SEASONAL ROOT CHANGES AND THEIR RELATIONSHIPS TO LEAF PRODUCTION AND PERSISTENCY OF GROWTH IN SOME GRASSES AND CLOVERS.

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SECTION I. INTRODUCTION.

The seasonal behaviour of pasture plant species is of fundamental importance to the farmer as one of the indices of value of any particular species, and the uses to which it can be put; and to the plant breeder, since it is his primary object to produce pasture plants which suit the farmer under his particular system of farming. To illustrate this point specifically, the example of the ideals aimed at in the breeding of short rotation ryegrass in New Zealand may be cited. Quoting Levy (1945), "... Italian ryegrass is a one- to two-year plant for the temporary pasture, and the true perennial ryegrass can be used widely in rotational and permanent pastures. There is, however, too large a gap between true perennial ryegrass and true Italian ryegrass, and many years of breeding and selection within these species accentuated the gap rather than reduced it." It can be seen that the short rotation system of farming, incorporating pasture leys of 3-4 years was not well served in the choice of a suitable ryegrass. Thus the plant breeding section of the Grasslands Division (Department of Scientific and Industrial Research) turned its attention towards breeding by hybridisation of perennial and Italian ryegrass, a type that would possess as far as possible the desirable qualities of these two species in combination; that is, it would combine some of the rapid establishment, high winter and early spring growth and palatibility of Italian ryegrass with an appreciable degree of persistency of perennial ryegrass. Seasonal growth thus played the major part in the objectives of the resultant programme from which short rotation ryegrass was produced. Comparative trials of yields of the three grasses, perennial, Italian and short rotation ryegrasses were conducted in different localities, serving a very important purpose in providing farmers with direct information regarding the seasonal characteristics of production of the new type. These trials, which are continuing, show that short rotation ryegrass conforms largely to the objectives aimed at, so that the requirements of the man farming under short rotation system have been met, to a great extent, by the work of the plant breeder.

While the result of any breeding project like the one described may be economically satisfactory, little knowledge is gained of the nature of these seasonal growth changes effected by breeding. The information regarding this is restricted to the foliage growth in terms of single plant observations during the progeny testing part of the breeding programme, and dry matter figures obtained from yield trials of the resultant product. Thus there arises a situation where a result is obtained with little reference to the cause. Hutchinson (1939) has stressed the need for increased knowledge of the physiological processes of plants if progress is to be made in breeding by improvement of methods of selection for quantitative characters. If this is true, more emphasis must be placed on investigations of the pasture plant as a physiological whole, whose seasonal growth is the result of the interaction of many environmental factors on the entire plant, root and above-ground parts.

It is at this point, that the present investigation hopes to supply some of the facts which link up seasonal production of grasses with one of the factors which, it would not be unreasonable to speculate, may be directly concerned, namely, seasonal root growth. Is is only comparatively recently that American workers have investigated this aspect of root development, using as experimental material both cultivated species and indigenous prairie grasses, the latter being easier to handle due to the relative thickness of the individual roots. There is a paucity of New Zealand work on root development of pasture plants, Jacques being the only worker to concentrate on the subject. No results of seasonal studies of root growth of grasses in use in New Zealand have been published, while such studies of clovers have been neglected both here and overseas.

The role of the root system cannot be divorced from any consideration of the seasonal growth of plants, since its physiological functions of absorption, transport and storage of nutrients so important to the plant, are so closely bound up with any changes which may occur in the top-growth of the plants. Thus this study entails the description of root changes such as weight, new growth, degeneration of older roots, colour, etc., with the seasons, and where possible, measurements of the same. In this respect, the objects of the work are somewhat ahead of New Zealand studies of above ground parts of pasture plants. Corkill (1947) in a paper read to the Genetics Discussion Group, Massey College, stated that more would have to be known of such considerations of leaf growth as morphological leaf characters, amount of tillering and seasonal influences, for improvement of selection methods in breeding, since yields from experimental plots by weight and individual plant observations without detailed descriptions were the only bases on which the pasture plant breeder worked in synthesizing new types.

