SCOTTISH PLANT BREEDING STATION

PENTLANDFIELD, ROSLIN, MIDLOTHIAN

REPORT

1958

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Scottish Plant Breeding Station

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DIRECTOR'S REPORT

Cereals.—The work of the Cereal Section during the year was carried on along lines similar to 1956. Material, both fixed and unfixed, was grown at selection centres in Argyll and Inverness-shire, plots at Pentlandfield representing a third environment. Yields from all three centres were lower than in 1956 but in spite of this the results obtained corresponded fairly closely with those of the previous year.

Investigations concerning the resistance of oat varieties to the stem eelworm *Ditylenchus dipsaci* which were begun in 1956 were continued and expanded. Confirmation that the variety Early Miller behaves as a resistant variety under field conditions was obtained from a trial laid down near Laurencekirk by arrangement with Dr Robertson of the North of Scotland College of Agriculture, in which the varieties Early Miller, Albyn Empress and Aa 732 were compared with Milford. A series of laboratory tests have been made to assess the reaction of the varieties Early Miller, Milford and *Avena ludoviciana*, and a routine examination of hybrid material at various stages of selection was carried out to determine whether any additional sources of resistance were available. While there was considerable variation in the degree of susceptibility none of the hybrids was as resistant as Early Miller and Milford, with the single exception of a hybrid between *Avena ludoviciana* and Milford which was superior to both the cultivated varieties. At the conclusion of the tests for resistance some 180 seedlings from a total of 21 resistant F_3 lines of this hybrid, Reference No. 0635, were transplanted and grown to maturity, and those plants which most resembled *Avena sativa* in type were crossed with a number of useful cultivars. Altogether 143 hybrid grains were obtained and it is hoped that F_2 generation seedlings will be available for further eelworm inoculation tests during the early months of 1958.

The year 1957 was particularly favourable to attack by stem eelworm in the field and no difficulty was found in obtaining material as a source of eelworm for inoculation experiments.

Thanks are due to those members of the staffs of the three Agricultural Colleges who located selection centres and sites for field trials, and material for the eelworm inoculation tests.

For details of the eelworm investigations the reader is referred to the Occasional Paper which appears on page 66 of this Report.

Herbage Plants and Genecology.—A considerable number of strains of grasses for arable land can now be readily obtained in this country providing the farmer with a fairly wide choice of both home and imported strains even within a given maturity type. There would, however, still seem to be room for a type that would give a good performance over a wide range of environmental conditions. The use of strains at present on the market as constituents in a modified form of the polycross has already been suggested in a previous report as a possible way of achieving such an increase in tolerance and it is hoped that the current experiments involving strains of the same maturity type but originating from different environments which are also geographically separated, will yield information bearing upon this point. Plants from first generation seed have been put out in randomised spaced-plant trials with their parents, and swards and rows have also been laid out. The first species to be treated in this way are perennial ryegrass and cocksfoot, seven early strains of the former and ten late strains of the latter.

The very erect type of late ryegrass which could be useful for silage cutting was given further attention and the progenies from new combinations of parents have shown valuable characteristics unusual in such a late type. The importance of highly specialised strains of arable grasses tends to increase rather than diminish with the refinement of methods of farm management and such strains which would most likely be of low inherent variability, would also provide useful constituents for the modified polycross should this method prove to be of value.

In 1956 this Station received tillers from a number of populations of Mediterranean cocksfoot, ryegrass and *Festuca arundinacea* as part of a plant collection and distribution scheme organised by F.A.O. in Rome. All-year-round observations in the field suggest that the characters of some of the cocksfoot types might be usefully combined and accordingly a number of plants from different populations were crossed in isolation under greenhouse conditions.

Rough grazings and, in particular, the problems presented by the extensive hill areas have periodically been occupying the minds of agriculturists for many years now and a fair volume of experimental work has been done though few conclusive results have been obtained. Probably the most attractive idea, particularly to those who are accustomed to arable farming, is that of adopting a wholesale programme of ploughing and reseeding with good arable strains regardless of the effect that it may have upon the relative values of untreated associated vegetations. As everyone knows, the average hill grazing consists of a number of different plant communities of varying usefulness and the possibility of improvement taking the form of intentionally adjusting the complementary balance of these communities is something that ought not to be overlooked. In previous experiments conducted by the Station it was shown that where potential arable land happens to lie adjacent to the open hill even very limited areas of an alien vegetation established after ploughing and reseeding can play a useful complementary rôle. There are, however, many places where the creation of arable conditions is out of the question but where other methods of changing the prevailing community pattern in a complementary direction might lead to an overall improvement. The article by Mr Grant of the North of Scotland College of Agriculture published in this report gives an account of experiments at present being carried out under poor conditions where surface seeding seems to offer the best approach. The grass seeds used are those of arable strains and as far as the work has proceeded, the results are very promising. Nevertheless, the environment of such areas is very different from the average lowland field and it cannot be overlooked that if surface seeding is going to be undertaken with a real chance of success, it may be necessary to find strains which are naturally better suited to the demands and rigours of these difficult environments. Such strains would be most likely to come from the species which occur normally under these conditions, and, therefore, the possibility of having to embark upon a breeding programme involving such a species as *Agrostis* must be kept in mind. The importance of *Agrostis*-fescue pastures for stock grazing on the hills is undisputed and while the presence of intraspecific races can be demonstrated, there is still need to understand the differences between the two related *Agrostis* species and between the two cytological races of fescue.

The genecological studies which are at present being undertaken at the Station are mainly concerned with the study of the constituents of hill vegetation and provide a logical approach to the problem of finding material suitable for a breeding programme. As far as the present trials of *Agrostis* species from different ecological habitats as well as different geographical areas are concerned, it is evident in several characters that though the variation within a habitat is considerable, making it unlikely that in sampling genotypic reduplication has occurred, there are also significant differences between habitat populations and they are maintained in a second year of measurement. This is even more true of *A. tenuis* than of *A. canina* possibly because *A. canina* is completely absent from very close grazings which naturally provide very extreme conditions. The populations considered show that there is a tendency for *A. canina* to keep a greater degree of winter greenness though the species is of somewhat slower and smaller growth than *A. tenuis* since within the second year there is a greater proportional increase in *A. canina* for such characters as Plant Height and Plant Diameter although *A. tenuis* remains the larger plant. It is interesting to note that although *A. canina* has an extremely erect habit of growth when flowering, it is indeed rather less erect than *A. tenuis* in the vegetative state. For purposes of a breeding programme the choice between the two species does not seem to be difficult to make on the basis of such results especially when supplemented by the knowledge that the radical leaves of *A. tenuis* are slightly longer and broader than those of *A. canina* in the same habitat and that total plant weight is greater.

The extent to which natural hybridisation takes place between the two species is not yet known though in this trial a high proportion of doubtful and possibly hybrid plants were excluded from each habitat population with the exception of one very heavily grazed population which was pure *A. tenuis.* Crossing takes place fairly readily under greenhouse conditions and the proportion of selfing which can be obtained is very small. Since this is the case the appearance of hybrid populations planted this year in the experimental garden for further examination suggests that whichever species provides the female parent, the progeny tend to resemble *A. tenuis* most closely.

The intensive genecological study of long-lived perennial grasses has raised problems of technique and further work on the risk of reduplication of genotypes in population sampling has confirmed that this can be a serious source of error in genecological studies. It is made the more serious by the fact that it is easily undetected, even when it is anticipated. Since the source of the trouble is the use of the within population variance as an error item, more reliable data of genecological interest could be collected if the trials were designed with an alternative error in mind. One such item is that variance derived as " between means of populations from ecologically similar sites." To use this item in trial work it is necessary to have a large number of populations, and if the trials are to be kept a manageable size this means reducing the number of plants per population sample. The obvious disadvantages of this scheme, principally the lack of precision in the individual population means, and the greater burden of field work at the collecting stage are probably more than compensated for in that any conclusions have only one known interpretation. Collecting for a trial of this type was completed during 1957, the work being shared between all members of the section. Altogether 226 sites were visited, principally in Southern Scotland, and material was collected of *Agrostis*, *Festuca ovina*, *Potentilla erecta* and *Plantago lanceolata*. While the within population variance must always be suspect as an error item it might be of great value and there is the chance that its use may be permissible if genotype reduplication is successfully avoided in sampling. From the evidence available it seems that this might be the case with *Festuca ovina* when sampled at intervals of about ten yards or more, and this procedure was adopted for the collection of all materials.

The study of the spread and occurrence of single extensive genotypes in natural communities is giving interesting evidence on their individual ranges of ecological tolerance. In most cases it seems that the individual genotype has the same ecological range as the local population, but there are a few cases indicating a slight, but as yet non-significant, trend towards a narrower range. This evidence is of value in assessing the adaptive differences of genotypes and the intensity of selection between them. For this type of study the apomictic *Taraxacum* is a particularly attractive material, and a small scale study of its genotype constitution in local populations has commenced.

The collection of genecological data from the thirteen taxa trial mentioned in last year's Report is now complete, and the results are being analysed. At first sight it appears to contain a great deal of information of genecological interest, all characters recorded showing highly significant differences between sites. However, it appears that these differences cannot be related to ecology and the conclusion is that they are of the spurious type associated with genotype reduplication in sampling. This means that each taxon is represented in the trial by a single local race, there being no ecotypic differentiation within the material.

The study of lead tolerance in *Festuca ovina* has continued, and most of the wild material collected has now been examined. An Index of Tolerance was constructed from the rates of root growth with and without the addition of lead to the solution and the populations fall into three groups when the mean values of the index are compared. The simplest explanation seems to be that there are three types of plant, showing high, medium and low tolerance, and that each population consists almost entirely of a single type. This was quite unexpected, and so far has not been explained in terms of adaptation to different soil types. This double discontinuity has not yet been established with certainty because it has proved difficult to make the technique sufficiently accurate to establish the precise tolerance of a single plant, but it seems very likely that the variation is in fact determined by a major gene system.

Many crosses were made between plants of differing tolerance and the progenies are now being tested. The lack of precision already mentioned makes the establishment of genetic ratios difficult, especially as the plant is tetraploid and the ratios likely to be complex, but it seems clear so far that tolerance is dominant to intolerance.

Of the plants collected from the Pennine and Lake District lead-mining areas only a few have so far been tested, but the highly tolerant form seems to be rare and most of the tips carry populations of medium tolerance.

A small but interesting observation was made when plants were being rooted in water prior to lead testing. From the middle of March rooting became more and more difficult until about the time of ear emergence, when the majority of the tillers produced no roots at all. The only ones which did root during this period were found to be completely vegetative—the development of flower initials led to complete inhibition of root production on that tiller.

The garden trial laid out to compare morphologically the plants of differing lead tolerance was examined in the autumn and some preliminary observations made, with rather unexpected results. Leaf length was found to vary markedly, but in a geographical manner and with no relationship to lead tolerance. Plants from Leadhills, whether tolerant or not, had much longer leaves than plants from Midlothian. Late in the season a rough score was made of the vigour of each plant. This trial looked much more uneven than is usual with *Festuca ovina*—some plants had died completely, and many others were showing only a few green leaves, most of the summer growth having withered. Analysis showed a clear association between poor growth in the trial and lead tolerance, suggesting that the tolerant plants might have developed a requirement for lead, or perhaps a susceptibility to poisoning by some normally innocuous substance in the garden soil.

The Pentlandfield part of an inter-continental trial of *Poa* species organised by the Department of Plant Biology, Carnegie Institution of Washington, was visited last summer by Dr Clausen of the Institution thus enabling scoring methods to be standardised.

In this Report we are fortunate in being able to publish as Occasional Papers genecological contributions by Dr Sinskaya of Leningrad and Drs Clausen and Hiesey of the Carnegie Institution. We are most grateful to them for consenting to prepare summaries of the important work for which their laboratories are responsible. Few if any genecologists can have a better appreciation of the significance to agriculture of this field of research than Dr Sinskaya, and the subject discussed by Drs Clausen and Hiesey, despite its obvious bearing upon plant introduction and variety testing, is only beginning to receive the attention it deserves.

Potatoes.—The work of the potato section comprises routine breeding for the production of new and improved economic varieties together with special investigations into fundamental problems of resistance to blight (*Phytophthora infestans*), the potato root eelworm (*Heterodera rostochiensis*) and potato viruses.

The experimental work concerned with field resistance to blight has been continued. This involved the raising and testing of new progenies bred specially for blight resistance and also the testing of all surviving seedlings raised in 1955 and earlier years. Race 1, 2, 3, 4 of the blight fungus was employed in the tests and the plants were grouped according to the following disease index classes: (1) Immune—no disease; (2) Highly Resistant—necrotic flecks and small lesions; (3) Moderately Resistant—larger lesions often spreading slowly; (4) Susceptible—active stem and leaf lesions with plants remaining alive; (5) Very Susceptible—plants dead or dying.

Since no definite line of demarcation between groups exists, this method of subdivision is arbitrary and individual plants are sometimes difficult to place with accuracy. However, this does not seriously detract from the value of the classification. It has been found that most commercial varieties and, in consequence, most seedlings bred from them, fall into group (4). Group (5) varieties are too susceptible to survive long in commerce. Blight susceptibility in this high degree was a feature of the hybrid progenies of the original eelworm resistant clones of *S. tuberosum* subsp. *andigena*. *S. vernei*, also valuable for eelworm resistance, appears to be considerably less susceptible to blight. A few varieties which have a reputation as blight resisters, *e.g.*, Champion, Ackersegen, Voran and Carmen, were classified as group (3). The new early variety Pentland Beauty and several promising selections were also classified as group (3). A few similar selections of possible economic value showed only necrotic spots and small lesions and were classified as group (2). No group (1) types were found in this material.

Amongst the seedling progenies bred specially for resistance to blight representatives of all groups were found. The frequency of the various resistant types present in these progenies was invariably related to the resistance exhibited by the parents and transgressive inheritance was frequently apparent, particularly in progenies bred from intermediate types of resisters. Since this material was not expected to be of commercial standard, selections from groups (1) and (2) were retained for observation in the field and for further breeding. A few similar selections of comparable origin raised in previous years gave heavy crops of tubers in the field plots and were successfully intercrossed and backcrossed in accordance with the programme. The wild species *S. demissum*, *S. stoloniferum* and *S. simplicifolium* were the main sources of field resistance in these experiments.

Since the type of potato root eelworm resistance present in C.P.C. 1673 (*S. tuberosum* subsp. *andigena*) has been made available for routine selection in the commercial breeding programme, the eelworm resistance of *S. vernei* has become the subject of the experimental breeding. As a parent species *S. vernei* has the advantage that its eelworm resistance remains effective against the "aggressive" eelworm populations which are known to overcome resistance in C.P.C. 1673. The fact that *S. vernei* is a wild diploid species does, however, present certain difficulties not encountered in breeding with subsp. *andigena*. The first of these difficulties, namely the initial hybridisation with commercial potato varieties, has already been overcome by chromosome doubling in certain *S. vernei* clones as a result of colchicine treatment. The proportion of resistant hybrids obtained, and the general level of their resistance, remains to be ascertained.

Since breeding from *S. vernei* is still at an early stage, it seems desirable to explore other sources of eelworm resistance at the present time, to discover possible alternative or complementary kinds of resistance and thus to gain more insight into the host/parasite relationship. For instance, *S. demissum* certainly possesses a degree of resistance to the "aggressive" eelworm population used for test purposes at Pentlandfield and the cross *S. vernei* × *S. demissum*, which has already been made, is expected to give tetraploid seedlings which may in turn be crossed with commercial varieties. Initially promising results have also been obtained with several other provisionally identified wild species and evidence for a delicately balanced host/parasite relationship is accumulating.

Small virus-tested stocks of twenty-four commercial potato varieties and seventeen advanced seedlings in various stages of multiplication were grown at Blythbank. Tests made during the season revealed a few plants infected with virus S in one commercial variety and a small number of plants infected with virus A in another. The affected plants were removed and no further infections were detected.

In work on virus S, the paracrinkle strain and our stock strain from Arran Victory were compared in their effects on potato varieties and in their rates of spread under field conditions. Of twenty-six varieties infected with the Arran Victory strain of the virus, none showed obvious symptoms of infection but, as in previous years, all infected plants matured earlier than their respective healthy controls. Plants infected with the paracrinkle strain differed widely. Some varieties were apparently symptomless while others were stunted and developed malformed leaves with chlorotic and necrotic areas. Tuber yields from the more severely affected plants were greatly reduced and many tubers showed cracks and internal necroses. There was no evidence of the natural transmission of paracrinkle from King Edward to any one of fifteen commercial varieties although there was a high rate of transmission from Arran Victory of the strain normally present in that variety.

