the result of hybridization between *C. rubra* and *C. flavescens* . . . "

This conclusion is based on a detailed comparison of the pattern of leaf morphological variation in the Kaiparoro population, with that of the putative parent species. The Kaiparoro plants range from forms typical of *C. rubra* to those typical of *C. flavescens* and the differences are largely under genetical control. The pattern of character variation is that expected of hybrids between *C. rubra* and *C. flavescens*.

Evidence supports the second premise of the working hypothesis, namely:

> ". . . that the distribution of the variants is influenced by some parameter of soil water conditions."

The plant characters correlate with soil oxygen diffusion rates. Although a causal relationship is not proved, such a conclusion is completely in accordance with the finding that the Kaiparoro *Chionochloa* are hybrids between *C. rubra*, which tolerates wet, and therefore poorly aerated soils, and *C. flavescens* which apparently does not.

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APPENDICES

FORTRAN PROGRAMS FOR I. B. M. 1620 COMPUTER

Appendix 1

```
C       HIERARCHAL ANALYSIS OF VARIANCE FOR THREE CHARACTERS
C       LAST CARDS FOR BENCHES AND POPULATIONS
        TYPE 2
    2   FORMAT (36H HIERARCHAL ANAL OF VAR  3 VARIABLES)
        TYPE 3
    3   FORMAT (30H SW 1 ON FOR BENCH DIFFERENCES)
        PAUSE
        DIMENSION SX(3),HOLD(3),SP(3),SG(3),X(3),SCPP(3,3),SCPG(3,3)
        DIMENSION CLOCP(3,3),GENCP(3,3),BENCP(3,3),SAVCP(3,3)
        DIMENSION SCPX(3,3),Q(3,3),CTCP(3,3)
C       INITIALISE VARIABLES TO ZERO
   60   DO 56 I=1,3
        DO 56 J=1,3
        SCPG(I,J)=0.
        SAVCP(I,J)=0.
        SCPX(I,J)=0.
        SCPP(I,J)=0.
   56   CTCP(I,J)=0.
        DO 57 I=1,3
        SG(I)=0.
   57   SX(I)=0.
        C=0.
        G=0.
C       READ 3 VARIABLES CHARACTERIZING EACH LEAF, GENOTYPE
C       IDENTIFICATION NO., RAMET PAIR NO., BENCH LAST CARD NO.,
C       AND POPULATION LAST CARD NO.
   20   READ 1, X(1), X(2), X(3), NO, LC, LB, LP
    1   FORMAT (F4.2,F2.0,F3.1,I3,I1,I2,I2)
        IF(LB-88) 18,27,500
C       CALC. SS AND CP THRU WHOLE POPULATION
   18   DO 22 I=1,3
        SX(I)=SX(I)+X(I)
        DO 22 J=1,3
   22   SCPX(I,J)=SCPX(I,J)+X(I)*X(J)
        C=C+1.
        IF(LC-2) 23,24,500
   23   DO 26 I=1,3
   26   HOLD(I)=X(I)
        GO TO 20
C       CALC. SS AND CP OF RAMETS-WITHIN-GENOTYPE SUB-TOTALS
   24   DO 45 I=1,3
   45   SP(I)=HOLD(I)+X(I)
        DO 61 I=1,3
        DO 61 J=1,3
   61   SCPP(I,J)=SCPP(I,J)+(SP(I)*SP(J))/2.
C       CALC. GENOTYPE TOTAL FOR BENCH (OR POPULATION)
```

```
      DO 25 I=1,3
   25 SG(I)=SG(I)+SP(I)
      G=G+1.
      GO TO 20
C     CALC. SS AND CP OF GENOTYPES-WITHIN-BENCH (OR POPULATION)
   27 DO 46 I=1,3
      DO 46 J=1,3
   46 SCPG(I,J)=SCPG(I,J)+(SG(I)*SG(J))/(G*2.)
      TYPE 12,G
   12 FORMAT (33H GENOTYPES IN BENCH OR POPULATION,2X,F4.0)
      DO 29 I=1,3
   29 SG(I)=0.
      G=0.
      IF(LP-99) 40,33,500
   40 DO 49 I=1,3
      DO 49 J=1,3
      SAVCP(I,J)=SCPG(I,J)
   49 SCPG(I,J)=0.
      GO TO 20
C     CALC. CORRECTION TERMS AND CORRECTED SS AND CP AT ALL LEVELS
   33 DO 47 I=1,3
      DO 47 J=1,3
      CTCP(I,J)=(SX(I)*SX(J))/C
      CLOCP(I,J)=SCPX(I,J)-CTCP(I,J)
      GENCP(I,J)=SCPP(I,J)-CTCP(I,J)
      BENCP(I,J)=(SAVCP(I,J)+SCPG(I,J))-CTCP(I,J)
C     TYPE OUT CORRECTED SS AND CP IN MATRIX FORM
   47 Q(I,J)=CLOCP(I,J)
      L=1
      TYPE 10
   10 FORMAT (//20X,6H CLONE)
      GO TO 100
  110 DO 58 I=1,3
      DO 58 J=1,3
   58 Q(I,J)=GENCP(I,J)
      L=2
      TYPE 14
   14 FORMAT (//20X,9H GENOTYPE)
      GO TO 100
  120 IF(SENSE SWITCH 1) 52,53
   52 DO 59 I=1,3
      DO 59 J=1,3
   59 Q(I,J)=BENCP(I,J)
      L=3
      TYPE 15
   15 FORMAT (//20X,6H BENCH)
  100 TYPE 101, Q(1,1), Q(1,2), Q(1,3)
  101 FORMAT (/3F16.4)
      TYPE 102, Q(2,2), Q(2,3)
  102 FORMAT (16X,2F16.4)
```

```
    TYPE 103, Q(3,3)
103 FORMAT (32X,F16.4)
    GO TO (110,120,53),L
 53 TYPE 13
 13 FORMAT (//21H LOAD NEXT POPULATION)
    PAUSE
    GO TO 60
500 TYPE 200
200 FORMAT (14H PROGRAM ERROR)
    PAUSE
    GO TO 60
    END
```