Again, it appears that the plant is considered merely in terms of the green matter it produces, without cognizance of the fact that the physiological processes that govern the growth of leaf, and the conditions which modify those processes must act in some way on the rest of the plant. This investigation hopes to show the influence of season on the growth and behaviour of the underground portions of various pasture plants, and how this is related to the seasonal behaviour of the above ground parts. It entails the description of morphological root characters and development of new roots as affected by season and thus parallels the studies that are mooted for the above ground parts of pasture plants.

The particular objects of this investigation are as follows:-

1. To set down seasonal changes observed in the root systems of the plants chosen for trial, and to measure these changes where possible.
2. To relate the different root changes in combination, to seasonal variation in production and persistency of growth of the pasture plants studied.

How useful work of this nature is likely to be to the plant breeder is a matter of pure conjecture, but it is apparent that, combined with the results of similar studies of the seasonal influences on top growth, a more adequate picture of the growth of pasture plants would be obtained.

SECTION 11: REVIEW OF THE LITERATURE.

The literature bearing on the seasonal aspects of root growth in pasture plants is not extensive. Investigations made on root systems have been more of the nature of enquiries into the effects of environmental factors such as moisture, temperature, light, aeration of the soil, soil fertility and different types of management of these plants on root development and growth. Prior to the work of Sprague (1933), seasonal growth studies on roots over a year or more had been made principally on fruit and forest trees by McDougall(1916), Harris (1926) and Collison (1935). It can be seen that the important details of seasonal root growth - development and changes in terms of extent, both lateral and vertical, weight, deterioration, and new rooting with the time of year have only recently received attention from botanists and agriculturists.

Early conceptions of the seasonal growth of pasture plants were probably founded on the ideas held by horticulturists. According to Goff (1898) it was common to find stated in horticultural publications, that fibrous roots of perennial plants die in the autumn and are renewed on resumption of growth in the spring. Goff indicates that this view was commonly held in reference to perennial pasture species. In the same paper (1898), he describes his investigations in which he found that root replacement did not occur in many species of forest trees, fruit trees and shrubs, but on the contrary, new growth of the fibrous roots generally began from the identical tips where it ceased in the autumn, the time, and development in terms of length, varying with the species. It was also found by the same author that the roots of Rubus species and Agropyron repens appear to make considerable growth before any visible growth of the plants above ground in the spring. The value of this work does not lie so much in its direct application to economic pasture plants, but rather in its early contribution to the appreciation of the fact that there is great variation in seasonal root behaviour among plant species.

The first direct investigation of the seasonal root growth of pasture plants was made by Sprague (1933) on two species of cultivated grasses, Kentucky bluegrass (*Poa pratensis*) and Colonial bent (*Agrostis vulgaris*). Two important findings were :

(i) in the spring, bluegrass formed new roots approximately two weeks earlier than bent grass.

(ii) maximum root weight for bluegrass occurred in early May and practically two weeks later for bent grass. The maximum root weight was approximately twice as great as at the beginning of the season, indicating that at least one half of the root system is newly generated by each spring.

Sprague ascribes the difference between species in the formation of new roots to a difference in the minimum temperatures at which growth can proceed.

The length of life of roots in five species of range grasses was studied by Stoddart (1935), who states that hitherto, no study of this nature had been made. Using the banding technique suggested by Weaver (1932), and subjecting the plants grown to all degrees of soil moisture from below wilting coefficient to saturation point, and to temperatures from $0^{o}F$ to $112^{o}F$, he came to the conclusion that roots of these species live for at least one year and many in excess of two. Here again, variation between species was found, this time in length of life of roots.

The roots of ten cultivated pasture species were placed in two classes, "Annual" and "Perennial" by Stuckey (1941), the former being those which the writer found to regenerate each year, the later being those in which maximum production of roots occurred during the first year and remained functional for more than one year. The following table is Stuckey's classification of the species studied :

"ANNUAL"	"PERENNIAL"
Timothy	Kentucky Bluegrass
Red Top	Canada Bluegrass
Meadow Fescue	Orchard Grass
Rough-stalked Meadow Grass	Crested Wheat Grass
Perennial Ryegrass	
Colonial Bent (probably)	

Most new root growth began in the autumn, continuing into the winter, ceasing during the coldest month (January), and resuming in early spring as the temperatures rose, maximum increase in the number of new roots being in the spring after the thaw for most species. Growth ceased in the summer, coinciding with periods of high soil temperatures. There was some considerable variation between species as regards time of onset and cessation of root growth.