A further collection of South American potato material was examined for resistance to virus S but none was found. When the same material was tested for reaction to viruses X and Y, immune, necrotic and tolerant responses were observed with each virus.

A field trial, laid down in 1956 to assess the behaviour of the German varieties Apta and Virginia towards leaf-roll and virus Y and to re-test S.S.R.P.B. seedling 1591(b)9, was completed. The number of infections recorded in fifty plants of each variety are given below together with corresponding data for the control varieties Arran Banner, Dr McIntosh and Craigs Snow White.

TABLE I.

Variety	No. of plants infected with	
	Virus Y	Leaf-roll
Apta	4	0
Virginia	6	0
1591(b)9	0	1
Dr McIntosh	2	8
Arran Banner	0	0
Craigs Snow White	30	0

It is clear that virus Y spread readily to Craigs Snow White but to a much smaller extent to Apta, Virginia and Dr McIntosh. The latter varieties are thus judged to be less susceptible than Craigs Snow White to virus Y, and more susceptible to the virus than either Arran Banner or 1591(b)9. Only Dr McIntosh showed any appreciable infection with leaf-roll and no comparison is possible between the other varieties.

A second randomised trial of a similar nature contained 100 tubers of each of five clonal stocks of Majestic which were tested previously in 1954. The results of both the 1954 and the 1956 trials are given below.

TABLE II.

Clone	No. of plants per 100 infected with			
	Virus Y		Leaf-roll	
	1954	1956	1954	1956
4A/48 .	0	12	5	9
46/53 .	3	13	6	12
46/67 .	9	11	7	12
46/78 .	6	11	9	5
46/104 .	3	7	3	10

In 1954 there were rather more infections with both viruses than in 1956, but there is little correspondence between the two years in the distribution of infections among the clones. None of the inter-clonal differences is significant and it is concluded that the earlier indication of clonal variation affecting susceptibility to leaf-roll and virus Y is not substantiated.

Genetical studies on various characters associated with resistance to potato viruses were continued. In *Solanum acaule*, it was established that immune, necrotic and tolerant reactions to virus X are determined by an allelomorphic series of three major genes. The reaction brought about by each gene was independent of strain among several strains of virus X tested. In this respect, the gene controlling necrotic reaction in *S. acaule* differs from the Nx and Nb genes found in *S. tuberosum* as the reactions incited by these genes are dependent upon strain. Material raised for a closer examination of the relationships between the genes controlling immune and necrotic reactions to virus X in *S. tuberosum* segregated in such a way as to provide conclusive evidence of a single gene in control of immunity but the data were insufficient to decide whether it was a dominant allelomorph of Nx or Nb or independent of them with epistatic effect.

The virus strain X^D is known to activate the gene Nx with lethal necrotic effect and it has been reported to kill, also, a few varieties which do not possess Nx. Investigation of this apparent anomaly has shown that the strain causes a non-lethal necrosis, distinguished as foliar necrosis, in the presence of the gene Nb and that this reaction is intensified to a near lethal level in material that is duplex for Nb. In the absence of Nx and Nb the virus causes no severe necrosis.

Experimental breeding has revealed that necrotic response to virus Y in *S. demissum* is always accompanied by necrotic reaction to virus A. A single gene controls this double response and joint segregation data from seedling progenies raised during the year show that this gene is dominant over, but allelomorphic with, a second gene which controls necrotic reaction to virus A only. These relationships between genes and viruses accord well with the previous discovery of virus strains having properties common to both virus Y and virus A (Rep. S.P.B.S. 1957) and suggest that the two viruses are themselves related.

An established, though hitherto unused, source of resistance to virus Y is known in the *S. chacoense* clone C.P.C.51b. From breeding experiments with this material, it was found that the resistance is due to gene-controlled hypersensitivity to the virus.

In the breeding programme for combining resistance to viruses, over 3,000 seedlings were raised from hybridisations between various wild species and commercial varieties. The whole of this material was tested for resistance to virus X and/or virus Y in the greenhouse, and only the survivors were retained for further testing and selection in the field.

Serological work on the differentiation of strains of potato virus X was continued but the preparation of specific antisera still presents difficulties and results obtained with the more specific antigenic fractions are rarely reproducible. In attempts to find a suitable technique for this purpose, two methods tested proved to be useful for the preparation of routine antisera to virus X. These two methods are discussed in an Occasional Paper (p. 77).

The results of work on the cucumber mosaic virus found in potato last year are recorded in another Occasional Paper (p. 75).

In 1957 the Registration Trials conducted by the Department of Agriculture for Scotland contained twelve of the Station's seedlings. Five of them have been recommended for further trial in 1958, three in the 2nd Year and two in the Final Year Trials. A further group of eight seedlings has been submitted for the 1st Year Trials in 1958.

The bulk stock of Pentland Beauty was grown in 1957 by the agents, Messrs B. Main & Co. Ltd., Perth, and covered approximately 3 acres. The crop was examined during the growing season and found to contain virus S. In view of the widespread occurrence of this virus in nearly all commercial varieties throughout the country and the desirability of building up virus-free stocks where possible, it was decided to discontinue the multiplication of this stock and to revert to the small virus-free stock maintained by the Society. Accordingly the virus-free stock will be multiplied in 1958 and then passed to the agents for further multiplication and distribution.

Root Crops—

Leafy Brassicas.—Several lines of investigation are being carried out with leaf bearing forms of *B. Napus*. Samples of Giant and Dwarf rape were sown along with kales on different dates in the spring and summer, and were examined for their speed of growth, maturity and leaf production after mowing. Later the plots were examined for rape plants which seemed to have held their green leaves longer into the winter than the others, and selections were taken. Intraspecific crosses were made between forms such as giant rape × hungry-gap kale for the study of growth rate and maturity. Interspecific hybridisation between forms of *B. Rapa* and *B. oleracea*, in the hope of obtaining a synthetic amphidiploid akin to *B. Napus*, has been practised on a small scale for many years. Using turnip as female and pollinating with a kale, shrivelled embryos and very occasionally a partly filled seed were obtained, but it was not till 1954 that a hybrid plant was successfully grown; the parents were The Bruce turnip × a hybrid kohl rabi × thousand-headed kale. A rather weak plant grew in 1955 and flowered in 1956, but despite colchicine treatments no seed was obtained that year. It was kept alive and multiplied by cuttings in 1957, and these were treated by applying drops of colchicine in different concentrations on axillary buds. Unaffected racines grew very long and bore flowers with normal petals but no anthers. Some treated racines had flowers close together with malformed petals, but full and dihiscent anthers. Fifteen seeds were obtained by bud-selfing and twenty-four by using the pollen on an emasculated hungry-gap kale (*B. Napus*). Cytological observations supported the supposition that the plant had $2n = 19$ chromosomes, and that parts affected by colchicine had $2n = 38$, the accepted number for *B. Napus*.

Most of the work on leafy brassicas, however, has been within *B. oleracea*, the cabbage tribe. The date of sowing trial, as well as the rapes mentioned above, included thousand-headed kale and marrow-stem kale samples, also three of the hybrid derivatives which will be discussed later. Observations on rapidity of growth, height, and loss of leaves were made during the season. The plants were grown in thinly sown but unthinned rows, and there was little side shoot development under these conditions. At 100 days from sowing, a strip was cut from a plot of each strain and samples from some strains sown on other dates were also taken. The stems and leaves per strip were weighed and the stems were measured individually to ascertain the variability. Samples of leaf were then separated into petiole-midrib and lamina and the dry matter in each determined. The recovery of leaves on the mutilated stumps, which is particularly good in rape forms, was also noted. These trials were unreplicated and were made in an attempt to devise suitable criteria for judging leafy brassicas. The rest of the work consisted of breeding and comparing lines bred from kales and other forms of *B. oleracea*.

Marrow-stem Kale type.—For certain purposes, such as the grazing of close spaced plants, the leafage of the marrow-stem kale may be of more value than the stem. An attempt to combine heavy leafage with a short stem was started in 1951 when plants of a short-stemmed strain were crossed with selections from a strain known as New Zealand "Hybrid" kale, which had a large amount of foliage persisting into the winter, but which had stems as long or even longer than the normal marrow stem. As the lines derived from these crosses became inbred they were intercrossed to restore vigour, and in 1956 some out-crosses with normal marrow-stem kale were also made. This year there were two trials, one of seed sown thinly in rows and left unthinned, and the other of plants transplanted from seed beds, the sowing dates in both cases being mid-April. In the unthinned rows the marrow-stem control had a modal stem length of about 37 inches by the beginning of September, and as usual it had the greatest gross weight ; its yield of stem was 2.8 kg. per yard of row, and its yield of leaf only 2.0 kg. The best of the experimental material was an F_2 of a cross between two of the hybrid lines, M45(ab), which had a stem length of 21 inches, only 1.1 kg. of stem, but 3.3 kg. of leaf per yard. Other lines were not so vigorous and the only new outcross in this trial, M64, yielded badly and had a very low ratio of leaf : stem.

The trial of marrow-stem transplants included a fairly comprehensive range of relationships, and may be worth considering in that connection. The trial consisted of five randomised blocks, and each plot contained 15 plants spaced at 24″ × 24″. Only three plants adjoined a path, and these were discarded when taking eight for individual measurement and weighing. Damaged and stunted plants were also rejected, and the results computed from average values of the plants in each plot are shown in Table I.

TABLE I.

MARROW-STEM KALES AS SPACED PLANTS

Name and Description	Stem length, inches	Ratio Leaf wt. : Stem	Mean Plant weight in kg.		
			Gross	Stem	Leaf
Normal Marrow-stem kale strains A	35	0.6 : 1	3.1	2.0	1.2
B	30	0.7 : 1	2.7	1.6	1.1
C	31	0.8 : 1	2.8	1.6	1.2
mean	32	0.7 : 1	2.9	1.7	1.2
M34, short-stem marrow kale, parent strain	14	2.1 : 1	2.2	0.7	1.5
M34 × N.Z. Hybrids					
M41Aa, F_3 gen. self.	11	2.7 : 1	1.7	0.5	1.2
M41(AC)a, F_3, cousins	15	2.4 : 1	2.1	0.7	1.5
M45a, F_2 (M41 × M38A)	12	3.2 : 1	2.4	0.6	1.8
M58, BX (M45 × M41A)	12	3.5 : 1	2.2	0.5	1.7
M57, F_1 (M44 × M41A)	12	4.2 : 1	2.3	0.5	1.9
Hybrids × Normal Stem					
M64, F_1, (M41C × M(i))	21	1.6 : 1	3.3	1.3	2.0
M61, F_1, (M45 × M(ii))	18	1.8 : 1	3.0	1.0	1.9
M60, F_1, (M40 × M(ii))	20	1.3 : 1	2.7	1.2	1.5
Significance (P = 1%)	3.6	..	0.59	0.31	0.43

There were three samples of commercial marrow-stem kales, which all showed the low ratio of leaf : stem weight, though strain A had better developed stems than the others. There was also a strain M34, selected from a short-stemmed marrow-kale stock which had been used as a parent in the crosses with New Zealand "Hybrid" kale. This line had a leaf : stem ratio of 2.1 : 1, its stem was less than half the length of the normal kale and was significantly lower in weight but despite inbreeding M34 yielded as much leafage as the control. The hybrid M41 is connected with several of the treatments. There were two F_3 samples, one of which, M41Aa, had been obtained by self-fertilising an F_2 plant, and the other M41(AC)a by crossing two F_2 plants which were cousins. The first mentioned was in very feeble condition, several plants in each plot being stunted or dead; while M41(AC)a was somewhat better and yielded rather like the parental M34. Both exhibited a tendency to side shoot development which was absent in the true marrow-stems, and of less frequent occurrence in the other treatments. The sample M45a was an F_2 descended from two of the original hybrids, of which M41 was one; M58 was a back-cross on M45 by an F_2 plant of M41, and M57 was an F_1 having the same M41 parent. All three had short stems, high ratios of leaf : stem, and good yields of leafage, but their gross yields were less than those of normal marrow-stem strains. Lastly there was a group of three F_1 constituents of crosses between the experimental lines and plants selected from a marrow-stem kale crop. Their stems were intermediate in length and weight between those of the parental types; their gross yields were well up to those of commercial kale, and their yields of leaf were comparable to those of the experimental lines and significantly higher than in commercial strains.

Thousand-headed Kale types.—Some Station strains and commercial varieties were transplanted and observed in a trial, but no weighings were made. Commercial seed was also sown and singled to provide a crop for selection, and samples were included in date of sowing trials mentioned elsewhere. The poor results obtained from some experimental hybrids in the past have been traced to the use of inferior plants of thousand-headed kale as parents. Commercial plants show a wide range of variability in the incidence, speed and extent of their side shoot development, and a careful examination was made of all available material. Those plants which appeared precocious were marked, and if they developed favourably they were selected and cuttings were struck for use in the greenhouse.

Broccoli × Thousand-headed Kale.—In 1957, F_3 progenies of selected plants had become available, and it was possible to see some effects of selection. There were two main groups of broccoli hybrids, one descended from Royal Oak, an April-heading type, and the other from Veitch's Self-Protecting broccoli which heads in the Autumn and is noted for its large leaves. To obtain information about the development of plants growing closely together, a trial was sown on 11th April in short rows which were cleaned of weeds but left unthinned. The full details of this trial, which contained both kinds of broccoli hybrid, and also kohl rabi × kale, cannot be given here, but a few points may be noted. The height to the top leaf in each plot was measured periodically; on 12th September the thousand-headed kale control was 37 inches high, and this was exceeded by only one of the hybrids, several of which were significantly shorter, the lowest being 25 inches. The thousand-head also had the greatest yield of stem, 1.4 kg. per yard of drill, and most of the hybrids gave about half that amount. The yield of leaf varied, however, for some hybrids yielded more and some less than the 3.1 kg. of the thousand-head. Individual measurements of stem length were made on the plants of each plot, so that variability could be compared.

There were two trials of transplants, one containing most of the Veitch's broccoli hybrids and the other the Royal Oak derivatives, one or two being interchanged to afford comparisons. The first trials contained a number of F_3 progenies of plants selected from several F_2 populations of Veitch's self-protecting broccoli × thousand-head. Selection had in most cases been directed towards segregates which had a short stem and numerous side stems bearing a compact rosette of narrow rigid leaves. This was the predominant phenotype of the progenies, whether descended from selfed plants, sib or cousin matings. Autumn flowering is the main trouble in this group, and the incidence of bolting was noted. In November all the plants which had flowered were dug up, and the remainder were kept till late January when a few were selected as winter hardy and late flowering types, but nearly all the plants were showing flower buds on that date.

TABLE II.

Thousand-headed Kale × Royal Oak Broccoli

Name and Description	Stem length, inches	Ratio Leaf wt. : Stem	Mean Plant weight in kg.		
			Gross	Stem	Leaf
Marrow-stem kale	37	0.5 : 1	3.00	1.98	1.02
Thousand-head	21	2.2 : 1	1.83	0.58	1.25
Cross $TH_4 : BR_2$					
F_2-generation parents were Sibs. –c–	8	5.4 : 1	1.65	0.27	1.39
–e–	12	3.6 : 1	1.71	.38	1.34
mean	10	4.5 : 1	1.68	0.32	1.37
F_2-gen. parents were cousins –(cm)–	14	3.7 : 1	2.08	0.45	1.63
–(cb)–	12	4.2 : 1	1.60	.31	1.29
–(ce)–	10	4.9 : 1	1.67	.29	1.38
–(bm)–	11	4.6 : 1	1.78	.32	1.46
mean	12	4.4 : 1	1.78	0.34	1.44
Backcross onto 1000-headed kale	18	2.9 : 1	2.30	0.60	1.70
Cross $TH_4 : BR_4$					
F_2-gen. from mass seeding –G	13	3.2 : 1	1.49	0.36	1.12
–H	13	3.4 : 1	1.71	0.40	1.32
mean	13	3.3 : 1	1.60	0.38	1.22
Significance ($p = 1\%$)	3.0	1.0	0.45	0.16	0.38

The second transplant trial of hybrids consisted of sixteen treatments replicated five times, and each plot contained twenty plants, only four of which bordered on paths. Two treatments, which had Veitch's broccoli ancestry were the only ones to flower, and these had to be discarded. In the rest of the treatments, ten undamaged plants were cut from the inner parts of each plot and recorded individually for weight and length of stem, development of side shoots and weight of leaves. The mean values shown in Table II. illustrate breeding within a small group, for all eight lines are descended from one thousand-headed kale plant, TH_4, which proved the best parent then available. It was crossed with two Royal Oak plants, BR_2 and BR_4, and in each case selected plants of the F_1 generation were seeded together and harvested separately. The two samples of $TH_4 : BR_4$ –G and –H at the foot of the table are F_2 progenies but the other group, $TH_4 : BR_2$ has progressed another generation by hand-crossing of brother and sister plants, *i.e.*, sibs, while the other four were obtained by intercrossing cousin plants. The backcross was onto an unrelated plant of thousand-headed kale. The hybrid lines were all shorter in stem length and lighter in stem weight than the commercial thousand-headed kale control; but the backcross had long stems which weighed as much as the control. This backcross and one line, –(cm)–, had significantly more leafage than the control, the other lines gave higher yields of leaf weight but the differences were not individually significant. The ratios of leaf : stem were higher in the hybrids than in the control, but the gross yields were not significantly different, except perhaps in the backcross. Comparing sib and cousin matings in cross $TH_4 : BR_2$, no significant differences are apparent, one of the sibs, –c–, was poorly with a short stem, but the other, –e–, was quite healthy. In leaf habit and general appearance these six hybrid lines looked very similar; the backcross resembled a thousand-head. Lines of the two crosses could be distinguished, and $TH_4 : BR_4$ gave the impression of greater height which was significant between groups but not for individual treatments. The leaf weight of $TH_4 : BR_2$ appeared to be greater than that of $TH_4 : BR_4$, but this was not significant at the 1 per cent level.

