AN INVESTIGATION

into the effect of

SEASONAL ROOT REPLACEMENT

upon

PLANT LONGEVITY

in

PERENNIAL RYEGRASS (LOLIUM PERENNE), ITALIAN RYEGRASS (L. MULTIFLORUM), TALL FESCUE (FESTUCA ARUNDINACEA) AND ANNUAL MEADOW GRASS (POA ANNUA).

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SECTION I.

INTRODUCTION

Any reader of the American news magazine, "Time", will know that the editors occasionally publish a "grass roots report" dealing with some current question of public interest. It takes the form of a survey of the fundamental issues involved, and attempts to assess their significance, in-so-far as those issues are known. It is a tacit recognition by a non-scientific body of opinion of the importance of the underground organs to the well-being of a plant, and that these organs are more essential, despite their hidden and inaccessible habitat, than is commonly recognized.

In any study of the growth of pasture plants, a realistic picture will only be obtained if the plant is treated as an entity. The common subdivision of plant tissues into either the aerial leaf and stem portion, or the underground root system, is artificial, for each part of the plant both depends upon and services other parts in many ways. In a discussion of the relative lack of knowledge of leaf chemical constituents, Melville (1954) has stated: "Of the dry tissue of a plant only about 10% comes in through the roots; the remaining 90% is derived from the air through the leaves which, quantitatively, are of very much greater importance as nutritional organs than are roots." A statement such as this does not indicate that the root system has little importance to the plant as a whole. Plant requirements for minerals and nitrogen are at high levels in productive pastures, and the uptake of those nutrients is effected solely through the root system. To a great extent, the 10% of the dry tissue taken in by the roots serves as a pace-maker for the level of activity producing the other 90%. For vigorous leaf production, not only must strong root system capable of fully meeting the plant's nutritional needs for minerals and nitrogen.

Too often, pasture plant studies completely ignore the underground tissues, despite the common knowledge that treatments applied to the leaves can have spectacular effects also upon the root system. The concept of physiological balance between the aerial part and the root system of the plant is based on this inter-dependence of the various plant tissues. In relation to the amount of literature published about top growth in pasture plants, there is a paucity of material dealing with root behaviour. Probably the best-known work has been that of J.E. Weaver and his associates with range grassland in the U.S.A. Weinmann (1948) has written a comprehensive review of the underground development and reserves of grasses, and a total of 125 references exhausts the literature in this field. In New Zealand, Jacques and a group of honours students at Massey College have published results on some aspects of root development in our major grass species. The experiment which is the subject of this thesis, was designed to contribute to this knowledge.

The seasonal nature of the growth of grass root systems is largely unknown, yet from the facts that leaf growth follows a seasonal pattern, and the whole plant is an entire unit with complete interdependence between its parts, it is safe to deduce that the root systems pass through a seasonal growth cycle. The evidence for this, from other studies, which is presented in the review of literature, is based on several different techniques. Some workers have used weight of roots in a fixed volume of soil as their sole criterion, but it is felt that little useful information can be gained in this way. Although a mixed pasture under normal grazing can be examined, it is not possible to measure accurately the contributions to the total weight made by the roots of the component species, while many environmental factors will remain unrecognized in their effects. The logical approach is the study of single entire plants, and this is adopted in the present experiment.

The aim of this work can be stated very briefly. It is to trace out the behaviour of the root systems of certain important grass species over a period of one year, and to determine whether or not this seasonal root behaviour can be related to persistency of individual plants. It is thought that in some species and at certain times of the year, the root system may not be capable of supplying the necessary nutrients in adequate amounts, with a consequent weakening of growth and a possibility of death. It is realized that an experiment of this nature, which is designed to survey a general field rather than to examine some particular character isolated from its complex in the plant, will pose more questions than it answers, and will open up new avenues for further study. Some of these avenues will become evident, or will be indicated, in the text.

SECTION II

REVIEW OF LITERATURE

This review will be presented in three sections, as follows:

(a) literature relevant to the top cutting treatments employed,

(b) literature describing the seasonal nature of growth of grass root systems, and

(c) literature dealing with the use of tetrazolium compounds for studying viability of plant tissues, other than the seed.

A. Top Growth.

The seasonal nature of top growth in grasses, and the control which can be imposed by various environmental factors, has been well established by numerous workers. It is correct to say that the seasonal nature of pasture growth is the basis of pasture management techniques, where the aim is to provide and to utilize herbage in the amounts and at the times when it is required by livestock, with a mininum of wastage.

