RHIZOME DEVELOPMENT IN PLANTS WITH SPECIAL REFERENCE TO ALFALFA (MEDICAGO SP.)

by

Charles Bernard Watson Rogers

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I. INTRODUCTION

With the recognition in recent years of the great economic value of "spread" or "aggressiveness" in forage types of alfalfa (*Medicago sp.*) the plant investigator should, logically, include in his considerations the underground structures of the plant. Of the gross earthy parts, roots, crown and rhizomes, perhaps the latter deserve the most attention. Rhizomes grow horizontally and may give rise to both aerial and underground parts. Their general habit, then, together with the fact that their development varies widely with genetic constitution and environment must give them a real value in determining the competitive relationships and coverage of any individual plant or strain. While the importance of rhizomatous types of alfalfa has been generally conceded no detailed study of their morphology and physiology has yet been made. The present report is an attempt to meet this deficiency.

Excellent material for study was available in the alfalfa plots at the University of British Columbia. With interest in the breeding program here centering on the selection of spreading forms, plants exhibiting wide variations in rhizome development were readily obtained.

Because of the general similarity of rhizome function in all plant species it seemed desirable to include in this report a rather complete bibliography and literature review of studies of other plants as well as of alfalfa. Wherever they are found rhizomatous structures function as organs of reproduction or serve as resting structures during periods of climatic adversity. A broad view is therefore justified. ?

However, one finds, actually, that rhizomes have been the subject of very little detailed study. This is rather surprising when it is considered that more than fifteen per cent of plants with perennial habit, including many of the most aggressive forage plants, and most noxious weeds, possess rhizomes.

II. REVIEW OF LITERATURE

(a) Some Introductory Considerations:

Many connotations of the term "rhizome" are to be met with in the current literature. The term was first employed by Ehrhart in 1787 but was not accurately defined by him. Asa Gray (1908) used the term rhizome in a general sense to designate it as "any prostrate or subterranean stem usually rooting at the nodes and becoming erect at the apex". The term has been used in a more specific sense. Jackson (1916), for instance, distinguishes between rhizomes, stolons, and runners. The rhizome, he believes, is any root stalk or dorsiventral stem of root-like appearance on or under the ground which sends off rootlets and whose apex sends up stems or leaves. He defines a stolon and runner as any "branch disposed to root". Holms (1929), in a plea for consistency, suggested that the term rhizome be applied to any subterranean stem and stolons and runners respectively to subterranean and aerial axillary shoots. This is the usage followed in this report.

Acceptance of this definition leads to the conclusion that some species in nearly every distinguishable habitat produce rhizomes. In turn, the wide distribution presupposes great variation in form and function. Medic species, for example, possess simple rhizomes useful in food storage; in xeric habitats rhizomes are frequently found functioning as water storage organs or in dispersal of the species; among aquatics there are found a wide variety of rhizomes ranging from the simple to the bizarre useful in anchorage, reproduction or storage. From the rain forests of the tropics to the tundra of polar regions rhizomes may characterize plant species.

(b) Some Morphological Features of Rhizomes in Alfalfa.

In the Common alfalfa of commerce (*Medicago sativa*) prominent development of rhizomes rarely occurs. However in at least two natural groupings in the genus *Medicago*, generally designated as *M. falcata* and *M. gaetula* rhizomes are formed characteristically. In these species the rhizomes are initiated in late summer and arise much as do the shoots in the Common alfalfa from the crown or more rarely from the hypocotylar tissues immediately below the crown. Unlike the shoots, though, which are from the outset positively geotropic and tend to grow upwards, the rhizomes initially are not actively geotropic but tend to grow horizontally below the ground surface for several months. Six to twelve inches of horizontal growth, the amount depending on the environment and genetic make-up, is commonly produced. In spring, growth becomes positively geotropic and from the rhizome apex and nodes, aerial growth commences. To a greater or less degree the rhizomes produce rootlets from the nodes. Apparently, these may be initiated any time.