Other hybridisations.—A few lines descended from kohl rabi × thousand-head were sown in the seed bed, but there were insufficient plants for a trial, so they were transplanted to observation plots. Compact rosettes of rather small leaves characterise these hybrids, and though short side stems may increase the leafage carried, the plant weight is generally rather light. Some of the lines still have a swollen stem, but this can be bred out, as was shown by Pease (1927, J. Gen. **17**). The use of "early" varieties of kohl rabi leads to considerable autumn flowering, but other varieties do not transmit this tendency. Since short stem types are not segregating from the Royal Oak broccoli crosses, and since heavy yields of foliage are not forthcoming from the kohl rabi × thousand-headed kales derivatives, the intercrossing of these hybrids might give better opportunities for recombination. Several of these "Triple" hybrids have been examined, but so far nothing very attractive has emerged. In order to widen the field of investigation a number of crosses between plants of thousand-headed kale and sprouting broccoli were made in 1956. Samples of the F_1 generations were sown in the seed bed and planted out in the form of a trial, but no weighings were made. The F_1 plant is large with a considerable yield of rather unattractive looking leaves. The stems are long and the side shoot development slow and inadequate but there was no Autumn flowering. Plants were chosen from three crosses in which there were no common parents, and cuttings were taken for greenhouse propagation. An F_1 generation of sprouting broccoli $\times$ marrow-stem kale was also observed. Attempts are now being made to obtain plants of late forms of cabbage in flower at a time suitable for crossing with thousand-headed kale, but the only cabbage crosses available for the trials were two F_1 generations of spring cabbage $\times$ thousand-headed kale. These hybrids formed loose hearts of cabbage-like leaves, and showed strong tendencies to flower in autumn. Before bolting the stems were relatively short, and side shoot development was rare.

Sugar Beet.—The programme included the arrangement of yield trials to assess bolting behaviour and to test yield in sugar beet bred by the Station, and also in lines and polycrosses bred by the Plant Breeding Institute, Cambridge, for their non-bolting strains, KNB and KLT ; two centres were used, one at Pentlandfield, and the other on a farm at Musselburgh. A seed bed trial for the production of stecklings was laid out with four strains of seed as sub-treatments in replicated plots sown on six different dates between mid-March and mid-July. Observations on bolting and plant size were made at Pentlandfield and certain groups of stecklings were sent to Cambridge for an investigation by that Institute. Seed was obtained from plants flowering in isolation plots in the neighbourhood, but this year it was very poor in quality ; better seed was harvested for the Station from isolation plots in Cambridgeshire. Plants subjected to light treatment during the spring of 1956 were transplanted to the field, and those least affected by bolting during the summer were selected for propagation. Further applications of light treatment were given to seedlings in the winter of 1956-57. Progenies of male-sterile mother plants which had been pollinated by a Station line were tested in a yield trial, and male-sterile and monogerm sugar beet plants were examined in the greenhouse where various hybridisations were made by bag-pollination.

At Pentlandfield the earlier sown trials afforded severe tests of resistance to bolting. Thus the Klein E control sown in the first trial on 15th March had 42 per cent of its plants bolted in September, while in the second trial sown on 27th March there were 21 per cent. In contrast the last trial sown on 16th April had only 2 per cent of the Klein E plants bolted. At Musselburgh the trials were all sown on 28th March, and Klein E showed 12-14 per cent of bolters on 10th September. The Cambridge lines were in trials at Musselburgh, and they showed quite satisfactory resistance. Station lines showed various grades of resistance, mostly good but a few sufficiently bad to disqualify them.

There were three groups of breeding material under observation, the first consisting of some fifteen lines bred from roots selected in 1948 at Logie Farm, Newburgh, on the basis of shape and quality but not foliage type ; secondly some male-sterile progenies and thirdly lines and progenies of crosses bred with a view to combining large foliage type with bolting resistance and yield. The sugar beet seed harvested in Scotland after the dry summer of 1955 was in excellent condition, and this was the first time that most of the Logie lines had been tested on comparable terms with the commercial controls. Fifteen lines were tested in the early sown trial at Pentlandfield and four of them were repeated in a trial at Musselburgh. Three of the Logie lines attracted attention with high yields and fair resistance to bolting, but unfortunately their tops were less than average weight.

Plants from an American stock of male-sterile sugar beet were exposed to pollen of the family Logie —10. Judging by its performance as shown in Table I., this family was not a fortunate choice, but it was fancied in 1953, and male-sterile plants selected from the progenies were again supplied with pollen from Logie —10 in 1955. Sixteen progenies of this backcross were included in a trial at Musselburgh, and in Table I. their range of performance is compared with values found in Klein E and in a sample of the male-parent family Logie —10. One line did equal Klein E in sugar yield, but in general the lower root yields were not sufficiently compensated by the higher sugar percentages. Resistance to bolting is shown by all the lines, but some had significantly large concentrations of noxious nitrogen which was somewhat high in Logie —10.

Many Scottish growers use beet tops for feeding, and more importance appears to be attached to a heavy yield of foliage than is the case in the South. It was, therefore, requested that attention should be paid to this requirement in conjunction with freedom from bolting. It is possible that the two characters may not be readily combined in the same plant, and this is suggested by many of the non-bolting lines which have been examined and found to have relatively small foliage.

TABLE I.

TRIAL OF MALE-STERILE PROGENIES AT MUSSELBURGH

Character	Klein E	Logie —10	Male-sterile lines	
			Greatest	Least
Sugar yield in cwt.	43.5	31.8	43.5	30.5
Root yield in cwt.	283	202	264	185
Sugar content (%)	15.3	15.8	17.1	15.8
Root weight in lb.	1.82	1.29	1.67	1.19
Sugar per root in lb.	0.285	0.206	0.273	0.194
Noxious nitrogen	38	46	73	36
% bolted on 24/8/56	8.1	0.5	1.3	Nil

The first group of selections for foliage size was chosen in 1949 from a standing crop at Scotscraig, Tayport. There was no opportunity, however, of selection for shape, size and quality of root, and some of the lines were very inferior in these respects. Most of these Scotscraig lines have been discarded as worthless, and only one was present in the 1956 trials. This yielded well, but was below average for weight of top. In 1955 crosses were made between plants of different varieties which had large foliage. One series was obtained by planting half roots of Bush and British S.K.W. together in pots which were isolated in different greenhouses. Six pairs of plants gave sufficient seed to be included in a trial. The offspring borne on a half-root was sown in four rows of twenty-four plants each in randomised blocks. Each mating was represented by reciprocal half-roots and in some cases all four half-root progenies were included, so that comparisons could be made of the variability between and within matings. Sown on 16th April, only 2 per cent of bolters occurred in the Klein E control, 3 per cent in Bush E, but over 12 per cent in British S.K.W. One mating bolted badly and was rejected, the other ranged from zero to 3 per cent. In October the plants were lifted, the foliage cut off above the crown and weighed individually. The root was then cleaned, weighed and tested for specific gravity of the juice. Two matings gave combinations of good-sized roots and heavy foliage, and the best plants of these were kept for propagation. It was noticed, however, that these two matings each showed 3 per cent of bolters, while a mating with deficient foliage had none.

Again with the object of obtaining large-leaved inter-varietal hybrids from which to breed lines, selected plants of four varieties were interplanted in a glasshouse compartment in 1955, and, after seeding together, were harvested separately. These were plants of Bush E and British S.K.W. selected from early sown plots, and also plants of the Italian varieties Cesana P and Italian 8, which had been sown later, and were selected in September when leaf growth was vigorous. Thirteen of the progenies were sown on 27th March 1956, in a trial at Pentland-field. Conditions were conducive to severe bolting, and the Klein E control, as mentioned above, had 21 per cent of bolters in September. A sample of Bush E showed 14.5 per cent and British S.K.W. had 41 per cent ; controls of the Italian varieties were not available. The progenies of the Italian mother-plants bolted freely, Cesana P having five progenies ranging from 24 to 32 per cent bolted, and Italian 8, two progenies, one with 33 and the other with 63 per cent. One progeny of a British S.K.W. mother-plant had 28 per cent, but another only 15.5 per cent. The progenies of Bush E mother-plants varied, two were resistant with only 7 per cent, one had 13 and the fourth had 26 per cent. Given material which was favourable in other respects, bolting might be reduced by light treatment, but the progenies of the Italian mother-plants tended to mature early, and though their leafage was very large during the summer, it had gone down by the time of harvesting. Thus three of the Cesana P progenies were below the average (1.63 : 1) for ratio of top : root weight, and only one was high at 2.01 : 1. This one had low root weight and sugar yield, but the other Cesana P progenies were at least average. The two progenies of Italian P had such small roots, and consequently low sugar yields, that the high ratios of 2.2 : 1 were of no practical value. One of the two British S.K.W. progenies combined relatively good root weight with a high top weight, giving a ratio of 1.81 : 1. The four Bush E progenies all had root weight and sugar per root above average, but the higher the yield the smaller the top and the narrower the ratio. With only four types of pollen available, it may be supposed that considerable numbers of the offspring would have parents of the same variety, especially if there were differences in date of flowering. The appearance of the foliage during the summer as well as the trial data quoted above, supports this and further generations of interpollination would be needed before a line became predominantly hybrid. One of the Cambridge-bred non-bolters, a line of Dhrobnovice, was thought to possess a large type of foliage, and lines were selected from a polycross population in which this had been the mother-parent. Nine of these lines were included in the above trial ; their top weights were not outstanding.

Publications

Black, W., and Gallegly, M. E. (1957). Screening of *Solanum* species for resistance to Physiologic races of *Phytophthora infestans*. *Amer. Potato Journ.*, **34** : 273-281.

A large number of clones and lines of *Solanum demissum* and other *Solanum* species maintained in the Commonwealth Potato Collection in Great Britain and by the Potato Introduction Station in U.S.A., were screened for resistance to potato races 1,2,3 ; 1,2,4 ; 1,3,4 and 1,2,3,4 of *Phytophthora infestans*. The results indicate that one or more unidentified R genes, different from the four now recognised, are present in the collections. In addition, very high levels of polygenic resistance were encountered making clear-cut genetic segregations difficult to obtain.

Species in which highly resistant individuals were found were *S. demissum, S. stoloniferum, S. pinnatisectum, S. bulbocastanum, S. guerreroense, S. oxycarpum, S. polyadenium, S. polytrichon, S. spectabile* and *S. verrucosum*.

Cockerham, G. (1958). Observations on the spread of virus X. *Proc. Third Conf. Pot. Virus Diseases, Lisse-Wageningen*, 1957.

In early experiments, the spread of potato virus X between infected and healthy plants in direct contact was found to be too low to account for much of the spread encountered in general farm practice. The spread of the virus was followed, therefore, over a period of four years, 1951-1954, in several field crops of the variety Majestic all of which were derived from a single stock but which were subsequently grown under different environmental conditions. It was found that in undisturbed crops grown in isolation the annual increase in the amount of virus was approximately twofold whereas in crops grown near to badly infected stocks the rate of increase was considerably greater due to infiltration of the virus from the outside sources. Circumstantially, the evidence pointed to the implements of cultivation as the main vehicles of transfer of the virus.

COCKERHAM, G. (1958). Experimental breeding in relation to virus resistance. *Proc. Third Conf. Pot. Virus Diseases, Lisse-Wageningen*, 1957.

Genetical data from breeding experiments within *Solanum acaule* and *S. demissum* are given. They show that in *S. acaule* reaction to potato virus X is controlled by three allelomorphic genes which, in descending order of dominance, determine immune, necrotic and tolerant responses. In *S. demissum*, genes controlling necrotic reaction to both virus Y and virus A, necrotic reaction to virus A but not to virus Y, and anecrotic tolerant reactions to both viruses are also shown to be alleles. An inference, drawn from the genetic relationships in *S. demissum* and supported by external evidence of similarities between the viruses, is that virus Y and virus A are themselves related.

DAVEY, V. McM. (1957). Brassica root crops in Scotland. *Genetica Agraria*, **8** : 143-150.

The chief root crops of Scotland belong to two species of Brassica, the common turnip (*B. Rapa* L.) with 10 chromosomes haploid and the Swede or Swedish turnip (*B. Napus* L.) which has $n = 19$ chromosomes. Since 1913 there has been a decline in the percentage of arable land growing these crops because of high labour costs and *Plasmodiophora* disease, but they are still very important. The swede is regarded as an amphidiploid containing genomes similar to those of the elemental spp. *B. Rapa* L. and *B. oleracea* L. Colour variations occur in analogous series, but some major genes are duplicated in the Swede while seemingly monohybrid in the turnip. The propagation methods used by seedsmen in Britain are described, and the results obtained by the author when line breeding and inter-crossing types of Swede are briefly summarised.

DUNNETT, J. M. (1957). Variation in pathogenicity of the potato root eelworm (*Heterodera rostochiensis Woll.*), and its significance in potato breeding. *Euphytica* **6** : 77-89.

Breeding for resistance to *Heterodera rostochiensis* was started in Scotland in 1952, using certain clones of *Solanum tuberosum* subsp. *andigena* as the source of resistance. Resistance was manifest by the almost complete failure of larvæ to mature in the roots of resistant seedlings, particularly those derived from the clone C.P.C. 1673.

In 1955 an eelworm population was found which overcame this resistance and was, for this reason, designated as "aggressive."

Resistance was also overcome in selfed and hybrid seedlings derived from subsp. *andigena* C.P.C. 1685 and C.P.C. 1690. Resistance in *Solanum vernei* was apparently maintained against the aggressive eelworm population which was studied. This aggressive eelworm population multiplied normally on commercial potato varieties.

In a first survey of potato root eelworm infested soils in Britain about 10 per cent of the eelworm populations sampled were found to differentiate little or no resistance in a test plant which was at least simplex for a resistance factor *H*, derived from C.P.C. 1673.

HARBERD, D. J. (1957). The Within Population Variance in Genecological Trials. *New Phytol.*, **56** : 269-280.

In a population study of *Festuca ovina* the individual plants were cloned, thus making it possible to partition the within population variance into its genetical and non genetical parts. The high genetical variation within populations, especially in view of the low one between populations, suggests that the characters recorded (which are traditionally regarded as of great adaptive importance) have little selective value.

It was demonstrated that genotype reduplication in population sampling can lead to serious errors in interpretation, making the significance of difference between populations spuriously high. By virtue of its habit of growth genotype reduplication seems unlikely in *Festuca ovina* and it is noteworthy that population differences were infrequent in this study.

HARBERD, D. J. (1958). A Spurious Significance in Genecological Trials. *Nature*, **181** : 138.

Further work on genotype reduplication in population sampling has indicated that it does sometimes reach very high proportions. One genotype of *Festuca rubra* accounts for one-third of the tillers of that species in an area of some nine acres of natural grassland, so that ordinary population methods would be unsuitable for its genecological study. It is pointed out that such a genotype must be both old and adaptable.

WILKINS, D. A. (1957). A Technique for the Measurement of Lead Tolerance in Plants. *Nature*, **180** : 37-38.

Calcium was found to reduce the toxicity of lead to roots of *Festuca ovina*. In the presence of calcium the rates of root growth with and without the addition of 25 ppm. lead (as nitrate) were compared under standardised conditions. An Index of Tolerance derived from this was used to distinguish tolerant from intolerant plants, and the results were found to agree reasonably well with the amounts of lead in the soils from which the plants came.