In New Zealand, the seasonal production of pasture growth under various experimental conditions at Marton and at Ruakura was reported by Hudson et al (1933). Department of Agriculture trials into grassland production levels, and the rate of growth and yield capacity of some of the major pasture species, have been reported by Elliott and Lynch (1942) and more recently by Lynch (1949). In perennial ryegrass at Marton, Lynch found that growth usually commences in early September, is at a maximum in October and November, falls away during December, January and February, and recovers during March or April to levels largely controlled by rainfall. Perennial ryegrass showed great variability between years in its summer and autumn production. Corkill (1950) has discussed the characteristics of the improved strains of grasses and clovers which have been released for commercial use under certification. He says: "...... it will be realized that the prime objective in the plant improvement programme has been to obtain increase in production and in the seasonal spread of that production."

A study of the effects that cutting of top growth has on root and leaf growth in perennial ryegrass and timothy was made by Roberts and Hunt (1936), who claimed that checks to root growth following top cutting were due to removal of stored reserves from roots to tops, especially at flowering, and that perennial ryegrass has its main storage reserves in the root system. They suggested that this may explain why perennial ryegrass stands more defoliation than timothy, where reserves are largely stored in the bases of stems. Jacques and Edmond (1952) studied the effect of various treatments upon top growth yields of perennial ryegrass and cocksfoot, and found that the yield was greater from (a) fortnightly cutting than weekly cutting, (b) 2-inch height of cutting than 1-inch or ½-inch heights, and (c) non-root-pruning than root-pruning. The effect upon yield from root pruning was greater than from height of cutting, which in turn was greater than the effect from frequency of cutting. These workers also found that, in general, the more lenient top treatments were associated with earlier spring growth and with better growth on into the summer. From a study of the effects of clipping upon plants of cocksfoot, bromegrass, ladino clover and alfalfa, Wagner (1952) concluded that damage to top and root growth, and to tiller, rhizome, stolon and leaf development, from grazing or clipping, could be more severe in older plants than in seedlings. The top:root ratio is a direct expression of the amount of top growth that a given root quantity must supply with water and nutrients, so that plants with a lower ratio would be better able to withstand adverse conditions than those with a higher ratio.

B. Seasonal Root Growth

An attempt to relate the seasonal root development of 5-year-old stands of *Poa pratensis* and *Agrostis vulgaris* to soil conditions in New Jersey, was reported by Sprague (1933). He found that the maximum root weight in both species was about twice as great as the quantity present at the start of the season, and concluded that at least one-half of the root system is newly generated each spring. Following the period of maximum root weights, a decrease occurred in both species at the time of heavy top growth; while in *Poa pratensis*, there was a gradual recovery in root weight as top growth became less abundant. Stoddart (1935) used a root banding technique to study longevity in individual roots of 5 range grass species, subjected to a wide range of soil temperatures and moisture levels. In each case, banded roots lived for at least one year, and many were still alive when observations ceased after two years. Some new roots were produced each season.

Using both perennial ryegrass and timothy, Roberts and Hunt (1936) showed that root weight increased in spring and summer, and that maximum root length was attained some time before maximum root weight was reached. During flowering in perennial ryegrass, shoot formation apparently took place at the expense of the roots, since root weight decreased during this period. Stuckey (1941) used mitosis as the criterion of root growth, and found that root tip cells were dividing actively at temperatures close to 32°F, while cessation of root growth during the summer months coincided with periods of high soil temperatures. Over 2 years, she observed that in unclipped timothy, meadow fescue, *Poa trivialis*, perennial ryegrass, *Agrostis vulgaris*, and redtop, the whole root system was regenerated annually, new growth commencing in autumn and increasing rapidly in spring. Most of the old roots disintegrated shortly after the new ones developed. With *Poa pratensis*, cocksfoot, *Poa compressa* and *Agropyron cristatum*, only a few new roots were formed after the first spring, and only a small percentage of roots disintegrated. Stuckey's conclusions about <u>Poa pratensis</u> and <u>Agrostis vulgaris</u>, based on evidence of cell division, are not in agreement with those of Sprague in the same species, based on root weight.

In a study of the effect of fertilizer placement on the yield of roots and herbage, Jacques (1943) found two periods during his sampling times when root weights decreased in perennial ryegrass. The first during June and July was of small dimensions and the cause was not determined. The second period followed a rapid increase in root weight lasting until the end of November, and continued to the end of February. This loss in weight was due to the death of some of the earliest-formed roots, and to the loss of cortical tissue from the older part of the roots. Weaver and Zink (1946) used a root banding technique on 3,424 roots on 181 plants of 10 range grass species, over a 3-year period. Root condition was determined by visual examination, living roots having a yellowish-white or brownish colour with good tensile strength. In all species, there was a high rate of root survival over at least the first 12 months, while even at the end of 3 years, many of the banded roots were still in good condition.

The effects of defoliation and root-pruning in cocksfoot and perennial ryegrass was studied by Edmond (1949), who found that the maximum production of herbage and new roots did not coincide. Following planting in March, root initiation was at its lowest in early June as herbage production was falling, but root numbers increased from June as herbage continued to decline. Maximum root initiation was reached in October at a time when a decided increase in herbage growth was becoming apparent. Root numbers then fell away sharply until the lowest point was reached in December. There was a slight recovery of root numbers in January, as herbage growth declined. Seasonal fluctuations were greater in perennial ryegrass than in cocksfoot.