Oliver (1913) examined alfalfa of many types for rhizome production. Grimm and Turkestan varieties, he noted, often branched laterally before reaching the surface but that the branches seldom produced roots. A high percentage, too, of Grimm, Turkestan, Baltic and Mongolian types, during periods of climatic extremes, were capable of withdrawing their crowns into the soil. In such varieties then many of the shoots, rhizome-like, would be initiated below the soil surface. Alfalfa varieties from northern Africa and from the Caucasus and Crimea were found to be free rhizome producers. In some of these types no tap root whatever was produced.

Oakley and Garver (1913) record an interesting example of root proliferation in a strain of M. falcata introduced from Russia (S. P. I 28071). Unlike rhizomes which are produced from the crown (which is morphologically stem tissue) the proliferations observed by these investigators were produced from the tap root or its branches fully ten to twelve inches below the ground. Like the rhizomes they developed horizontally. At irregular intervals along the lateral roots swellings occurred usually about twice the root in diameter and $\frac{3}{4}$" - $1\frac{3}{4}$" in length. New buds formed on these swellings which gave rise to new plant or remained undeveloped. Fibrous roots are sparsely produced on the proliferations and the swellings are as a rule devoid of them. Individuals which develop from these special roots grow connected to the parent for some time and may attain a considerable size before developing roots from their bases.

In plants other than alfalfa the rhizomes are often quite different in nature. Holms (1929) has attempted to classify them into three types. (1) horizontal homogeneous (2) vertical homogeneous and (3) horizontal heterogeneous. The first type is commonest perhaps being best represented in the rhizomes of Tripsacum dactyloides L. Many other monocots and dicots possess this type, among them alfalfa. The vertical homogeneous rhizome is rare, is found only in Gentiana villosa L., Symplocarpus, spp., Panax and a few others. The third type, composed of slender internodes with alternating tuberous internodes is found in Dentaria laciniata and many orchids.

Further specific examples might serve to illustrate the diversity of type in rhizomes of different plant species. Not infrequently rhizomes are swollen and tuberous in nature. Good illustrations of this are to be found in Helianthus tuberosus (Zimmerman and Hitchcock 1938) (Wettstein 1938) and Sweet Potato (Impomea botatas) (McCormick 1916). Jurica (1924) gives an interesting description of vertical rhizomes in Ernygium yuccifolium which form a series of bulb-like parts beneath the ground. The bulbs grow one above the other each year for four years but in the fifth and sixth years form flower stalks. After anthesis two secondary buds are initiated and the cycle is repeated. Old plants present thus the appearance of a dense cluster. Jurica notes a similar phenomenon in Smilax herbacea and Podaphyllum peltatum where however annual bifurcation of rhizomes was reponsible for the appearance of a dense cluster in plants of same age. Decay may develop at the posterior end of these clusters, resulting in eventual break-up.

An unusual rhizome formation is encountered in Helianthus scaberrimus native of the prairies of the Western United States. The "fairy rings" produced by this species are found by Cooper and Abraham (1931) to be due to the fact that regenerative buds are found only in the apices of the rhizomes and that the old growth tends to die each year. Thus death of the tissue proceeds outwards from the fecus of the colony and new growth is usually seen as a ring.

In the grasses likewise rhizomes develop in various ways. In Kentucky Bluegrass (Poa pratensis L.) Evans and Ely (1935) found that the rhizomes originated from buds at the base of above-ground shoots. Development was at first downwards with later penetration horizontal. In Canada Bluegrass (Poa compressa), however, the same writers found rhizomes being initiated at the underground nodes of old rhizomes. Quackgrass (Agropyron repens L.) Red top (Agrostis alba L.) and Reed Canary Grass (Phalaris arundinacea L.) it was found spread in this same way. Evans and Ely (ibid) found, as in alfalfa, rhizome production in these grasses of Northern Ohio to be most active during June, July and August. Tuckey (1940) indirectly confirms this observation.

For further information on morphological diversity in rhizomes the works of Kalmykova (1935) Kannenberg (1931) Vystotskii (1928) Weaver (1930) Heath and Luckwill (----) Vladmirov (1937) Bower (1935) and Almeida (1928) should be consulted.