INVESTIGATIONS ON THE COMPOSITION OF ECOTYPICAL AND VARIETAL POPULATIONS

A brief survey of some of our works published in Russian

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Our pre-war investigations on ecotypical populations were summarised in the book: 'Dynamics of Species' (1948). These investigations were conducted on geographical and zonal lines mostly with wild plants.

After the war we resumed our explorations of zonal variability of species of the genera *Onobrychis* (sub-section *Vulgatae*), *Bromus*, wild species of *Secale* and others and have continued them up to the present day. The following vertical belts of *Onobrychis*-populations are clearly distinguished in the Northern Caucasus:—

1. The high mountain belt. Under cultivated conditions, plants from this belt do not blossom during their first year of life and in some cases not even in the second year but when they do come to flower in the second and following years they are earlier than the plants from the lower belts. The high mountain belt is one of hardy, slow-growing, early flowering, winter perennials.

2. The middle-mountain belt where only very few plants come to blossom during the first year of their life. In the following years they begin to flower later, and their stems are considerably higher.

3. The sub-mountain belt where the majority of plants come to blossom during the first year of their life though their flowering is relatively late. These plants are spring tardi-florous perennials.

4. The belt of adjacent steppes where are distributed plants which all come to blossom in the first year of their life. They are more or less drought-resistant and may be named spring perennials.

In this series all the morphological characteristics change very gradually: from the upper to the lower belts the size of the flowers and pods diminishes; the degree of toothyness of the pods (legumens) gradually diminishes too; the colour of the petals becomes less bright; the leaflets become broader and so on. It is, therefore, extremely difficult to draw a distinguishing line of morphological characters between the *Onobrychis* species, so that the Caucasian *Onobrychis* species are really "botanicorum crux et scandalum." It is much easier to draw a distinguishing line between vertical belt-populations and to establish new species corresponding to these belts of populations, on the basis mainly of biological and ecological characteristics.

The distribution of biotypes along the vertical belts of *Onobrychis*-populations follows the law of zonal population variability (with respect to young species and with more or less continuous distribution—without effective barriers). This law was formulated in our book 'Dynamics of Species' as follows: "The course of the successive variability in the population compositions in the process of zonal ecotype (and species) formation is expressed by the system of spiral intergrading series in which some variants are gradually eliminated, and others gradually change in the direction of the change in environmental conditions. At the same time, analogous variants (ecoelements) in neighbouring (zonal or vertical) series are not wholly identical and express successive stages of natural selection" (Sinskaya, 1948).

Later we establish that such a regularity also occurs in ecological-geographical investigations of those cultural plants which are widely distributed. For a period of two years A. M. Gorsky, under our guidance, studied the composition of the populations of a great number of samples (200) of local, and breeding, varieties of winter wheat and rye in four geographical localities. The varietal populations of winter wheat and rye in conditions of unusually late spring sowing, fall into three main ecoelements (definite groups of biotypes): (1) with early emergence of ears in the year of sowing; (2) with late emergence of ears in the year of sowing; (3) those remaining in the vegetative stage in the year of sowing. The third ecoelement differs from the others in that it has the longest stage of jarovization. The local and even the so-called "pure line" varieties show the same mode of composition. The first ecoelement with early emergence of ears predominates in the South and can be met with there also in a pure state in the population—without admixture of two other ecoelements. The third ecoelement predominates or is met with in a pure state in the North. The most complicated populations are concentrated in the middle latitudes—in the intermediary zones. These complicated populations present the greatest possibilities for selection. The analogous biotypes (ecoelements) in the populations originating from remote geographical regions are only similar, but not wholly identical. The plants belonging to the third ecoelement (which do not ear during their first year of life) have the longest stage of jarovization, especially when they originate from districts with comparatively mild climate and deep snow cover. The plants belonging to the third ecoelement of North-Western districts of the European part of U.S.S.R. have a longer stage of jarovization than typical Siberian winter wheat. The plants belonging to the first ecoelement (with early emergence of ears) taken from Southern varietal populations are usually more heat-resistant and less hardy in winter in comparison with analogous biotypes from more Northern localities.

The ecological classification of wheat and rye (and other cultivated plants) should be determined more specifically by investigations of the composition of varietal populations. The differences in their composition become manifest not only by comparing remote geographical localities but also by comparing properly chosen ecological habitats situated not far from each other. A. M. Gorsky under our guidance observed the variability of the population-compositions of winter wheat, barley and rye in the North-Western Caucasus at the Maikop Experimental Station. His observations were conducted in valleys (at about 350 m. (800 ft.) above sea level) and on hills (at about 600 m. (1,950 ft.) above sea level). Many sowings were carried out every five days all the year round (with the exception of periods of more or less deep snow cover). The recording of plants showing ear emergence was made every five days. In this way the varietal population was differentiated into fractions according to the time of ear emergence. The number of such fractions was different according to the times of sowing.

In general wheat (variety 'Novoukrainka') and barley (variety 'Krasnodar' 2929) show many more fractions in the valley plot than on the submountain one.

THE COURSE OF EAR EMERGENCE OF BARLEY 'KRASNODAR' 2929 AND WHEAT 'NOVOUKRAINKA' IN THE VALLEY AND IN SUBMOUNTAIN FIELDS.

Fractionation of Varieties according to date of ear emergence	Barley		Wheat	
	valley field	submountain field	valley field	submountain field
Maximum number of fractions on one plot of a definite time of sowing	7	2	6	2
	Percentage of the total number of plots			
Number of plots of different times of sowing with "isochronal" ear emergence (omitting the first fraction)	16.7	84.6	17.0	12.3
With two fractions	8.3	15.4	17.0	87.5
,, three ,,	8.3	0.0	0.0	0.0
,, four ,,	8.3	0.0	0.0	0.0
,, five ,,	25.0	0.0	17.0	0.0
,, six ,,	16.6	0.0	33.0	0.0
,, seven and more ,,	16.6	0.0	0.0	0.0

The prolonged period of ear emergence shown by many sowings in the valley field *is caused by the irregular course of spring here*: the warmer weather alternates with returning coldness and here we have the same effect as when applying "partial" jarovization, *i.e.*, when the same population is subjected to jarovization many times for different periods of days. In the submountain field, on the other hand, low temperatures are for longer periods, the temperature is more uniform and thus all components of the population pass through the stage of jarovization more or less simultaneously.

In order to analyse the composition of experimental populations it is more convenient to grow them in the valley field. The period of times with "isochronal" ear emergence, when it is difficult or impossible to single out fractions, continues in the valley field from July till December (times of sowing of winter crops). In this period all the biotypes of the varietal population manage to pass through the stage of jarovization. The temperature of this period is a "stabilising" factor with regard to times of ear emergence. This same period appears to be the optimal one for the growth of the stem in both localities for all the times of sowing within it. However, the amplitudes (differences between the maximum and minimum heights on one plot of a definite time of sowing) are also greater just in this period. Thus this period cannot be called stabilizing in relation to the height of plants, though it is optimal with regard to this feature. Therefore, if one needs to select the hereditary variants which differ among themselves in the height of the stem, it is convenient to do this work just in this period. On the contrary it is very difficult or impossible in this period to single out biotypes distinguished in their biological properties, firstly in the length of the stage of jarovization. It is much easier to carry out this selection on the plots sown at times outside this period where the population presents itself in a disintegrated state with regard to this feature.

Numerous times of sowing may be substituted by differential jarovization (during one, two, four, five and so on days). It is easier to choose one definite length of jarovization when the majority of biotypes are not entirely jarovized. This was observed in the following experiment carried out by us with Timothy grass. The population of a polar ecotype from the Murmansk region showed in the North as many fractions as regards times of panicle emergence as in the South. Both at Pushkin and at Maikop Stations we had four fractions. However, at Pushkin there were no early fractions during the period from the 1st to the 20th of July. It appears that at Pushkin the selection of the earliest spring biotypes from the perennial population is much more difficult than at Maikop.

On the contrary constant spring forms are more easily singled out in Maikop out of two earlier fractions. This conclusion was confirmed by subsequent selectional work by Borkovskaya and Sharapova. The jarovization influenced the course of panicle emergence in the conditions of the North (at Pushkin) and produced no effect in the South (at Maikop). The North-Caucasian ecotype of Timothy grass did not react to jarovization either at Pushkin or at Maikop. Thus the effect of jarovization depends on environmental conditions and on the nature of the ecotype.

THE COURSE OF PANICLE EMERGENCE IN THE POLAR ECOTYPE OF TIMOTHY GRASS

Place of growing and mode of seed-treatment \ Times of Recording	Percentage of plants showing panicle emergence in the first season of life						
	10/VII	20/VII	30/VII	10/VIII	20/VIII	30/VIII	Percentage of all plants in generative phase
Pushkin (near Leningrad). Jarovization of seeds during 33 days at 0–4° C.	11.2	14.6	7.8	27.0	9.0	9.0	78.6
Pushkin. Without jarovization	0.0	0.0	27.6	17.0	10.7	16.0	71.3
Maikop Experimental Station (North Caucasus). Jarovization of seeds as at Pushkin	7.8	29.6	6.6	10.1	0.0	0.0	46.5
Maikop Experimental Station. Without jarovization	7.9	23.5	7.0	11.1	0.0	0.0	49.5

Inter-reaction among the components of the population is realised in different ways: (1) directly (interpollination, excretion through roots, phytoncides, concretion (adhesion by growth) of root systems and so on); (2) indirectly (by means of changes in phytoclimate, soil composition, drying, shading, accumulation of dead remains of plants and so on).

The interpollination of components of the population (pollination-regime) is the most important and interesting factor and plays an extremely important part in the process of population formation and in sustaining it at a definite level of vitality; it is a factor which in particular controls the relative stability of the population-composition. However, notwithstanding the importance of this factor, it has still been unsatisfactorily studied. Its rôle can be properly understood only in connection with the influence of environmental factors. This state, we think, is well illustrated by our investigations of the composition of the varietal populations of the sunflower.

These varieties were formed by mutual interpollination within the group of plants specially chosen by the plant-breeder according to their useful biological and economical characteristics. Each such group is being grown on the isolated plots for successive selection. In this method of plant-breeding mutual interpollination results in the relative levelling up of the variety according to the time of flowering and maturity, height of the plants and economic characteristics. Interpollination, moreover, maintains the population at the needful level of vitality and health. This factor gives the varietal population an ecological plasticity in the sense of adaptability to the local complex of environmental conditions and to those such seasonal climatic changes, which are characteristic of a given region.

However, in the region, where the variety was formed, the heterogeneity of the population-composition appears only in definite and comparatively narrow limits. Notwithstanding the varietal population represents a biological entity, and not simply a mixture of accidental forms. Thus the environmental complex of regional conditions, where the given ecotype or cultivar (variety) has been created, appears stabilising for the given variety.

The transferring of the cultivar (varietal population) or the ecotype into extremely different conditions often leads to its completeness being broken with a consequent loss of uniformity. The Krasnodar varieties of sunflower on trial plots in Siberia sometimes show greatly prolonged periods of flowering and such a diversity in the height of the plant that they require repeated harvesting and even lose their value as economic varieties. However, the analysing (widening of the limits of the visible variability of the population) of conditions, has made the selection of various biotypes easier.

It is very important to understand the analysing or stabilising significance of separate factors. The analysing influence of the length of day has been particularly effective in such varietal populations of the sunflower where the "long-day" plants and "short-day" ones are included. The very fact of the co-existence of such different physiological components in one varietal population is interesting. It became possible to reveal this quite unexpected fact only by applying the special method elaborated by us, but this method cannot be outlined in this brief survey.

Experiments carried out by us in collaboration with D. S. Nezchezeret show the existence of several types of varietal populations of sunflower. These types more or less correspond to zonal ecotypes :—

1. Varietal populations which include only "long-day" biotypes ;
2. Populations which consist of later "long-day" biotypes and comparatively early "short-day" plants ;
3. Populations including later "short-day" biotypes and comparatively early "long-day" ones. Such a type of population-composition was discovered among late silage varieties ;
4. Populations consisting only of early "short-day" biotypes—up till now only one variety of such composition has been found.

More complex populations representing various combinations of the above-mentioned types with "neutral" biotypes were met with.

The natural length of the day in Krasnodar stabilises the varietal population of the sunflower, while the "short-day" disintegrates it into separate components.

The higher temperature during spring and summer accelerates the development of all or the majority of biotypes of sunflower. The dates of their blossoming become closer to each other, the period of flowering of the whole population is much shortened so that all biotypes easily interpollinate and thus the pollination is achieved by a most diverse mixture of pollen. Such a pollination-regime is the richest and the most favourable for reproducing the whole varietal population as an entity at the highest level of its vitality. Thus the conditions of late sowing may be called stabilising as regards the composition of the given variety and the best for its seed reproduction.

When the complex of environmental factors is very unfavourable to all or to the great majority of biotypes of the population, visible differences among the components of the population usually diminish and consequently selection becomes extremely difficult or even impossible. Severe and long droughts often influence the composition of the population in such a manner, and even natural elimination in such circumstances is of no selective significance. In many of our experiments with different plants sudden changes of an effective factor *were much more drastic in comparison with the constant action of the same factor*. The permanent (throughout the whole life of plants), 8 or 10-hour "short-day" is less effective as a factor in disintegrating sunflower-populations than the "short-day" regime applied only 25-30 days after the appearance of seedlings. The "long-day" factor applied before bud formation (before the end of the "light" stage of development) divides all biotypes of sunflower into "short-day" and "long-day" ones according to whether "long-day" or "short-day" regime has an accelerating influence on the development of plants. The "short-day" regime applied *after* bud formation accelerates the development of all biotypes of sunflower and then its disintegrating ability becomes greatly diminished or is practically nullified. Thus the disintegrating capacity of each factor depends also on the stage of development and the age of the components in the population.

In our experiments with one variety of oil plant *Perilla ocymoides* continuous "short-day" regime appears as a stabilising factor, the whole population being represented by *quite uniform*, early-flowering dwarfs. The population of the same variety of *Perilla* growing throughout its life in conditions of 24-hour day also consists of quite uniform plants. After some months they all come to blossom simultaneously. These plants are quite unlike the constant "short-day" plants —they are tall and their development is extremely slow.

The same population of *Perilla* growing from the seeding-phase in "short-day" conditions during 15-20 days and then transferred into natural or constant 24-hour day shows the very striking picture of a fabulous disintegration into several morphobiological types. Certain features of these plants are beyond the limits of our present-day knowledge of the variability of the given species and even family (for example, the bow-shaped nervature of the leaf instead of the reticulate one).

Such a mode of disintegrating the populations (beyond the limits of their own norms of reaction and those of their components) should be distinguished from the state of falling into ecoelements which are the initial points or nodes of natural selection activity.

It is possible to establish the most effective method for analysing (disintegrating) or for the relative stabilisation of the population of the given unit (species, ecotype or cultivar). Investigations in this field are very important for the development of the theory of plant-breeding and for a better understanding of processes of species and ecotype formation.

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PHENOTYPIC EXPRESSION OF GENOTYPES IN CONTRASTING ENVIRONMENTS

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The general field known under the names of genecology, race ecology, ecological genetics, experimental taxonomy, and biosystematics has many ramifications and emphasis. This field is still developing vigorously, opening new approaches at various points that eventually may become important sub-fields. One of the latest developments is in comparative physiology. A driving force in these various fields of research is a realisation that neither genetics, ecology, physiology nor plant systematics can exist alone but must be related to the others and to the kinds of environment that exist on our planet. In this brief note it is impossible to review all the ramifications of genecology, therefore the discussion will be limited to the phenotypic expression of genotypes.

Recent interchanges of ideas and methods between the fields of genetics and experimental ecology have clarified and expanded the concepts of both and have opened new vistas into an understanding of the nature of variation of living things. Some of these aspects are presented by the authors in a recent publication on the genetic structure of ecological races. It seems appropriate to review some of these conclusions in a report from the Scottish Plant Breeding Station, because plant materials and ideas have for a long period of years been exchanged between workers at that station and those at the Department of Plant Biology of the Carnegie Institution of Washington, stationed at Stanford in California.

(1) *The phenotypic expression of genes and genotypes.*—A conspicuous gap in our knowledge of genetics is the lack of information on the phenotypic expression of genes and genotypes over a range of environments. *Gene* is a concept in genetics, and *environment* a concept in ecology, fields that historically have employed opposite methods of research. In the early development of genetics, Wilhelm Johannsen clearly stated the premise that the phenotype is the product of the

genotype and the environment. Phenotypic modification is an accepted principle in genetics, but the mechanisms involved in modification have never been properly investigated, nor has it been ascertained to what extent modifications can be attained and whether all organisms modify to the same degree.