According to Stuckey, new root growth in the "annual" type of root is followed by disintegration of the old roots as soon as the former have become established, while in the "perennial" type only a small percentage of the old roots disintegrate and only a few new roots develop.

The length of life of the roots of ten species of perennial range and pasture grasses was studied by Weaver and Zink (1946) All these grasses showed a high rate of root survival in the first year. No losses or replacements of root systems were reported, so that the results were in agreement with those obtained by Stoddart (1935), who also worked with prairie species. From these investigations it appears that there is a sharp difference between the seasonal root behaviour of the native prairie species of the United States and the European or cultivated species. The latter demonstrate annual replacement of roots by new growth, varying with species in quantity and time of growth, while the former have root systems which are more permanent in nature.

There seems to have been no work done directly on the seasonal growth of grass roots apart from that summarised above. Pavlychenko (1942) mentions that root development of *Agropyron cristatum*, *Agropyron pauciflorum* and *Bromus inermis* was greatly retarded or ceased entirely 180 days during the autumn, winter and spring, between the first and second seasons of growth, but pursues the subject no further.

It will be observed that no literature concerning the seasonal root growth of clovers has been cited. The only reference that could be found on the subject is that of Erith (1924) in connection with her work on white clover. She makes several pertinent statements regarding the development of the adventitious root system. Plants grown from seed sown in April and examined in July possessed few adventitious roots, but on the stolons of the same plants in September (late summer) there were numerous roots. In wild clover plants one adventitious root is produced at each node of the stolon, and occasionally two roots appear at the same node. These roots often grow to considerable length (4-6 inches) before forming lateral branches. Erith states also, that later, the adventitious root system from each node resembles a typical tap root system. She does not mention the behaviour or the fate of the primary root system.

The deficiencies in present knowledge of seasonal root growth are obvious. The differences between species alone offers wide scope for detailed study, and while the time of the onset and new root growth in some species of grasses has been recorded, there is a paucity of data on root deterioration and its nature. Jacques (1941) in reference to perennial ryegrass states: "... there is deterioration in the cortex and the absorbing surface of the more superficial or older portions of the roots before the dry weather sets in..." The relationship between seasonal growth of roots (including new growth and deterioration, increase in length, spread, branching, etc.) and leaf growth or 'production' has not been determined, although some work has been done on the effect of environmental factors on the root-shoot ratio of trees and some grasses without reference to changes in these factors due to season, e.g. Austin (1923) - influence of pruning, Weaver and Himmel (1929) - effect of illumination, etc. However, the knowledge gained from botanical studies on the effects of environmental factors and agricultural studies on the effects of different forms of management on root growth, will aid greatly in the interpretation and understanding of the various phenomena associated with the seasonal behaviour of roots.

SECTION III. PLAN: OF INVESTIGATION.

(i) Materials.

It was decided that the investigation would be conducted on the following ten pasture plants cultivated in New Zealand:

Clovers.	Trifolium pratense	(a) Montgomery red clover. (b) Broad red clover.
	Trifolium repens	(a) No.1 N.Z. white clover. (b) A poor strain of white clover.
Grasses.	Dactylis glomerata	(a) C23 cocksfoot. (b) A Danish type cocksfoot.
	Lolium westerwoldicum	- Western Wolths ryegrass.
	Lolium multiflorum	- Italian ryegrass.
	Lolium perenne	- Perennial ryegrass

Short rotation ryegrass - a selection from a cross of L.perenne and L.multiflorum produced by the Plant Research Bureau, N.Z.

The contrast of types in both clovers and grasses is fairly wide. In the red clovers, the broad red may be considered a temporary type, while Montgomery red is more persistent; in the white clovers, the range is extreme from a poor strain to the best type of permanent pasture white clover.