Appendix 2

```
C       ANALYSIS OF VARIANCE FOR THREE VARIABLES
C       USE RAMET AVERAGES CORRECTED FOR BENCH DIFFERENCES
C       LAST CARD AFTER EACH POPULATION (LP=88)
C       LAST CARD AFTER FINAL POPULATION INCLUDES LEND (8899)
C       PRINTS NO. IN EACH POPULATION, TOTAL, MEAN, TOTAL-,
C       BETWEEN-, AND WITHIN- SS AND CP
        DIMENSION SX(3),SSX(3,3),SXP(3),SSXP(3,3),CT(3,3),X(3)
        DIMENSION TSS(3,3),BSS(3,3),WSS(3,3),Q(3,3),AMEAN(3)
        PRINT 12
   12   FORMAT (//34H ANALYSIS OF VARIANCE, 3 VARIABLES)
        PRINT 3
    3   FORMAT (/21H POPULATION    NUMBER,3X,6H TOTAL,5X,5H MEAN)
C       EQUATE VARIABLES TO ZERO
   47   N=0
        P=0.
        G=0.
        DO 41 I=1,3
        SX(I)=0.
        SXP(I)=0.
        DO 41 J=1,3
        SSX(I,J)=0.
   41   SSXP(I,J)=0.
C       READ 3 VARIABLES CHARACTERIZING EACH LEAF, GENOTYPE
C       IDENTIFICATION NO., POPULATION LAST CARD NO., AND FINAL
C       CARD NO.
   33   READ 1, X(1),X(2),X(3),NO,LP,LEND
    1   FORMAT (3F10.4,I4,I2,I2)
        IF(LP-88)32,34,500
   32   DO 30 I=1,3
C       CALC. SUM OVER ALL POPULATIONS, SUM FOR EACH POPULATION,
C       AND SS AND CP THROUGH ALL POPULATIONS
   30   SX(I)=SX(I)+X(I)
        DO 31 I=1,3
        DO 31 J=1,3
   31   SSX(I,J)=SSX(I,J)+(X(I)*X(J))
        DO 39 I=1,3
   39   SXP(I)=SXP(I)+X(I)
        G=G+1.
        P=P+1.
        GO TO 33
C       CALC. SS AND CP FOR POPULATION TOTALS
   34   DO 35 I=1,3
        DO 35 J=1,3
   35   SSXP(I,J)=SSXP(I,J)+(SXP(I)*SXP(J))/G
        N=N+1
        PRINT 2,N,G
    2   FORMAT (4X,I4,F12.0)
```

```
C       CALC. EACH POPULATION MEAN
        DO 62 I=1,3
   62   AMEAN(I)=SXP(I)/G
        DO 61 I=1,3
   61   PRINT 101,SXP(I),AMEAN(I)
  101   FORMAT (20X,2F12.4)
        DO 36 I=1,3
        AMEAN(I)=0.
   36   SXP(I)=0.
        G=0.
        IF(LEND-99)37,38,500
   37   GO TO 33
C       CALC. CORRECTION TERMS FOR SS AND CP
   38   DO 40 I=1,3
        DO 40 J=1,3
   40   CT(I,J)=(SX(I)*SX(J))/P
C       CALC. CORRECTED TOTAL-, BETWEEN-, AND WITHIN- POPULATION SS
C       AND CP
        DO 50 I=1,3
        DO 50 J=1,3
        TSS(I,J)=SSX(I,J)-CT(I,J)
        BSS(I,J)=SSXP(I,J)-CT(I,J)
        WSS(I,J)=TSS(I,J)-BSS(I,J)
C       PRINT IN MATRIX FORM TOTAL-, BETWEEN-, AND WITHIN-
C       POPULATION SS AND CP
   50   Q(I,J)=TSS(I,J)
        L=1
        PRINT 4
    4   FORMAT (//20X,6H TOTAL)
        GO TO 100
   44   DO 42 I=1,3
        DO 42 J=1,3
   42   Q(I,J)=BSS(I,J)
        L=2
        PRINT 5
    5   FORMAT (//20X,8H BETWEEN)
        GO TO 100
   45   DO 43 I=1,3
        DO 43 J=1,3
   43   Q(I,J)=WSS(I,J)
        L=3
        PRINT 6
    6   FORMAT (//20X,7H WITHIN)
  100   PRINT 7,Q(1,1),Q(1,2),Q(1,3)
    7   FORMAT (/3F16.4)
        PRINT 8,Q(2,1),Q(2,2),Q(2,3)
    8   FORMAT (/3F16.4)
        PRINT 9,Q(3,1),Q(3,2),Q(3,3)
    9   FORMAT (/3F16.4)
        GO TO (44,45,46),L
```

```
 46 PRINT 10
 10 FORMAT (//20H PROCESSING COMPLETE)
    PAUSE
    GO TO 47
500 PRINT 11
 11 FORMAT (14H PROGRAM ERROR)
    PAUSE
    GO TO 47
    END
```