One of the principal reasons why these questions have largely remained unanswered is that the study of the mechanisms of interaction between genotype and environment require fairly complex techniques. A major requirement is that the heredity remain constant while the environment varies, but this demand largely limits the experimental organisms to those that can be cloned, which excludes most animals and annual plants. Another demand is that the environmental changes should surpass seasonal and periodic annual fluctuations in the native environment to which the organism already is evolutionally adjusted. Such experimental conditions are seldom achieved. A third requirement is that the genes that operate in at least one of the environments are fairly well known, but it so happens that the organisms that are best known genetically are those that are not suited for vegetative propagation.

(2) *Pathways of gene action.*—Some suggestion concerning the nature of the mechanisms that produce phenotypic modification comes from the modern comprehension of gene action: genes regulate processes through various pathways, biochemical and physiological, leading to growth and to morphological development. These processes are affected by fluctuations in the environment. The gene-regulated processes of an organism are adjusted to the environment in such a way that the organism can survive and compete. Although the details are still unknown, it is evident that pathways of causal effects exist within the organism connecting the controlling influence of the genes with the various impacts of the environment. A concomitant effect of the integration of pathways is that the intensities and direction of development of the life processes shift with temperature and other environmental factors.

An example of environmental modification of a morphological character regulated by specific genes was observed in the petal shape of *Potentilla glandulosa* (Clausen and Hiesey, 1958). A subalpine individual having notched petal tips was crossed with a foothill plant having entire petals. The parents, F_1, and all F_2 plants were cloned and grown simultaneously near sea-level at Stanford in the California Coast Ranges, at

4,500 feet altitude in the Sierra Nevada, and at 10,000 feet near Timberline. At all three altitudes the subalpine parent had deeply notched petals, and the foothill parent entire petals.

The F_1 was slightly notched at all stations, although the degree of notching was greatest at the high, and least at the low altitude. At the lowland station in certain years almost all petals of the F_1 were entire, while in other years there would be a mixture of flowers ranging from slightly notched to entire. At the high altitude the petals of the same individual varied from slightly to moderately notched. Such fluctuations in expression of the notch character from flower to flower at one station combined with station-to-station modifications indicate the existence of a delicate balance between different regulating mechanisms within the F_1 as compared with the much more stable internal systems in the parents.

The frequencies of phenotypes in the F_2 and F_3 progenies indicated that at least three pairs of genes regulate the character notched petals, one pair promoting and two pairs inhibiting notch. The F_2 population consisted of 575 plants that were cloned and transplanted at the three contrasting altitudes. With regard to notch, the parental phenotypes were recovered in 171 F_2 plants which had entire petals at all three stations, and in 93 individuals that were fully notched all three. The entire-petalled F_2 plants were genotypically of two kinds: those lacking a gene for notch and those having 1-2 notch genes in addition to 4 inhibitors. The fully notched F_2's were interpreted to possess either 2 genes for notch with 0-2 inhibitors, or 1 notch gene and no inhibitor.

A third group, consisting of 57 F_2 plants, had fully notched petals at the high altitude though fully entire at low. Based on the frequency of this group, this situation was interpreted to arise from the interaction of 1 notch gene with 3 inhibitors. Finally, the major class of the F_2 plants, 254 individuals, had partially notched petals at all three altitudes, the notch varying in intensity from flower to flower on the same plant, from station to station, and between plants at the same station. This class was interpreted to possess either 1 notch gene with 1-2 inhibitors, or 2 notch genes with mainly 3 inhibitors, so that the notch and inhibitor genes were fairly evenly balanced.

The segregations of the notch character make it obvious that it is genically controlled and that the genes have opposite effects. It is equally obvious that an environmental effect is connected with the expression of the genes. Moreover, the inhibitory effect predominates at the lower altitudes, and is much weakened at Timberline. One might suppose that the production of an inhibitory chemical is favoured at the low altitude more than at the high.

Notch is a morphological character. It is unusual in *Potentilla glandulosa*, but it is the normal situation in *Potentilla gracilis* of a different subgenus. Its expression in hybrid plants of *Potentilla glandulosa*, varying from flower to flower, from season to season, and from station to station, is the kind of penetrance to be expected if the regulating action is provided by substances of opposite effects, the amounts of which vary with the immediate environment.

(3) *Activation of latent genes.*—Phenotypic modification can also be caused by the activation of latent genes. Genes may be latent for several reasons. They can lack complementaries, can be inhibited or suppressed by other genes, or the environmental conditions may not be favourable for their expression.

Many chemical and biological processes have temperature thresholds above and below which they do not function. Similarly, genes are known that have thresholds at which they become activated; possibly all genes have such thresholds. Scattered experiments have indicated that living things of various kinds may possess latent or residual genes that become activated in environments that differ radically from those in which the organism evolved and where long-range natural selection has occurred. Even a particular gene of a polymeric series may have its own threshold.

The high-altitude subspecies *nevadensis* of *Potentilla glandulosa* possesses a gene regulating anthocyanin coloration of stems and leaves that was phenotypically expressed only at the Timberline station at 10,000 feet altitude (Clausen and Hiesey, 1958). At altitudes of 4,500 and 100 feet, plants having only this particular anthocyanin gene are green. This gene is number 4 in an epistatic series of four regulating these pigments. The other three genes occur in subspecies and ecotypes from lower altitudes. They are expressed at all three stations, although in different strengths inasmuch as all produce more intense anthocyanin at the highest altitude. The "penetrance" of all four genes changes therefore with altitude, and the most hypostatic gene of the series has practically no penetrance at the two lower altitudes.

A striking example of the activation of a latent gene is known from milo of *Sorghum vulgare*, a grass from tropical latitudes, in which Quinby and Karper (1945) described the effect of a maturity gene Ma_1. This gene is inactive at a 10-hour photoperiod, where the Ma_1Ma_1 biotype is indistinguishable from the recessive ma_1ma_1. At a 14-hour photoperiod the Ma_1 gene has become active, and the effect is dramatic, inasmuch as under these conditions the flowering in the Ma_1 biotype is delayed about 2 weeks over that of the ma_1, the stem is lengthened, and the number of leaves below the panicle is increased by 11.

Another example of activation of latent genes is known from Wimmera ryegrass of *Lolium rigidum*, a spring annual that does not require cooling for initiation of flowering. Cooper (1954) found that seedlings of this strain that had germinated and were grown out-of-doors in the cool English spring uniformly developed inflorescences at the 6th to the 7th node from the base. When grown in a heated greenhouse and subjected to a 24-hour photoperiod, a great deal of residual variability was disclosed and the plants initiated flowering at a range varying between the 5th to the 21st leaf. When the Wimmera strain was vernalised at 3° C. for 6 weeks and thereafter grown at a 24-hour photoperiod in the heated greenhouse, the variability was again concealed and flowering was uniformly initiated at the 4th to the 6th leaf. The omission of moderate pre-cooling, combined with a 24-hour photoperiod, disclosed the latent genetic variability in this otherwise uniform agricultural strain. Selection had never taken place in this kind of environment.

Another example of latent variability was found in the tufted hairgrass, *Deschampsia caespitosa*, which in its native habitats is non-viviparous in contrast with the highly viviparous *D. alpina* of a different ecological zone. Viviparity is the morphological character that is used in distinguishing the two species. Seedling cultures of *D. caespitosa* from the 56th latitude in southern Sweden, from the 60th in southern Finland, and from the 68th in Swedish Lapland were grown at Stanford in California at 38° N. Here they became much more variable than in their native environments ; each culture was composed of mixtures of viviparous, non-viviparous and non-flowering plants that varied in height from 20 to 80 cm. (Lawrence, 1945). The viviparous plants varied in degree from highly to slightly viviparous (Table I). Flowering began in late June, but the inflorescences did not start developing viviparous plantlets before August and September when the days had shortened. A species that appears to be highly uniform in one environment can therefore become highly variable in another that is radically different.

TABLE I.

Latent variability in degree of viviparousness and in ability to flower in *Deschampsia caespitosa* as disclosed by transplantation to a low latitude. The three populations were uniformly floriferous and non-viviparous in their native habitats.

FREQUENCIES AT STANFORD AT 38° N. LATITUDE

Origin of population	Strongly viviparous	Moderately to slightly viviparous	Non-viviparous	Non-flowering	Total
3301, 56° N., Skäralid, Sweden .	10	16	3	6	35
3302, 60° N., Ruotsinkylä, Finland .	6	9	10	10	35
3303, 68° N., Abisko, Lapland .	1	..	1	6	8

These examples indicate that latent genes exist. They are probably far more common than is generally realised because their study so far has been very limited.

(4) *The gene systems of contrasting ecotypes.*—Ecotypes from contrasting climates differ greatly in the composition of their genes and are especially rich in unexpressed genes. Such ecotypes have evolved independently, possibly for long periods of geologic time, and their adjustments to climate have been achieved in diverse ways. Their genes are nevertheless completely interchangeable.

A high degree of variability becomes evident when the heredities of interecotypic hybrids are recombined, revealing great differences in the genic composition of the parental races. Inactive genes from one race may combine with their complementaries from another. Genes repressed by inhibitors in one race may be released by another race which has neither. Many characters are also regulated by genes of epistatic sequences in which the hypostatic genes are unexpressed. Recombinations may free the hypostatic genes to expression, but it may require several generations to release all the possibilities.

Most gene systems are composed of several kinds of genes, such as those having complementary, cumulative and oppositional effects. The expression of a character often depends upon the balances of such a system, and it may vary with the environment as discussed above. The phenotypic expression of the gene system that regulates a character in wild plants is usually sufficiently fixed so that the character varies little from year to year. When such plants are moved to a radically different environment, however, striking modifications may occur such as those in *Deschampsia*.

(5) *Hybrid vigor in relation to environment.*—When contrasting ecotypes are intercrossed, the F_1 may exhibit some hybrid vigor. If sufficiently large F_2 and later populations are grown, the variability is highly increased and is often accompanied by transgressive segregation. An increase in vigor over the parental ecotypes may be retained, or even increased, in later generations. This is in contrast to the hybrid vigor of maize and rye in which the vigor of the F_1 is lost in subsequent generations and is replaced by inbreeding depression.

The kind of hybrid vigor that is expressed in growth must be assumed to result from the combination of growth-promoting genes contributed from both parental sources. Such a result may be obtained by combining genes that have either additive or complementary effects, or by the removal of genes that have inhibitory effects on growth processes. In maize it appears that both growth promoting and recessive, growth-reducing genes or sublethals are located fairly closely together on the chromosomes, so that crossovers are rare. Such an arrangement would tend to favor heterozygosity.

Hybrid vigor changes with the environment, for the gene-controlled growth-promoting and growth-inhibiting processes vary in intensity with the environment, and otherwise inactive genes may become activated in new environment. An especially striking example was reported in the F_2 of the cross between subspecies *borealis* and *gigantea* of *Achillea borealis* (Clausen, Hiesey, and Nobs, 1955). At the lowland station at Stanford there is no conspicuous hybrid vigor in the F_2 of this cross. At the mid-altitude Mather station, however, the high-latitude *borealis* parent tends to die during the summer, and the low-latitude lowland *gigantea* tends to become killed during the winter. The F_1 plants survive with great vigor, and more than 50 per cent of the F_2's are even more vigorous than the F_1. The parental characters complement each other in both the F_1 and the greater part of the F_2, producing more vigorous growth and greater tolerance to both winter and summer seasons in this environment.

Strains of agricultural crops that were developed in contrasting climates parallel natural ecotypes. Some strains of this kind could be expected similarly to have evolved distinct genetic systems that may complement each other when recombined after crossing.

(6) *Apomixis as a means of cloning.*—A study of phenotypic expression of a genotype over a range of climates is often limited by the physical difficulties in transplanting live propagules of plants over great distances, such as over many degrees of latitude, or from one continent to another. Certain groups of plants, especially among grasses and sunflowers, can produce seed apomictically, so that genotypically the progeny are exact copies of the maternal plant. In such plants a group of cells near the egg apparatus develops into an embryo without reduction in chromosome number and without fertilisation. Although the sexual process is retained in some degree in most apomicts, the individuals resulting from sexual fusion are few in number, highly variable and in wild plants weaker than those grown from seeds of the apomictic type. Consequently, the sexual progeny tend to disappear in competition (Clausen, 1953).

Plants that can reproduce apomictically thus provide convenient materials for the study of identical genotypes in contrasting environments at great distances. Hybrids of the grass genus *Poa* have produced highly diverse apomictic biotypes that have been tested in environments in various parts of the world ranging in latitude from 30 to 68° N., and in altitude from sea-level to 10,000 feet.

Apomictic species of the western North American bunch-grass section of *Poa* were crossed with other species of the rhizome-bearing *Pratensis* section (Clausen and Hiesey, 1958; Clausen, 1952, 1954). Each parent species is highly polyploid and the chromosome pairing in the hybrids is relatively good (Grun, 1952, 1955). The hybridisation changed the balance between the apomictic and sexual systems of reproduction found in the parental species to the extent that many F_1 hybrids become sexual. These sexual hybrids were fairly fertile, and segregated moderately in the F_2. New apomictic types may appear in such F_2 progeny, and by judicious selection it is possible to obtain a number of diverse new apomictic *Poas* from a single hybrid plant. Each new apomict represents a different genotype that propagates as a seed clone and can be tested with the parental apomicts in diverse environments.

When the individuals of an apomictic progeny are space planted, each individual of the apomictic biotype stands out as a replicate of the maternal parent, and the few progeny resulting from sexual reproduction appear as weak aberrants. In dense plantings usually only the apomictic type is seen. Spaced plantings of commercial varieties of *Poa pratensis* often reveal that they are mixtures of several apomictic strains. In dense plantings of commercial varieties some of the apomictic biotypes may be suppressed in one kind of environment, and others in a different one, so that the surviving biotypes may differ with the environment.

Local natural populations of apomictic *Poa pratensis* are likewise usually mixtures of apomictic biotypes that differ in morphological characters, in chromosome number, in time of flowering, and in susceptibility to diseases. Other subspecies of *Poa pratensis,* such as *alpigena, irrigata,* and *artica,* vary also in chromosome number within the local population (Nygren, 1950; Löve, 1952).

Studies in only one kind of environment are not always sufficient to determine whether or not a progeny is apomictic. Occasionally, a hybrid progeny of *Poa* appears to be constant and uniform in one environment although actually it is genotypically variable and sexual as disclosed when brought to a highly different climatic zone. Apparently certain genes are not brought to expression in the first kind of environment, or they counterbalance there.

Apomictic processes enable plants to accumulate heredities from vastly different sources into constant, self-producing lines and thereby increase the hereditary buffering within the genotype. To even a greater extent than sexual plants, apomictic plants contain great amounts of unexpressed variability that can be released if the apomictic mechanism breaks down and the sexual mechanism takes over. Sexuals among the apomicts have passed through the same process of accumulating and compounding genomes.

Conclusions.—The hereditary structures of living things that successfully have been able to populate climatically diverse regions of the world are so constituted that they contain a great deal of unexpressed variability. Some of the hidden variability can be made evident by moving the organism to a highly different environment, where some of the inactive genes become activated. Another part may become manifest when genes of ecotypes from contrasting environments are brought to expression through crossing and recombination. Distinct species undoubtedly carry vast amounts of unrecognised variability, because only differences that can be genetically analysed can be identified. Living things are flexible in their environmental responses, but our knowledge of the potentialities of such responses is limited because only few investigations have been undertaken to study organisms in highly diverse environments.

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PROGRESS AND PROSPECTS IN GENECOLOGY

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It is a well known biological fact that different members of a species are not identical one with another ; they are sufficiently similar to be grouped together, but they have an individual distinctiveness. The species is variable in most, and one might indeed say in all, characters since even in respect of the so-called constant characters there are the occasional aberrant individuals which the taxonomist would include without hesitation. Whereas a considerable amount of this intra-specific variability is environmentally imposed, it is easily demonstrated that a great deal of it is genetical in origin. The basis of genecology is the observation that genetically controlled intra-specific variability is not randomly dispersed over the territory ; it is distributed in such a way that neighbouring plants tend to resemble each other.

One of the principal dogmas of genecology may be stated as "differentiation of populations depends on the balance between selection and gene flow." When contrasting environments adjoin, it is assumed that selection will tend to encourage divergence of the local population, but that gene flow will resist it. Clearly differentiation will depend on the relative strengths of these two forces, and in most cases we are as yet only able to guess at which will predominate. The general belief is that the habitat population is in dynamic balance with its environment ; by selection each population is attuned to its own circumstances. The common observation that adjoining populations are significantly different when examined by orthodox genecological techniques fosters this belief. At the other extreme there are those who place the emphasis on isolation believing that population differences owe their existence very largely to chance occurrences. While it is only the coarser differences which show a clear correlation with environmental factors, comparative studies have suggested that few local populations have their exact counterparts in other localities, and it is sometimes inferred that the smaller differences must also be related in some way to equally small differences of habitat.