Similarly in the grasses, both short lived and persistent types are included. The former are represented in the ryegrasses by Western Wolths and Italian, the latter by perennial ryegrass, while the short rotation ryegrass can be regarded as intermediate between the two. The status of Danish cocksfoot in New Zealand is not determined by any production figures from the Plant Research Bureau, but can be arbitrarily classified as fairly persistent if subjected to management favourable to its survival, but less persistent than the New Zealand C23 strain.

Because uniformity within each species could be virtually assured, it was thought advisable to plant single tillers of one plant of each species rather than use seed to obtain plants for examination. While no objections could be raised towards adopting this method in the grasses, the clovers presented a problem in that, by taking clones, tap-root formation might not be similar to that when the plants are propagated by seed. Weaver (1926) states, "In the case of certain clovers which propagate by runners, the roots of the new plants arising from these are entirely adventitious." The white clovers fall into this

Figure 1. Diagram of randomisation of plots for planting and lifting.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	PW 5	It 10	H1 3	C23 9	IW 3	PCx 6	BR 14	MR 9	Per 7	WW 13	MR 5	H1 9	BR 10	It 9	Per 5	WW 10	PW 1	C23 14	PCx 13	IW 4
B	H1 12	MR 8	C23 1	It 7	PW 14	Per 1	1W 1	PCx 10	WW 3	BR 11	BR 1	PCx 11	Per 4	WW 11	C23 12	IW 5	PW 10	It 3	MR 13	H1 1
C	H1 7	PCx 12	BR 3	MR 4	Per 3	WW 12	It 13	1W 6	C23 4	PW 12	PCx 7	PW 8	It 11	Per 14	H1 8	IW 13	C23 11	BR 7	WW 4	MR 6
D	WW 6	It 2	BR 13	IW 10	C23 2	PCx 1	H1 13	Per 9	PW 4	MR 12	IW 11	BR 2	Per 2	MR 1	H1 2	It 8	WW 2	PW 9	PCx 4	C23 8
E	BR 6	MR 10	WW 9	C23 5	PW 13	It 4	Per 8	H1 10	PCx 7	IW 8	Per 13	WW 1	PW 7	BR 12	C23 6	H1 11	IW 12	PCx 5	It 5	MR 7
F	BR 4	PCx 9	WW 5	It 12	PW 2	MR 3	C23 13	H1 4	Per 11	IW 9	C23 3	It 1	BR 5	IW 7	WW 8	H1 5	PCx 14	MR 11	Per 10	PW 3
G	H1 14	IW 14	It 6	BR 8	PW 11	C23 7	MR 14	Per 6	WW 14	PCx 3	MR 2	It 14	H1 6	PCx 2	PW 6	Per 12	WW 7	IW 2	C23 10	BR 9

Each square represents a 12" glazed pipe sunken into the ground, twelve inches from its surrounding pipes. The upper symbols in each square represent the species or strain which each pipe contains and the lower symbols indicate at which lifting the plants were examined.

<u>KEY</u>

Species or Strains

- BR – Broad red clover
- MR – Montgomery red clover
- PW – Poor strain of white clover
- 1W – No. 1 white clover
- PCx – Danish Cocksfoot
- C23 – C23 N.Z. Cocksfoot
- WW – Western wolths Ryegrass
- It – Italian Ryegrass
- Per – Perennial Ryegrass
- H1 – Short Rotation Ryegrass

Time of Lifting

- 1 – Mid-Nov. 1946
- 2 – Early Dec. 1946
- 3 – Mid Jan 1947
- 4 – Early Feb. 1947
- 5 – Late Feb. 1947
- 6 – Mid March 1947
- 7 – Early April 1947
- 8 – Late April 1947
- 9 – Mid May 1947
- 10 – Early June 1947
- 11 – Late June 1947
- 12 – Mid July 1947
- 13 – Early Aug. 1947
- 14 – Late Aug. 1947

class and since there was no guarantee that the tap root would develop normally when the plant was obtained by planting runners, and also that it is difficult to obtain uniformity in the cuttings (as regards number of nodes from which roots form), it was decided to grow the plants from seed. The rooting habits of red clover being unknown, it was considered a necessary precaution to use seed also.