Possibly because the plant breeder has been the chief investigator interested, the gross morphology and not the histology of the alfalfa rhizome has been given the most attention. However, a few studies of the microanatomy have been made. Miss Holliday (1932) clearly indicated the stem-like nature of the rhizomes she studied. Hayward (1938) also notes that in common with the aerial stem "the rhizome possesses well-defined casparian strips in the endodermal layer followed later by a pronounced lignification of the inner and tangential walls". The cells of the rhizome pericycle, unlike those of the stem, function as a phellogen producing a periderm. He observes further that the rhizome will live for three or four years while the stem dies at the end of each season.

While they did not specifically investigate the rhizome Wilson (1913), Poczosky (1917) and Winter (1932) have contributed much to the general histology of the alfalfa plant. Wilson's observations were not of a detailed character. Poczosky, on the other hand, endeavoured to trace the development of the root system in relation to vegetative reproduction and to winter hardiness. Winter studied mainly the root-stem transition system. Mention might also be made of the contributions which were in part histological of Simonds (1935) and Jones (1928) on the structure of the crown and root of lucerne.

In other plants rhizome structure has received more detailed consideration than in alfalfa. As in alfalfa, however, most investigators find these organs to have in the main a stem-like histology. Careful work bearing out this contention is found in the paper of Thoday and Woodhead (1932). They note the stem-like nature of the stele but note modifications such as the extensive lignification following cambial activity in older tissues. Wille (1926) has given us a detailed study of the microanatomy of the principal groups of the Cyperaceae. Fuchs and Zeigenspeck (1926) have described the anatomy of the stems and rhizomes of many orchids. Niki (1927) and Carlson (1938) emphasize the histological differences of stem, root and rhizome in other studies.

The origin of rhizomes in the stem tissue and the origin of the adventitious roots arising from the stem-like rhizome have been the subject of much controversy. To judge from the literature studied one may safely conclude that the pericycle and cortex are the chief initiating tissues. Which happens to be functional in a particular case depends largely on the species. For details, the papers of Chang (1927) and Hayata (1929) should be consulted. Chouard (1934) also gives an interesting description of the origin of rhizomes from buds produced in the axils of parent bulbs in Scilla adlonis.

Contractility of rhizomes, a feature of importance in many plants, has been studied from the histological viewpoint. Ziegenspeck (1927) finds that in Hedysarum comosum the sudden activity of the cambium crushes adjacent tissues inducing rhizome contraction. According to De Vries the shortening of contractile roots, and presumably of rhizomes, is due to growth in parenchymatous cells with anisotropic walls which are more extensible transversely than longitudinally. Thoday (1931) in Oxalis wearnata and Brodiara lactea finds rather that contraction is purely physiological and may be explained by the withdrawal of sap from the cells involved.

Other papers of histological interest are those by Browne (1933), Leandri (1928), Lauder-Thomson (1933), Sumpstine (1931), Cross (1931), Chakraverti (1939), Smith (1931), Carlsen (1927), Howe (1931), Johannson (1929), Wilson (1927) and Taylor (1926). Of especial interest is a paper by Goette (1931) comparing the anatomy of rhizomes of Corwallaria majalis from different habitats.

(c) The Functions of the Rhizomes in Alfalfa.

Interest in alfalfa improvement has led slowly to the realization of the many and varied functions which the rhizomes may serve. The feature which first awakened interest was its relation to spread and coverage. Important adaptations of alfalfa in its native semi-arid Eurasia are its well developed tap root and the longevity of the individual plant. With the introduction of alfalfa to cultivation, however, has come the desire for dense stands in new and often very different habitats. In the new areas alfalfa is no longer so long lived. Disease and adverse conditions of climate and soil thin the stand. Little replacements or compensations occur and grasses and weeds compete vigorously for the areas lost. Accordingly, plant breeders seeking to adapt the plant to new conditions sought plants which would ensure continued dense stands. Rhizomatous alfalfa seemed to be the goal of the quest.