It is, however, possible that these apparent differences are not always genuine examples of differentiation but artefacts resulting from the techniques of population sampling adopted. In this connection the consequences of genotype reduplication in population sampling is receiving attention at this Station. For instance, in one trial samples of nine populations of diploid *Festuca ovina* were grown. This particular trial differed from the ones most commonly employed in genecological studies in that every plant had been divided into five pieces and all five pieces were planted in the same lay out. Each of the nine populations was represented originally by a 30-plant sample. The first analysis was carried out on the basis of these 30 plants and showed that two significantly differentiated groups of populations existed but that intra-group differences were absent. When, however, the analysis was repeated on the basis not of 30 plants per sample but of 150, the standard error was considerably reduced with the result that now differences between population samples within the two groups reach significance. The repetition of genotypes in this second analysis by reducing the standard error had made it appear that population differences did exist whereas in fact they did not.

It seems probable that in perennial species, liable to spread vegetatively, genotype reduplication in sampling could be a serious source of error, leading to erroneous conclusions. For instance, in the above example had the reduplication been accidental and undetected the population differences found might well have been interpreted as evidence of localised ecotypic differentiation whereas the evidence from the 30-plant samples indicates a regional differentiation only, and one that may have little or no ecotypic meaning. A further series of nine tetraploid samples from contrasting habitats were all indistinguishable from each other, having neither ecotypic nor regional differentiation. It should be pointed out that the perennial *Festuca ovina* has very little power of vegetative spread so that genotype reduplication in population sampling is unlikely.

Unfortunately, it is impossible to prove that two plants in a population sample are of the same genotype, but failure to distinguish them under circumstances which usually permit the recognition of different genotypes is at least suggestive of genotypic similarity. When in addition the plants under investigation happen to be self-incompatible, differences between the seed set of crosses with apparently identical plants and with observably distinct plants can supply useful corroborative evidence.

Using these two methods we now have a fairly clear idea of the spread of single genotypes in natural communities in a few species. Thus it is found that *Festuca ovina can* spread for more than a yard. At the moment of writing the evidence is as conclusive as it could be for one genotype having a spread of 2½ yards, while it seems very likely that a spread of 4 yards will be established. In *F. rubra* with a much more efficient means of vegetative spread we have a well documented case of a single genotype having a spread of 250 yards. Furthermore, it is not easily missed in a population sample since over this range it forms about one-third of the total tillers present. In fact, at one site it accounted for 17 of the 23 tillers collected. Clearly in such a case population sampling can scarcely avoid genotype reduplication so that genecological investigation by the normal methods is almost bound to result in misleading significance.

Another trial was laid out comprising thirteen species (*Agrostis tenuis, Festuca rubra* and *ovina, Poa pratensis* and *trivialis, Holcus mollis, Anthoxanthum odoratum, Achillea millefolium, Trifolium repens, Cerastium vulgatum, Luzula campestris, Galium hercynicum* and *Carex caryophyllea*), from 80 stands of the bent fescue community. The sites were small, being no more than four square metres, to ensure a reasonably high ecological uniformity within site, and all material was collected vegetatively. Every character measured in every species has shown highly significant differences between sites. However, in several cases it has been possible to "recognise" a plant during scoring as being the same as one scored already, and on examining the randomisation plan it is found that the two plants originated from the same site. As a result we can expect some of this significance to be of a spurious type.

It is too early yet to say that population differences of the finer type (which have not been shown to be related to ecology) invariably, or even commonly, result from genotype reduplication. But the work has gone far enough for it now to be necessary for a worker to give reasonable evidence that no genotypes are reduplicated before he can claim to have demonstrated population differentiation ; and this is an even more difficult task than collecting evidence that two plants are the same.

Such an obstacle does not arise when a correlation with environmental factors can be shown. Normally a large number of populations are required for such a demonstration. This approach can well be illustrated in *Potentilla erecta*. The late Dr F. Earnshaw, working at this Station, laid out a trial with this species shortly before his death. On analysis it is found that there are significant differences between populations. In this case there can be no reduplication of genotypes since the population samples were raised from seed. However, the same sort of spurious significance would occur if too many of the seedlings were derived from the same parents. On grouping the populations according to their origin it is found that those which came from peat communities (*Calluna, Nardus*, etc.) are composed of significantly larger plants than those from bent fescue communities on mineral soil. This difference is of real genecological interest. There are small differences within the two groups but these have not been related to ecology and may well be of a spurious nature.

A similar method has been used in the case of the 13 species trial mentioned above to see whether the site data are of genecological interest. The 80 sites concerned were subjected to a fairly detailed ecological examination and it has been possible to group them into ten variants of the bent fescue community. The ten variants form approximately a series, the three principal stages being :—

I. Wet sites of moderately high pH (5.5–6.5) including such plants as *Cardamine pratensis, Poa* (esp. *trivialis*), *Ranunculus repens, Rumex acetosa, Sagina procumbens*. Very little *F. ovina*, abundant *F. rubra* ; *Agrostis* represented by *tenuis*, but also *stolonifera* and a little *canina canina*.

II. Drier sites of slightly higher pH, species rich with many calcicoles—*Prunella vulgaris, Euphrasia* sp. *Plantago lanceolata, Briza media, Thymus serpyllum, Viola riviniana, Lotus corniculatus*. Both *F. ovina* and *rubra*. *Agrostis* not so abundant and all *tenuis*. Both I. and II. are heavily grazed and lawn like.

III. Low pH (4.5–5.5) dry sites. Sp. list almost limited to *Agrostis* (*tenuis* and *canina montana*), *F. ovina* with little *rubra, Anthoxanthum odoratum, Potentilla* *erecta*, *Galium hercynicum* and *Luzula campestris*. A much rougher, often badly grazed community.

Whereas all characters in all thirteen species have shown highly significant differences between sites, not one of them is related to this ecological scheme. Had any one character been distributed according to some ecological trend then we should have expected that the sites in one group would have tended to yield low values, and those of another high values. It seems clear that in this case there is no genecological differentiation between the 80 sites in any of the thirteen species.

It might be argued that the sites are very similar, all being forms of the bent fescue community, and that therefore ecotypic differentiation is not to be expected. However, the point at issue is that had any two adjoining populations been compared regardless of the others, the difference found might have been assumed to be an ecotypic one since spatial isolation was obviously not involved.

By demonstrating that there is no genecological differentiation in this case, we have also demonstrated that selection, if present, is not powerful enough to overcome gene flow. This does not mean that selection can never differentiate between two adjoining populations, and, in fact, it seems most likely that this has happened in the case of lead resistance in grasses (Bradshaw, 1952 ; Wilkins, 1957). But lead is lethal to susceptible grasses ; there is apparently little evidence of a character of more moderate disadvantage being selected against. The genecologist has tended to accept without evidence the statement that "It is obvious that so long as there is genetical variation present, selection will take place." This does assume a very rigid relationship between genotype and phenotype, whereas it is very well known that a single genotype is able to grow successfully under a wide range of environmental conditions, adopting a range of morphological forms at the same time. In fact, our experience with collecting single extensive genotypes in the wild indicates that such genotypes have the same wide ecological range as the local population. Thus, though we have demonstrated (for instance, in the East Anglian fescues (Harberd, 1957)) that populations have a high genetic variability in such characters as leaf length and time of ear emergence, which are generally supposed to have great adaptive importance, there appears to be very little genetical variation in ecological tolerance—so little, in fact, that we should not expect selection to take place. The great adaptability of a race is able to cope with a wide range of ecological circumstances. It is, in fact, conceivable that selection acts to maintain this wide ecological tolerance, over-specialisation being traditionally regarded as a dead end in evolution.

At this point we might again consider the East Anglian sheep's fescue. It has already been pointed out that the nine tetraploid populations were indistinguishable. They were gathered from a series of strongly contrasting sites but selection had not caused them to differentiate in respect of those characters observed. Following the discussion of the previous paragraph this should not be surprising. By contrast, there were among the diploids two morphologically distinct population types. Both types had been gathered from grazed lands and from dense *Calluna*, and several other strongly contrasting sites. The only meaningful distribution of the sites seemed to be the geographical one between an eastern and a western area. In this district such a geographical distinction carries very little climatic difference. There is much greater ecological difference within either of the areas than there is between them. The morphological difference cannot be explained by selection ; it has more the appearance of a phenomenon of chance.

From a consideration of the age of genotypes in these natural communities there is evidence that selection has not had the opportunity to differentiate populations on such a scale as has been generally supposed. Some of the clones recorded must be hundreds of years old to cover such an acreage. They have not been overwhelmed by new gene combinations, and so far as these particular genotypes are concerned the populations may be regarded as static. This in itself is contrary to the concept of a selectively sensitive dynamic balance within populations. In any case they must be relatively few generations removed from the original colonisers after the Ice Age. In terms of generations there has not been time for selective differentiation, except with very drastic selection pressures.

Since both the findings of genecological investigation and the interpretation of these data will depend to a considerable extent on the characteristics of the material employed it is not at all surprising that opinions differ regarding the relative rôles of selection and isolation in occasioning the patterns of infra-specific variation. At this Station we are now particularly interested in plants of natural grasslands and especially those characterised by being out-crossed, wind pollinated and long-lived, longer apparently than we had realised. From the evidence so far derived from this material certain points emerge and these are tentatively listed as follows :—

1. Frequently there is no intra-specific differentiation within a region. Even where a species occurs in a wide range of ecological conditions, the local form is sufficiently adaptable to occupy the habitats without change in genetical composition.
2. Where differentiation is demonstrated within a region the total number of distinguishable races is very few, and perhaps only two, though in some cases these may be connected by intermediates through hybrid swarm formation.
3. Material gathered from places widely separated geographically is more likely to show differentiation than material gathered from within a smaller region. On further investigation it may be found that distant geographical places are connected by intermediates in the form of a cline, or that the races remain distinct and constant.

Consideration of the situation presented by these suggestions reveals a close similarity to that which might be expected if isolation were the main source of differentiation, and selection a secondary one. Thus, if a new territory has been colonised by only one race of a species, there will be little differentiation if any and it will be related to selection in either of two ways. In the first place, there may be rare pockets of territory of the lead spoil type which are poisonous to plant life and consequently left bare. Should a genotype arise capable of growing on this medium then a differentiated population will almost certainly develop. In the second place differentiation may spring from the fact that distant parts of a large and continuous territory are isolated one from another. Both isolation and selection could then act to produce differentiation in the form of a cline. Within any region the local race would be adapted to the prevailing major environmental factors, such as climate, day length and perhaps the predominating soil type or vegetation, but no intra-regional differentiation would be observable except as a very small and possibly non-significant segment of the cline. In either case there would be a marked emphasis in differentiation towards adaptive characters.

But if the territory were colonised by more than one race there would be in addition to these two features an overriding pattern determined by the characteristics of the races and their geographical sources. Thus, if the races differed in their morphology but were ecologically equivalent or nearly so, they might mutually exclude each other from territories colonised. Something of the kind has already been suggested by Gregor and Watson (1954). Alternatively perhaps with short-lived perennials, gene flow across the boundary would establish clines and this is the explanation that Woodson (1947) puts forward to account for his results with *Asclepias*. If, however, the races did show ecological differences there is the possibility as a result of hybridisation of ecocline formation, many of the characters though correlated with the environmental factors having in themselves no adaptive value.

The age of grassland plants has been used as evidence against the possibility of differentiation by selection in situ. It could just as well be used to argue that differentiation is impossible in isolation. However, it is very probable that there is a rapid turnover of generations during colonisation. Any large territorial advance must be by seed dispersal, since vegetative spread would be too slow. The seed would mostly be derived from the nearest plants and these, of necessity, would be the youngest, and probably few in numbers. Thus the requirements for differentiation—many generations of small size, in circumstances approximating to isolation—could all be satisfied at the invasion front. The new territory might be divided into sectors like an ever mutating fungus on a petri dish.

Undoubtedly colonisation of a territory would not have been a simple matter of seed dispersal. There may well have been several advances and retreats, or virtual extinction save for a few strongholds followed by recolonisation from the separate points. The actual history of colonisation may affect the present-day picture and be readable from it. Godwin's Quaternary researches give very good reason to believe that after being universal the grassland species were restricted to small "refugia" from which they have spread out again. The refugia would have supported small populations and may well have been isolated. Many of them were characterised by unstable rooting material so that constant re-establishment from seedlings might have given a sufficient turnover of populations. Once again the requirements for differentiation in isolation would be satisfied.

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IMPROVEMENT OF ROUGH GRAZINGS BY SURFACE SEEDING

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Methods of improving rough grazings are many. During the last war reseeding after ploughing received the greatest emphasis and the development of heavy ploughs helped to further a system of direct reseeding on a heavy flat furrow. However, on most hill farms areas which can be easily ploughed are limited.

Manuring of the natural vegetation is therefore of topical interest and much experimental work has been done in which the Scottish Agricultural Colleges have taken their share. Results have not been encouraging for on only a few types of hill swards has there been a visible improvement giving even a promise of an economic return. There may, of course, be improvement in the grazing which cannot be detected by the eye; in fact some evidence of the preference of stock for treated pastures suggests this. Yet New Zealand experience encourages further experiment, although it would be necessary to bear in mind that even if striking results from manuring were demonstrated one would have to think carefully before embarking on an extensive programme of manuring on an open hill. Problems of stock movement and herding would have to be considered as well as the possibility of recovering from the stock the extra rent paid in manures. One could easily intensify the pasture improvement programme to an extent which the stock and system of farming applicable to the land could not support.

In this article I have been asked to deal particularly with the improvement of hill pastures by surface seeding and manuring. The practice is not new. Before the last war Mr Allan at Auchinleck and my colleagues Dr Clouston and Mr Angus Macleod in North-East Scotland and in Lewis had pioneered the system. Little was done during the war years. Emphasis was then on ploughing and subsidies were restricted accordingly. In post-war years Government grants for surface seeding and official encouragement of sheep and cattle rearing have provided the necessary incentives. The North of Scotland

College of Agriculture has over the past ten years carried out an extensive programme of surface seeding. The bulk of the work has been done in the Western Islands and Western mainland where in co-operation with crofting townships areas of common grazing, usually about 20 acres in extent in each case, have been set out as demonstration and trial fields. Recently the College farm at Achany, Lairg, has been developed as a centre for these investigations. At first crofters were slow to follow these demonstrations, but now, particularly in Lewis, many townships have surface seeded sections of their common grazings.

Conditions on the West Coast and in the Islands are usually very suitable for surface seeding. The climate is mild and the rainfall generous. This is ideal as autumn growth is usually prolonged and there is little danger of drying out which is the greatest risk in establishing a new pasture. The natural sward is usually of sparse heather over a soil of eroded, "skinned" or deep peat. Heather which is open in the bottom allows the seeds to reach the soil easily and offers little competition to the new grass seedlings. A grassy sward even if it can be burnt is usually too dense for successful seeding without cultivations of some kind. Grass can be easily established in deep peat bogs but provided there is moisture either lying on the soil surface or from frequent rain, shallow peat or mineral soil is more suitable. Haulage of materials is easier over a firm soil and the established sward will dry out to a firm pasture more suitable for stock grazing.

Little or no preparation of the site is necessary or even possible. Where the old vegetation can be burnt off this is usually worth while but when the site is on a slope or inclined to be too dry short heather may offer useful protection to the seed and prevent washing off by heavy rain. That the latter may be serious is often well demonstrated by the thick growth of grasses and clovers in ditches leading from a seeded area.

Provided the land is sufficiently dry to allow transport of seeds and manures there should be no drainage in the first year. Even on very wet land little drainage may be required in subsequent years as when the new sward is established it helps to dry it out. Initial establishment will be better on the wetter patches but if surface water persists it should be drained off as grass will tend to get slimy and die out and, of course, stock will not thrive well on wet land.

It is usual to fence before or soon after seeding, not necessarily to protect the seedlings which at first will really benefit from being trampled into the soil, but rather to prevent the hefting of hill stock from being upset through their being drawn towards the young grass.