To avoid heterogeneity in the seed as far as possible, nucleus stock seed of Montgomery red clover (Type Aa.581) and No.1 White clover (Type 4.W13) were obtained from the Plant Research Bureau. From the same source, broad red clover (Type Aa.580) and a poor strain of white clover (Type Aa.2541) seeds were acquired.

Single plants of the two types of cocksfoot, perennial ryegrass, Italian ryegrass, and short rotation ryegrass were obtained from the control rows of increase plots at the Plant Research Bureau, and a plant of Western Wolths was also obtained from there. From these plants, tillers were obtained for planting.

(ii) <u>The Plots - Planting and treatment.</u>

A plot was prepared on the Massey College dairy farm in April, 1946, on a paddock that had been recently sown down to pasture. The area was 50 feet by 30 feet, being free draining, free from shade and yet shielded by hedges from strong winds. Planting was randomised (Figure 1) to minimise the effects of soil heterogeneity, and the actual planting was carried out on May 11, 1946. The plants were planted in glazed drain pipes, 12 inches in length and 12 inches in diameter. Glazed pipes were preferred since they reduce the tendency of roots to congregate around the sides, a feature that is most marked in the unglazed type, as Jacques (1941) noted. The pipes were sunk to just under ground level 12 inches apart, in rows 12 inches apart as in Fig.1. Clover plants, one month old, grown from seed in boxes were selected for uniformity and transplanted directly into the pipes, two of each type per pipe, there being 14 pipes for each type of clover. The single plants of the grasses were divided into single tillers of uniform size and planted, three of each type in a pipe, there being 14 pipes for each species or strain. What was left of the grasses and clovers was planted in the soil, not in pipes, away from the sunken pipes as a check on type. The method is that adopted by Jacques (1937, 1941) and may be termed a modification of what Pavlychenko (1937) calls the "Soil-containers Method" of root development study.

At planting, $^{1}/_{5}$ oz. of superphosphate was added to each pipe and mixed with the top inch of soil. This represents a dressing of between three and four hundredweight of superphosphate per acre. No fertiliser was subsequently applied. The plants were cut to $1\frac{1}{2}$ - 2 inches above ground every three weeks, approximately, the object being to maintain them as nearly as possible to the condition of plants growing in the field, without, however, inducing checks to tillering as described by Roberts and Hunt (1936), or to rooting as described by Jacques (1937) by too frequent cutting of the tops. The pipes and surrounding ground were kept clear of weeds throughout the trial.

(iii) <u>Observations</u>.

Observations were begun on November 15, 1946, and carried on at 20 day intervals until September 1947, extending over a period of 10 months and entailing the observation of 14 pipes of each species or strain. The observations consisted of two processes:

(a) Lifting of pipes from the soil and washing away of the soil surrounding the roots.

(b) Examination of the plant in the laboratory.

(a) <u>Lifting</u>.

Since one pipe of each species or strain was required, ten pipes were lifted at each observation. Each set of observations took about five days to complete. The lifting order for each type was randomised to assure that no selection was exercised in event of any variation in top growth between pipes containing the same species. This order is shown in Fig.1.

The actual lifting procedure was as follows: Firstly the pipe was dug out of the ground, then taken clear of the occupied portion of the plot where it was immersed in water for about half an hour. This immersion made the subsequent washing-away of the soil within the pipe less tedious than direct washing after lifting. The lower end of the pipe was then sprayed with water from a $\frac{1}{2}$" hose to remove the soil from the roots gradually. No pressure nozzle was used on the hose since enough force could be obtained by restraining the flow of water from the mouth of the hose with the operator's finger. After washing, the plants were kept moist by enclosing them in damp calico bags suitably labelled until the lifting was complete and they could be transferred to the laboratory.

(b) Laboratory examination.

As soon as the plants were brought to the laboratory, the root system was immersed in water contained in rectangular glass jars while awaiting examination. The following table of results was made at each lifting of the clovers:

Section A. Plant examined entire.