Appendix 3

```
C       CALCULATE THREE WEIGHTING COEFFICIENTS
C       LEAD CARD WITH HIGHEST LATENT ROOT, FOLLOWED BY V THEN E
C       MATRIX EACH ON THREE CARDS AND READ ROW BY ROW
        DIMENSION V(3,3),E(3,3),ER(3,3),D(3,3)
C       READ HIGHEST LATENT ROOT
   32   READ 1,HLR
    1   FORMAT (F12.6)
        READ 2,V(1,1),V(1,2),V(1,3)
    2   FORMAT (3F10.5)
        READ 2,V(2,1),V(2,2),V(2,3)
        READ 2,V(3,1),V(3,2),V(3,3)
        READ 2,E(1,1),E(1,2),E(1,3)
        READ 2,E(2,1),E(2,2),E(2,3)
        READ 2,E(3,1),E(3,2),E(3,3)
C       MULTIPLY ALL ELEMENTS OF E BY THE HIGHEST LATENT ROOT
        DO 30 I=1,3
        DO 30 J=1,3
   30   ER(I,J)=E(I,J)*HLR
C       CALC. DIFFERENCE BETWEEN (E*HLR) AND V AND CALL RESULT D
        DO 31 I=1,3
        DO 31 J=1,3
   31   D(I,J)=V(I,J)-ER(I,J)
C       CALC. VALUES OF WEIGHTING COEFFICIENTS.  ELIMINATE THIRD
C       ROW OF D, EQUATE FIRST COEFFICIENT B1 TO 1 AND SOLVE
C       SIMULTANEOUSLY FOR B2 AND B3
        B1=1.
        Q=(D(1,2)*D(2,3))-(D(1,3)*D(2,2))
        B2=((-D(1,1)*D(2,3))-(-D(2,1)*D(1,3)))/Q
        B3=((D(1,2)*(-D(2,1)))-(D(2,2)*(-D(1,1))))/Q
C       INSERT B1, B2, AND B3 IN D AND CHECK THAT ALL ROWS EQUAL
C       ZERO
        T1=D(1,1)+(D(1,2)*B2)+(D(1,3)*B3)
        T2=D(2,1)+(D(2,2)*B2)+(D(2,3)*B3)
        T3=D(3,1)+(D(3,2)*B2)+(D(3,3)*B3)
        PRINT 4
    4   FORMAT (//12X,23H WEIGHTING COEFFICIENTS)
        PRINT 5
    5   FORMAT (6X,3H B1,12X,3H B2,12X,3H B3)
        PRINT 6,B1,B2,B3
    6   FORMAT (3F15.6)
        PRINT 7
    7   FORMAT (//16H TEST SHOULD = 0)
        PRINT 8,T1,T2,T3
    8   FORMAT (3F15.6)
        PAUSE
        GO TO 32
        END
```