In the majority of surface seeding schemes no cultivations either to prepare a seed bed or to cover seeds have been attempted. Usually the land surface has been so soft and uneven that it could be crossed only with a track-laying tractor and cultivations involving equipment on that scale have been thought uneconomic and unnecessary, but where some attempt at surface cultivation has been made it has undoubtedly led to a better establishment of grass. At centres in South Uist, Benbecula, North Uist and Skye a Parmiter harrow was used. There the original swards tended to be rather too grassy and harrowing helped to open them up and gave seeds a better chance. The same effect has been achieved at Achany on a small area where the land was very wet and almost too boggy to cross with a tractor. This year at Lubcroy, Sutherland, Mr Thomson has successfully seeded rough hill land after discing the surface. Any machines which will break the soil surface and give a light cover to the seed will help germination and help to prevent washing off of seed, but their use is usually limited by the rough nature of the land and the knowledge that on a suitable site given suitable weather no cultivations are needed.

The better catch of grass in wheel marks and tracked areas has suggested that seed can be more effectively embedded and covered if it is distributed as a first operation so that it may have the benefit of the packing and cover resulting from the subsequent distribution of lime and fertilisers. There is considerable evidence to support this particularly where the lime used is a wet bulky shell sand. A most effective cover can be had by treading in by sheep herded on the treated land as soon as other operations have been completed.

Amounts of lime and fertilisers used have varied. In our earlier trials we used lime, heavy dressings of Ground Mineral Phosphate or Basic Slag and little or no nitrogen. Generous liming is essential and there is evidence that after an initial dressing of 2 to 3 tons Ground Limestone per acre lighter maintenance liming should be considered after three to four years. Potash has given little obvious benefit. Nitrogenous manuring is essential and gives a much quicker establishment. The fertilisers most commonly used have been 2 to 3 tons Ground Limestone or the equivalent of wet shell sand, 5 cwt. Ground Mineral Phosphate and 4 cwt. High Nitrogen fertiliser per acre. The use of Ground Mineral Phosphate had been suggested by the fact that we were generally working in high rainfall areas and by the subsidised transport of "straight" fertilisers. Trials have, however, been indicating that its use is not justified and that for the first few years it is more satisfactory and economic to use only superphosphate to supply phosphate.

In earlier years seed cleanings were mainly sown but although there is a case for their use in some circumstances to-day the rise in other costs and the present subsidy schemes justify a seeds mixture of first quality. A simple mixture of Perennial Ryegrass, Timothy, New Zealand White Clover and Kent Wild White Clover does well. Crested Dogstail has been sown as a bottom grass but it is much too difficult to control. S.23 Perennial Ryegrass and White Clover are the essential items. Timothy usually establishes very well. This may be partly because it is a bare slippery seed which finds its way quickly into the soil. When seed cleanings are used Yorkshire Fog is usually prominent in the pasture. It establishes quickly and grows strongly and in some circumstances should be considered a useful grass. At North Dell and North Brager in Lewis two 20-acre fields were sown with mixtures of Yorkshire Fog and Wild White Clover and they developed into most useful pastures for that area.

July is usually a wet month and we have in most cases aimed at getting the advantage of this by sowing grass seeds at the end of June—drought is the greatest risk in surface seeding. Spring sowing may also be successful and is to be recommended if lime and fertilisers have been applied earlier. Particularly if there is grass in the old sward, sowing of grass seeds must follow immediately after as the fertilisers will increase competition from the grasses and give a more dense ground cover. Autumn sowing is too risky even under West Coast conditions.

Great care must be taken to protect the pasture from overgrazing in the first autumn and winter, but, as with any pasture, it is wise to graze off proud growth which would go down with frost. On firm soil, too, grazing may do little harm but where grass has been established in moss, grazing sheep will pull it out and do much damage in a short time.

There are many advantages in having such areas of reseeded land on a hill farm. It is sometimes said that they upset the stock and draw them off the hill. If the reseeded areas are fenced there is no need for this to happen. The stock can be selected and the pastures used for special purposes and for special classes of stock.

A more important and difficult problem is that of the extent to which intensive improvement of grassland is justified. At least three points arise and have to be set against the revenue from the new pastures :—

1. The initial cost which, allowing for subsidies available at the present time and excluding fencing, might be £10 per acre.
2. The cost of maintenance.
3. The cattle stock which the farm can carry and have available to control summer grass. The cattle stock will be limited by the amount of winter keep and grazing available in late spring and autumn at which time cattle and sheep interests may conflict.

The farmer or crofter must decide whether it is worth while to improve his own particular hill or outrun and, if so, which special section and what proportion should be tackled. At present there is most promise in the system of fencing off and improving smaller areas which could be intensively treated and exploited for special purposes of high value to the farm. In such enclosures a complete renewal of the pasture by some system of reseeding can be practised. There can be no generalisation, but each farmer must decide knowing the area and quality of the grazing, the proportion of rough to inby and cultivated land, the likely effect on his stock and his ability through his stock to turn the improvement to profit.

RESISTANCE IN OATS TO ATTACK BY THE STEM EELWORM *DITYLENCHUS DIPSACI* (KÜHN)

The cultivars Early Miller, Milford and the hexaploid species *Avena ludoviciana* Dur, as sources of resistance

D. Cameron and D. W. Speed

Since Goodey (1937) reported differences in susceptibility to attack by the stem eelworm (*Ditylenchus dipsaci* Kühn) amongst oat varieties, the problem of resistance has received increasing attention from plant breeders. Seasonal differences in the degree of attack by stem eelworm in the field, and the difficulty of obtaining a uniform level of infestation, led to the commencement at Pentlandfield in 1956 of a programme involving the injection of a suspension of eelworm into oat seedlings using the method described by Seinhorst (1952) in his work on rye. At the outset the material chosen for test consisted of (*a*) varieties known to be susceptible; (*b*) the varieties Early Miller, Albyn Empress and the unnamed Aa 732 which were believed to have a measure of resistance, both the latter having Early Miller in their parentage; (*c*) the variety Milford, known to be resistant in the field; and (*d*) an F_2 population of the hybrid ref. no. 0635, *Avena ludoviciana* × Milford. Subsequent tests included selected F_3 lines of this hybrid and its other parent *A. ludoviciana*, in addition to repeating (*a*), (*b*), and (*c*) above.

The scope of the present paper is to give an account of the accumulated evidence in support of the conclusion that Early Miller behaves in the field as a resistant variety, to describe the symptoms exhibited by the material under test in response to the presence of eelworm, and to relate observations made in the field to the symptoms observed in the course of the above tests.

Until recently the only cultivars grown in Britain known to be resistant to stem eelworm were winter oats derived from Grey Winter or from European Oats of the Grey Winter type. McLeod, Golightly and Price (1954) reported the spring oat variety Milford to be resistant, which was confirmed a year later by workers at Aberystwyth, Jones, Griffiths and Holden

(1955), and Aberdeen, Robertson (1955). Milford, bred at Aberystwyth from a cross between Victory and S. 172, also derives its resistance from Grey Winter (Griffiths, Holden and Jones (1957)).

In 1947 workers at the Scottish Plant Breeding Station, Corstorphine, had their attention drawn to Early Miller as a possible source of resistance to attack by stem eelworm in the field, when by chance a replicated trial containing 25 varieties was attacked by this pest (S.P.B.S. Rep. (1948)). It was apparent that all the varieties in the trial had not suffered to the same extent and an examination of the incidence of attack revealed that out of nine varieties with Early Miller in their parentage seven were unaffected or only slightly damaged. A similar occurrence in 1950 confirmed this impression. In that year a trial of 23 varieties had two out of four replications damaged by eelworm, while the other two were virtually unaffected (S.P.B.S. Rep. (1951)), as also was an adjoining plot of Early Miller. Seven varieties were of Early Miller parentage and six of these were amongst the twelve to be harvested, the remainder being so severely attacked that the plots containing them were ploughed in midsummer to prevent the multiplication of weeds. Part of the land occupied by the Plant Breeding Station at Corstorphine was heavily infested by eelworm, nevertheless on no occasion had an attack of stem eelworm on Early Miller been recorded, nor could any evidence of attack be obtained from other districts in Scotland in which the variety had been grown. Unfortunately, Early Miller had not been included in resistance trials such as those carried out in Kincardineshire, Robertson (1955). The evidence in favour of Early Miller as a resistant variety was therefore largely circumstantial, based on the behaviour of a number of varieties derived from it.

When the inoculation tests revealed that Early Miller and the two varieties derived from it were able to tolerate a large population of eelworm it was appropriate to have them tested in the field against Milford. Seed was accordingly sent to Dr Robertson in Aberdeen to be included with Milford in a trial near Laurencekirk, in a field with a history of eelworm attack. Under the conditions prevalent in 1957 in that area, from which a number of cases of total crop failure due to attack by stem eelworm were reported,* patches of typical

* Private communication from Mr G. M. Mackintosh.

"Tulip root" were found in both Albyn Empress and Aa 732, the former being the more severely affected. Both Early Miller and Milford were free from such patches but on close examination a few plants with swollen secondary shoots were found, indicating the presence of eelworm in the tissues, such shoots being less conspicuous in the latter.

The inoculation tests were carried out in the following way. The oat grains were husked and the kernels placed overnight in corked tubes with "Panogen" at the rate of 1/300 c.c. per 20 grains. Paper germination pads 3 mm. thick and measuring 16 cm. by 8 cm. were prepared by cutting 20 slits 1 cm. long and 8 mm. apart, parallel to the short sides of the pad, and approximately 5 mm. from the upper edge. The kernels were then inserted in the slits so that they were buried in the pads, leaving only the germ protruding at the bottom of each slit. The pads were placed in aluminium holders, covered with two layers of thin filter paper, soaked with Knopp's solution, and suspended in racks. Each rack held 30 pads, kept moist by having their lower edges in water.

After germination, the seedlings when 1-1½" long were injected by means of a hypodermic syringe with a suspension of eelworm in an aqueous solution of carboxy-methyl-cellulose at the rate of approximately 300 eelworm per plant. In the 1957 series of tests, which confirmed the results obtained in 1956, injections were made on each of two consecutive days at the rate of approximately 150 eelworms per plant per inoculation. Control inoculations in which seedlings were injected with an aqueous solution of carboxy-methyl-cellulose were made in both years. Two assessments, at intervals of 20 and 40 days after inoculation were made. At the second assessment selected seedlings were potted for growing on to maturity.

In all the material tested the eelworm inoculated plants were both shorter and thicker than the controls. There were, however, considerable differences in degree of both shortening and thickening. Photographs taken at the first assessment were projected and measurements made of plant length and of the thickness 1 cm. from the base. These are set out in Table I. together with the means of inoculated plants expressed as percentages of the controls, and descriptions of the inoculated plants at the second assessment.

Four distinct types of reaction to the presence of eelworm in the tissues were detected at the first assessment. (1) The

TABLE I.

Material tested	*	Year	20 days after inoculation						40 days after innoculation
			Plate No.	Number of plants examined	Mean length cm.	Mean thickness cm.	Inoculated as % of control		Appearance of inoculated plants
							Length	Thickness	
Milford (Victory × S. 172)	C	1956		20	11.6	.22			Basal region — slightly thickened. Lower leaf—little change. New Growth — similar in appearance to controls.
	I	,,	1.*c*.	20	5.2	.28	44.8	127.3	
	C	1957		5	15.9	.20			
	I	,,		15	8.3	.23	52.2	115	
Early Miller (Potato × Record)	C	1956		20	12.8	.20			Basal region — markedly thickened with irregular dilated patches giving a crinkled puffy appearance. New Growth—vigorous.
	I	,,	1.*b*.	20	11.0	.36	85.9	180	
Aa 732 (Abundance × Early Miller)	I	1956		20	9.2	.38	71.9†	190†	Similar to Early Miller. New Growth—slightly less vigorous.
	C	1957		5	16.0	.20			
	I	,,		15	11.2	.34	70	170	
Albyn Empress (Early Miller × Progress)	I	1956		20	10.2	.36	79.7†	180†	Similar to Aa 732. Basal region } rather more New Growth } distorted.
A. ludoviciana	C	1957	1.*a*.	5	12.4	.16			No external symptoms.
	I	,,	1.*a*.	15	10.4	.18	83.9	112.5	
0635 F_3 (*A. ludoviciana* × Milford)	C	1957		28	13.8	.17			Similar to *A. ludoviciana*.
	I	,,		79	11.4	.19	82.6	111.8	
C104 (Albyn Donside × Star)	C	1957		5	14.6	.20			All plants dead or dying and considerably distorted.
	I	,,	1.*d*.	15	5.0	.44	34.2	220	

* C = Control. I = Inoculated with *D. dipsaci* (Kühn). † = Measurements obtained from Early Miller controls used as a standard in this series.

type found in *Avena ludoviciana* and in hybrid 0635 in which the plants were neither stunted nor dilated (Plate I.*a*.); (2) the Early Miller type in which the plants were dilated, but not stunted (Plate I.*b*.); (3) the Milford type in which the plants were stunted but not dilated (Plate I.*c*.); and (4) the type of C104, a typical susceptible variety, in which the plants were both stunted and dilated (Plate I.*d*.).

Plants are regarded as "stunted" where the length is reduced to less than $\frac{2}{3}$ of the control and as "dilated" where irregular thickening gives a puffy and crinkled appearance (Plate I.*b*.).

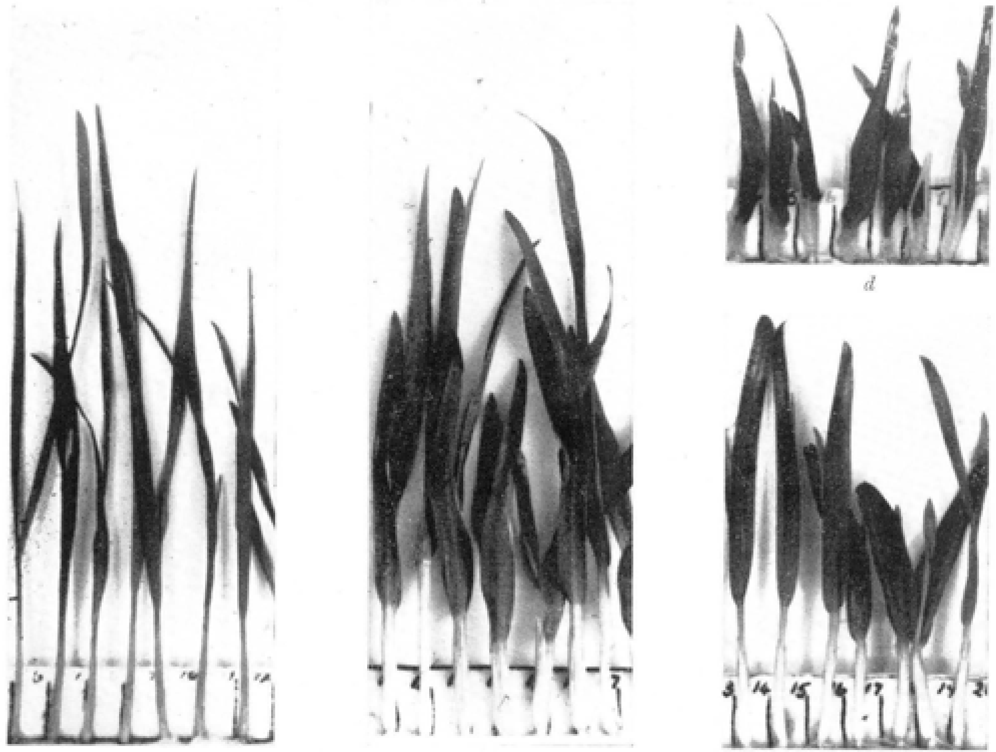
The Early Miller type of reaction was also exhibited by Aa 732 and Albyn Empress, which were scarcely distinguishable from Early Miller at the first assessment. At the second assessment however, when sufficient time had elapsed for multiplication of eelworm in the tissues to take place, the development of Aa 732 and Albyn Empress had been disturbed, particularly in the amount of dilation at the base of the plants. The order of merit at this second assessment was Early Miller, Aa 732 and Albyn Empress. When the selected plants were compared at maturity the varieties were again placed in that order.

Milford, which at the first assessment was slightly thickened at the base without dilation, and severely stunted, had recovered from the initial shock by the second assessment. Though retarded in comparison with the control, the plants appeared to be growing normally.

In completely susceptible varieties dilation of the base was frequently associated with brownish lesions in the tissues, and by the second assessment the plants were either dead or dying.

Avena ludoviciana and hybrid 0635 were neither stunted nor dilated at the first assessment and maintained this appearance at the second assessment, being only slightly less vigorous than the uninoculated controls, and growing to maturity with no external signs of eelworm damage.