1. Length of root system - taken from the point of attachment of the root to stems, to the tip of the longest root.
2. Condition of the cortex - Colour; nature of deterioration, if any; extent of such deterioration.
3. Visual comparison between root systems of the different clovers.
4. Number of tillers or stolons per plant.
5. Conditions of leafage.
6. General notes.

Section B. Examination of roots after separation from the remainder of the plant at the point of attachment.

1. Number of laterals of the first order.
2. * Extent of branching of the laterals (1st, 2nd, 3rd, etc., order).
3. Diameter of the tap root (1 inch from the point of attachment).
4. * Diameter of branches of the first order (½" from point of attachment).
5. Root hair development on tap root
   - * laterals
   - * branches of 1st, 2nd, 3rd, etc., order.
6. Length of longest root hair found.
7. New rooting. Nature of new rooting, amount, branching, root hair development.
8. General notes.
9. Root weights - Roots air dried and weighed at constant humidity in humidity room. All weighed Sept.2, 1947, at relative humidity of 66%.

* Indicates those observations in which samples of 20 roots were taken at random from the root system and the average figure taken.

The following table of observations was made at each lifting of the grasses.

Section A. Plant examined entire.

1. Length of root system - taken from the point of attachment to the tip of the longest root.
2. Condition of the cortex - Colour and nature and extent of deterioration, if any.
3. Visual comparison of roots between species.
4. Number of tillers per plant.
5. Condition of foliage.
6. General notes.

Section B. Examination after the roots had been separated from the rest of the plant at the point of attachment to the tillers.

1. Number of roots.
2. Diameter of the largest white root (1" from point of attachment).
3. Diameter of the smallest white root (1" from point of attachment).
4. * Number of laterals in the top 6 inches of root length.
5. * Extent of branching (1st, 2nd, 3rd, etc., order).
6. * Diameter of branches of 1st order (½" from point of attachment).
7. * Root hair development on main white roots and branches of 1st, 2nd, etc., order.
8. Position of the greatest frequency of root hairs.
9. Length of longest root hair observed.
10. New rooting - Numbers, nature and branching, etc., of new roots.
11. General notes.
12. Root weight. As for clovers.

* Indicates those observations in which samples of 20 roots were taken at random from the root system, and the average figure taken.

The nomenclature adopted in the care of grasses is that used by Jacques (1939).

Many of the measurements made were found to have no application to the subject, whilst factors such as cortex colour and condition, deterioration, new rooting, for example, were of utmost importance in considering the seasonal root growth of pasture plants. Some measurements such as length of root had to be most carefully considered as far as its effects were concerned, since in the ground, the roots extended only into the top twelve inches of soil, and the figures for this measurement were often over 20 inches, due to lateral spread and entanglement, which could not be measured since the plants were grown in restricted areas.

Some difficulty was encountered in measuring deterioration in both clovers and grasses. In the former, the tap root was found to be the main consideration. The end point of deterioration was the death of the tap root, which was manifest by its rotting away. The intermediate steps were difficult to measure objectively, so that a system of arbitary units was set up.

A - First signs of deterioration.

B - Portions of roots showing deterioration - under half the length of the tap-root.

C - Portions showing deterioration - over half of the tap-root and showing plainly on the branches.

D - Tap-root entirely blackened.

E - Disintegration of the tap root.

Root deterioration per plant in the grasses was measured by the number of roots affected by sloughing of the cortex, but whether the roots died or not after this was difficult to determine. Weaver and Zink (1946) assert that there is no completely satisfactory test to determine whether grass roots are dead or alive. For such determinations, the afore mentioned workers used tests of tensile strength, colour, etc., with species of which they had had much experience. The method used in this investigation was to attempt to combine Weaver and Zink's qualitative tests with a quantitative one. This latter was done by first obtaining counts of roots per tiller of each plant in the first few liftings before the plant produced new roots. Then at all subsequent observations, the number of old and new roots per tiller were taken, as well as the number of new tillers. Thus it was thought that the rate of survival, or more properly, the death of old roots through the period of the trial might be determined. However, the problem of recognising all the new tillers on different plants of the same species at different liftings made the determinations difficult and the verity of the resultant figures questionable.