Evidence on the physiological behaviour of the biennial medium red clover and the short-lived perennial mammoth red clover, was sought by Smith (1950). Carbohydrate root reserves were diminished during winter dormancy, and both carbohydrate and nitrogen root reserves were reduced by early spring growth as well as by new top growth after each cutting. Restoration of root reserves occurred whenever the photo-synthetic area was sufficient to allow storage. The possession of a taproot by legumes should make the storage and movement of reserve metabolites a more prominent feature of plant growth than it is in the monocotyledonous grasses. Troughton (1951) examined the seasonal root development in permanent pastures containing perennial ryegrass, <u>Poa trivialis</u> and <u>Agrostis spp.</u>, with smaller amounts of timothy, Yorkshire fog and white clover, in three heavy clay fields at Aberystwith. The amount of root material was lowest in November with a consistent increase, until May or June, followed by a gradual decrease, partly due to root death and decay, from July to November. This general trend was modified in one field where there was a decrease from December to February while the pasture was under heavy grazing; while in a second field, a decrease in root weight from May to June was thought to be due to heavy calls on root reserves as the pasture was closed for hay and came into flower.

C. Tetrazolium Staining of Plant Tissues.

There is a steadily growing literature on the use of tetrazolium salts as indicators of reduction-oxidation potentials in biological material. Its major use has so far been as a rapid indicator of seed germination capacity, and nearly all the papers examined dealt either with the application of the compound to seed germination problems, or with the chemistry of the reactions involved. Very little information is available yet from studies made on other plant tissues.

The reduction of 2, 3, 5 - triphenyl tetrazolium chloride at the sites of plant meristematic tissue was examined by Roberts (1951), with the conclusion that the lack of specificity for this reaction makes it probable that no one reductase system is responsible for the reduction of the salt by plant tissues. She says: "It seems more likely that a general redox potential level, maintained by the operation of several physiologically active systems, brings about the reduction of tetrazolium." In an earlier paper, Roberts (1950) pointed out that tetrazolium differed from the majority of redox indicators since it forms an insoluble formazan in its reduced state, and the reaction is therefore irreversible; it is visible in minute quantities so that the reaction is very sensitive; it readily penetrates plant tissues and is not adsorbed; nor does it diffuse from the site of its precipitation. Microscopic sections of fresh material were used for staining, while weak or sluggish reactions were improved by putting the sections into a dessicator attached to a vacuum pump to aid penetration of the tissues by the test solution. The freezing microtome was unsuitable, since the characteristic reduction was either greatly changed or completely absent after freezing.

Actively growing root tips in all the plant species (Allium cepa, Capsicum annuum, Cucurbita maxima, Phaseolus vulgaris, Raphanus sativus, Ricinus communis, and Vicia sativa) examined by Roberts (1950) showed some degree of reducing activity. The strongest reduction was in the zone of cell division, with continuing reduction of progressively less intensity from the apex back, in the outermost and innermost regions of the cortex. Reduction by the pericycle at the sites of secondary root origin, but before there was any histological evidence of secondary root initiation, was also observed in the monocotyledon, Zea mays. Such reduction patterns offer a means of determining physiological differentiation which may exist within tissues, by detecting regions of high metabolic activity.

The influence of tetrazolium salts upon the growth and cytology of onion root tips was studied by Sonnenblick et al (1950). Reduction of 2, 3, 5 - triphenyl tetrazolium chloride occurred in excised root tips, but the effect was not so specific as from the newer tetrazolium salts that were also examined. With the chloride, the entire root is coloured pink to red with the meristem staining deeply, and formazan began to diffuse out of the cells some one to two hours after the start of a test. Ordinary dehydration and clearing procedures with microscopic sections resulted in the loss of most of the precipitated granules.

As a result of a study of tetrazolium staining patterns in pea seedlings, Stafford (1951) suggested that selective adsorption by certain tissues may be involved in the apparent localization of reduced tetrazolium salts. A major source of error in enzymatic cytochemistry is due to diffusion of the coloured indicator compounds after their formation to other sites with a high selective affinity for them. Dyar (1953), using rapidly-acting, light-stable "blue tetrazolium" in a study of the Hill photo-chemical reaction in green plant tissues, claimed that it is improbable that an appreciable amount of such an insoluble compound as reduced "blue tetrazolium" could diffuse from the site of its formation to localized regions within the matter of minutes involved in the reaction. This argument would not apply to the earlier-known tetrazolium salts, such as 2, 3, 5 - triphenyl tetrazolium chloride, because the speed of the reaction is so much slower,