Subsequent to the conclusion of the tests a further differentiation in respect of degree of resistance appeared to have taken place in the pots, and plants which had been selected for resistance were dissected and examined to compare the level of eelworm infestation in the different types at maturity. To arrive at a satisfactory basis for comparison the basal 4" of each of the two lowest available leaf sheaths were fixed and stained in cotton blue lactophenol (T. Goodey, 1949, and J. B.



a b c d

PLATE 1.—OAT SEEDLINGS 20 DAYS AFTER INOCULATION AT THE RATE OF 300 EELWORM PER PLANT × $\frac{1}{2}$.

a. *Avena ludoviciana*. One control plant is included at left of picture.
b. Early Miller. All plants inoculated.
c. Milford. All plants inoculated.
d. C.104. All plants inoculated (very susceptible).

Goodey, 1957), and the eelworms in the tissues were counted. The results are given in Table II.

TABLE II.

Material examined	No. of plants	Mean No. of eelworms per inch of leaf sheath at maturity
0635	147	below 4
	19	4 to 17
	14	17 to 45
A. ludoviciana	5	below 4
Milford	4	17 to 50
Early Miller	5	56 to 130
Aa 732	4	300 to 500
Albyn Empress	3	over 1,000

While the figures arrived at in this manner must necessarily be subject to a large experimental error, differences in numbers of eelworm in the tissues agree with the order of merit in which the inoculation tests and field observations place the varieties. It is probable therefore that these differences are due to factors governing the rate at which eelworm can multiply in the tissues. The extent to which these factors determine the expression of the visible symptoms is not at present clear. It will be observed that the numbers of eelworm recovered from *Avena ludoviciana* and Milford were within a comparatively small range, and that the numbers of eelworm observed in 180 selected F_3 plants of the hybrid 0635 lay between the limits found in the two parents. In 147 of these plants, however, there were between 0 and 4 eelworms per inch of leaf sheath which corresponds to the numbers observed in *ludoviciana*. This suggests that in selecting the plants which appear most resistant, those in which eelworm were least able to multiply were automatically chosen. In order to obtain a strictly satisfactory comparison of the rates of eelworm multiplication in the different material it would be necessary to fix seedlings of the different types at the time of the second assessment. This was not done as the material which survived the test was required for breeding purposes, but will be the subject of further study.

It is interesting to compare the appearance of Early Miller in the inoculation tests with the description of Record published by Goodey (1937), Record being one of the parents of Early Miller. Goodey drilled a number of varieties in April 1936, and of Record collected on the 10th June he writes "some of the plants showed typical but not very marked symptoms. One plant examined had a main stem about 18" high with 3 basal tillers all of which were swollen. There was not much brown discoloration of the tissues, but 150 to 200 adult *A. dipsaci* were found."

The inoculation experiments described above show that a close correspondence exists between the results obtained in the course of these tests and observations made in the field. An explanation is now offered as a result of these tests for the anomalous position of Early Miller, which in Scotland seems to behave as a resistant variety, while in tests at Aberystwyth it was regarded as susceptible.

Goodey (1937) classified varieties as resistant, susceptible and very susceptible, on the basis of the numbers of eelworm found in the tissues of seedlings collected during the months of May and June. Griffiths *et al.* (1957) give an account of tests in infested plots at Aberystwyth, varieties showing signs of swelling at the base in the seedling stage being classified as susceptible. Both the above methods relied on assessments at the seedling stage, and did not take into account the ability of a variety to tolerate an eelworm invasion of the tissues and grow to maturity in spite of it. Prior to the appearance of this latter publication it was thought that an explanation of the discrepancy lay in the possible existence of a number of eelworm races, and while this possibility cannot be excluded, it seems more probable that the reason is to be found in the different methods of classifying the varieties for resistance.

The numbers of eelworms injected into the seedlings corresponded to those found some eight weeks after sowing, in the varieties classified by Goodey as susceptible. In naturally invaded plants of this age one or more generations of eelworm would have multiplied in the tissues.

Even in "resistant" varieties a high level of infestation accompanied by unfavourable environmental conditions in the early part of the season could well result in the failure to survive of many seedlings, or by retarding them lay them open to attack by frit fly or fungal diseases. The thinning out of Milford in the Aberystwyth tests, Jones *et al.* (1955) attributed to lack of winter hardiness, may in fact be due to the type of reaction in which large numbers of eelworm severely retard the growth.

Early Miller and the varieties derived from it are not greatly retarded in their early growth when inoculated with a large number of eelworm, and this type of reaction is regarded as one of tolerance. This conclusion has been anticipated in the S.P.B.S. Rep. for 1957. In addition, however, Early Miller shares with Milford, though to a lesser degree, the capacity to inhibit the rapid multiplication of eelworm within its tissues. From a comparison of the tolerant Early Miller, Aa 732, and Albyn Empress it is concluded that inhibition of eelworm multiplication is essential to resistance in the field, and when environmental conditions favour an eelworm attack tolerant varieties exhibit symptoms of tulip root in proportion to the extent to which eelworm are capable of multiplication in their tissues. Under conditions which would seriously affect a susceptible variety Early Miller remains virtually undamaged while Aa 732 and Albyn Empress escape serious damage.

Confirmation of the resistance of *Avena ludoviciana* as revealed by the inoculation tests at Pentlandfield is provided independently from Aberystwyth, Griffiths *et al.* (1957). The Pentlandfield tests have shown this species to be both inhibitant and tolerant since it exhibits neither swelling nor stunting and only few eelworm are recovered from the mature tissues. *A. ludoviciana* appears to combine the different types of resistance found in Early Miller and Milford, but is superior to both, in that the rate of eelworm multiplication in the tissues is lower than in either of the two cultivars, which are themselves superior in this respect to the others tested.

Selections from a hybrid between *A. ludoviciana* and Milford were more resistant to stem eelworm than Milford.

Avena ludoviciana crossed readily with varieties of *A. sativa* and investigations in progress are designed to determine whether the resistance found in *A. ludoviciana* can be transferred to oats of commercial value, whether the types of resistance found in Early Miller and Milford are of the same genetic nature as in *A. ludoviciana*, and whether the two types of resistance can be combined in a hybrid between Early Miller and Milford.

The nature and degree of resistance to stem eelworm is readily detected in the course of inoculation experiments, the results of which can be related to behaviour in the field. This being so, the inoculation technique provides a convenient and sensitive method of testing oats for resistance, independent of the vagaries of climate, which when applied to progenies of hybrids, involving Early Miller, Milford and *Avena ludoviciana*, should lead to the development of new resistant Spring Oat varieties.

Acknowledgments

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Fig. 1

Fig. 2

Fig. 3

Fig. 1.—Yellowing of inoculated leaf of Craigs Royal potato 20 days after sap inoculation with cucumber mosaic virus.

Fig. 2.—Systemic leaf yellowing in Arran Crest potato following sap inoculation with cucumber mosaic virus.

Fig. 3.—Leaf-withering and leaf-drop in Arran Crest potato following sap inoculation with cucumber mosaic virus.

A NOTE ON THE OCCURRENCE OF CUCUMBER MOSAIC VIRUS IN POTATO

A. W. Macarthur

The natural occurrence in potato of cucumber mosaic virus has been reported in England (1) and also in Scotland (2). This short note deals with the virus which was detected in 1956 (2) in a potato seedling grown in a field of a private raiser.

A potato plant showing unusual symptoms was noticed in a plot of seedlings. The plant was chlorotic and showed patches of a blistering mottle. The leaflet apices were unusually elongated and the leaflet margins were distinctly wavy. All tests of the foliage for the common potato viruses were negative. From the results of sap inoculation to *Datura stramonium*, *Lycopersicon esculentum*, and *Nicotiana tabacum*, and of cross-protection tests in tobacco with a derivative of Price's yellow variant strain of cucumber mosaic virus, it was concluded that the virus was a strain of cucumber mosaic virus. This was confirmed by cross-protection tests in *Zinnia elegans*.

The responses of commercial potato varieties to infection with this virus were examined. One virus-tested plant of each of 22 commercial potato varieties was inoculated with sap from infected White Burley tobacco. The course and severity of the disease in potato varied slightly from variety to variety but usually the inoculated leaf began to yellow 2-3 weeks after inoculation (Plate 1, figure 1); the yellowing spread slowly to the base of the petiole and was followed by complete collapse of the leaf which remained suspended by the withered petiole. Yellowing symptoms were shown in turn by each leaf, above the site of inoculation, as it was invaded by the virus (Plate 1, figure 2). At this stage the symptoms resembled those of leaf-drop streak due to virus Y (Plate 1, figure 3). In a few varieties the inoculated leaves yellowed but this was not followed by systemic invasion. The virus could be recovered, by sap inoculation to tobacco, only from those parts of the potato plant which were showing symptoms of infection.

All inoculated potato plants produced tubers and one tuber from each plant was grown for further examination. The resulting plants appeared healthy and sap inoculation to tobacco failed to recover the virus.

In the conditions under which the tests were made there was no evidence of the virus being transmitted through the tuber and, according to a previous report (3), only a small proportion of artificially infected potato plants produced infected progeny. It is unlikely, therefore, that the disease could be of economic importance.

I am grateful to Dr D. C. Graham, Scientific Services, Department of Agriculture for Scotland, for making the cross-protection tests in *Zinnia*, and to Dr D. A. Govier, Pentlandfield, for the photography.

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THE PREPARATION OF ANTISERA TO POTATO VIRUS X

D. A. Govier

Current practice in preparing antisera to plant viruses is to inject the antigen (virus) intravenously, either in a single dose, or in a number of doses spaced at intervals of 2-7 days. A given quantity of virus produces a higher antiserum titre when injected in a number of small doses than when injected in a single dose (Matthews, 1957, p. 69) and, therefore, the second method is used when high titres are required. The first method may be used when the virus can be highly purified and concentrated, particularly when antisera with a high concentration of the minor antigens are required for cross-absorption tests (Matthews, 1957, p. 96). Potato virus X can be purified, with some difficulty, but the resulting suspensions are highly variable and not especially suitable for preparing antisera to be used in cross-absorption tests.

In attempts to find a technique suitable for preparing antisera to virus X with a high concentration of strain specific antibodies, two methods were tested which, although they have not been evaluated for the purpose in hand, should prove useful for the preparation of routine antisera to potato virus X. They give high-titre antisera following the injection of very small quantities of virus.

In the first method (C in Table I.), the rabbits were given two intravenous injections of partially purified virus suspension, the second injection (4 ml.) being made 4 weeks after the first (2 ml.). The rabbits were bled 10 days after the second injection, when the antiserum titres had reached a maximum; bleedings taken 2 days later showed no increase in titre and with three of the rabbits there was a slight decrease.

In the second method (D in Table I.), the partially purified virus suspension was emulsified in an equal volume of Difco Bacto-adjuvant Complete (Freund) and 1 ml. of the emulsion was injected intramuscularly into each of the hind legs of the rabbit. Bleedings were taken at intervals after the injection and the antiserum titre was found to reach a maximum after about 4 weeks (Fig. 1). Adjuvants have been used previously for the preparation of antisera to animal viruses (Salk and

TABLE I.

Immunisation procedure	Rabbit No.	Antigen		Maximum antibody concentration	Antibody units per antigen unit	Mean antibody units per antigen unit
		Concentration	Total units injected			
A	64 70 75 80 85	16,384	81,920	256 256 256 128 128	0.003 0.003 0.003 0.0015 0.0015	0.0024
	41 47	2,048	4,096	64 64	0.016 0.016	0.016
	43 46	256	512	48 48	0.106 0.106	0.106
B	68 73 79 83 89	164	984	256 512 512 512 128	0.260 0.520 0.520 0.520 0.130	0.390
C	49 51 52 53	512 and 256	2,048	8,192 16,384 8,192 8,192	4.000 8.000 4.000 4.000	5.000
D	65 71 76 81 86	16,384	16,384	4,096 4,096 2,048 4,096 4,096	0.250 0.250 0.125 0.250 0.250	0.225
	63	128	128	2,048	16.000	16.000

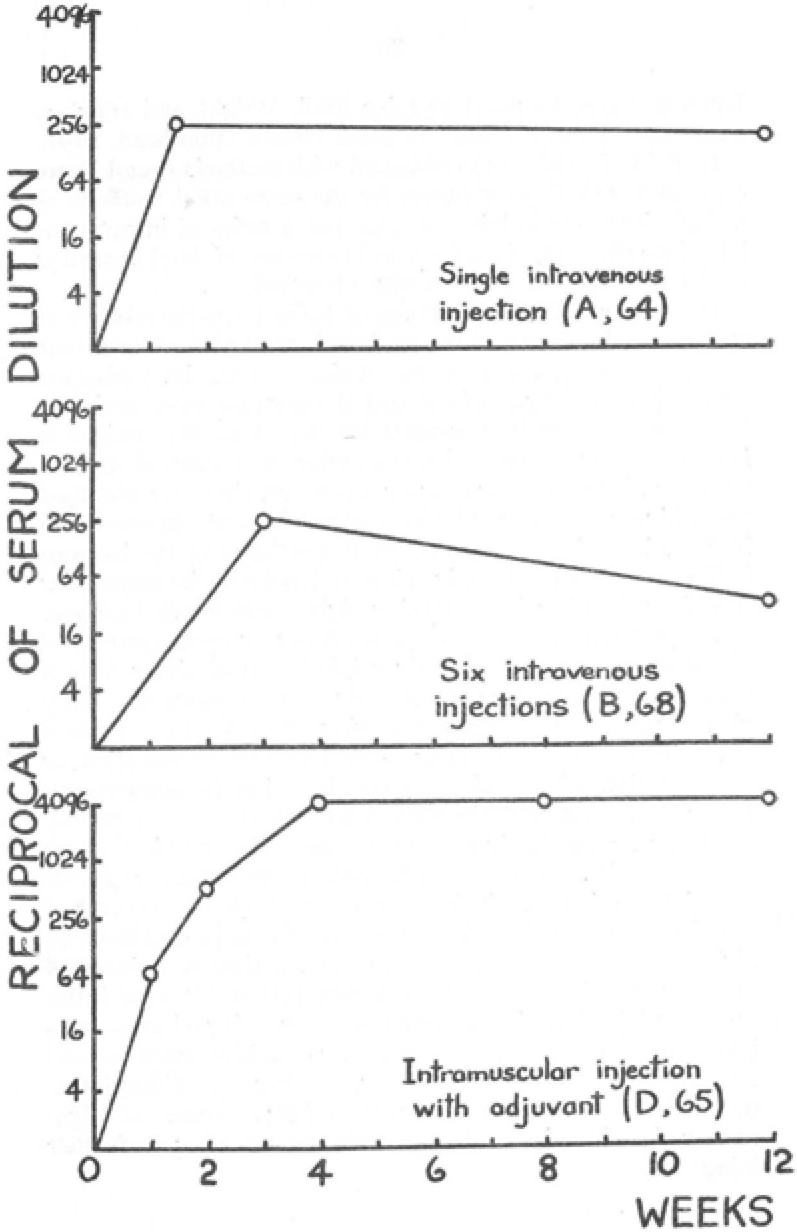

FIG. 1.—DEVELOPMENT AND PERSISTENCE OF ANTIBODY IN RABBITS AFTER IMMUNISATION WITH POTATO VIRUS X.

Note.—It is unlikely that the graphs represent the true rate of development of antibody, as an insufficient number of points is plotted.

Laurent, 1952), to plant proteins (Gell, Wright and Hawkes, 1956) and, on one occasion, to plant viruses (Moorhead, 1956).

In Table I., the results obtained with methods C and D are compared with those obtained by the more usual methods of a single intravenous injection (A), and a series of intravenous injections (B). The data for A and D are sub-divided according to the concentration of the antigen injected.

The figures in the sixth column of Table I. are the number of antibody units produced by the injection of each antigen unit and are, thus, a measure of the efficiency of the immunisation procedures. The figures for A and D show that more antibody is produced per unit of antigen injected when the antigen is injected at low concentration than when it is injected at high concentration. Although higher antiserum titres are obtained when highly concentrated virus suspensions are injected, the increase in antiserum titre is not proportional to the increase in the concentration of antigen injected and with intramuscular injections little is gained by using highly concentrated antigen.

Methods C and D produce a much higher concentration of antibody for each unit of antigen injected than either A or B when the concentrations of the antigen are comparable. They give high antiserum titres following the injection of small amounts of virus and, using these methods, purification or concentration of the virus before injection is unnecessary. Injecting intramuscularly with adjuvant (D) has a further advantage. The antigen used to immunise rabbit 63 was heat-clarified tobacco sap which produces unpleasant reactions when injected into rabbits intravenously. No ill-effects resulted from intramuscular injection of this material and a satisfactorily high titre was produced. The antiserum titre is maintained at a near maximum level over a long period following intramuscular injection with adjuvant, or after a single intravenous injection, but the titre falls off more rapidly when several intravenous injections have been given (Fig. 1). Using intramuscular injection with adjuvant, a large volume of high-titre antiserum can be obtained without resorting to further injections.

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