Southern alfalfas, Peruvian, Provence, etc., invariably have small crowns. Individual plants of Grimm, Cossack utilize areas somewhat more efficiently. Ladak and Turkestan are almost semi-rhizomatous and spread appreciably. Mention has been made of types of falcata and gaetula which are truly spreading. Oliver (1913), for example, has found plants of the falcata type from North Africa which produce rhizomes three and one-half inches in length. From the shallow soil regions of the Caucasus and Turkestan have come other plants with phenomenal spread. Illustrations of how these spreading types are proving of value in present day breeding programs will be given later. (Moe (1928), Myers and Kirk)

The capacity to send out rhizomes is recognized as a valuable attribute in many other plants of economic value. Swards of Kentucky Bluegrass (Poa pratensis), (Kannenberg 1931a, 1931b), Bromus inermis, Trifolium subterraneum , various Calamagrostis spp., Agrostis alba, etc., are directly related to rhizome production.

Rhizomes may function in the production of new plants from a parent plant. In alfalfa this is not commonly seen, but the observations of Oakley and Garver (1913) on its occurrence should not be neglected. Trifolium subterraneum, a native of New Zealand, sets little seed but is largely dependent on rhizomes for dissemination. This is true also of Calamagrostis rubescens, and is partly true of Smilax herbacea, Eryngium (Jurica 1924), Scilla adlami (Chouard, 1934) and Helianthus tuberosus (Zimmerman and Hitchcock, 1938). Age, apparently, has a very important bearing on the regenerative powers of rhizomes. Froese (1938), among others, found rhizomes which were several years old to be most active in *Lathyrus pratensis*. In *Agropyron repens*, Ennvere (1937), found regeneration to be directly related to dry matter content. Scavone (1939) states that ramie rhizomes propagate best at three years of age.

An additional reason for special interest in the rhizome of alfalfa is its association with resistance to extremes of heat and cold. The early plant explorers of the U. S. D. A., Hansen, Meyers and others, noted that rhizomatous alfalfas were found in the extreme heat of the north African rocky soils and in the frigid areas of Siberia, where the subsoil scarcely thawed in summer. Hansen (1909) records *falcata* types growing at Verhoyansk, Siberia, latitude 68° N., where minimum temperature experienced is -90°F. Leschenko (1930) found that in Krassnokutsk in Russia, after the severe winter of 1927, all American, European, and Ukrainian alfalfas killed out with the exception of one native yellow-flowered rhizomatous species. Schubert (1931) was able to separate hardy and non-hardy strains on the basis of their tendency to form rhizomes. Graber (1927) has stated that the hardiness of Grim, Ontario Variegated and Hardigan is related to their production of rhizome initials with dense cytoplasm. Klapp (1932) at Zwätzen, Jena, Germany, says that "forms distinguished by winter hardiness are also distinguished by a branching of the roots and in hybrid lucernes the forming of rhizomes is not a rare occurrence." Garver (1922) in a thorough study of hardy and susceptible alfalfas finds that "there are outstanding differences between the root systems of southern grown common and yellow-flowered (*falcata*) alfalfas in the prominence of the tap root, the development of the branch roots and the number of rhizomes, and in the number and place of the most profuse production of fibrous roots. Extreme forms could be told apart by their underground structures.

The histological relationships of winter-hardiness in alfalfas have been ascertained by Jones (1928). Young protoplasmic structures such as rhizome and shoot initials seldom show any winter injury, whereas the pith parenchyma, xylem and phloem parenchyma show marked susceptibility. A histological explanation then for the hardiness of young and rhizomatous alfalfas may be concluded. Southworth (1921) also has contributed to these studies.

In Common alfalfas the tap root is the primary storage structure. Of necessity in rhizomatous alfalfas the rhizomes must undertake this function. Food reserves are stored mainly as carbohydrates. Morozov (1939) finds that inulin-like compounds and hemicelluloses predominate. Of the simple sugars, sucrose is the most abundant. Smelov and Morozov (1939b) carried their investigations further by determining the carbohydrate reserve separately for roots, rhizomes and stems. The study showed that roots contained mostly hemicellulose, the rhizomes mainly soluble carbohydrate and the stems both hemicellulose and soluble carbohydrate. In another investigation (1939a) they found during tillering and anthesis to seed setting, rises in reserve carbohydrates. This parallels the experience with other plants, Bromus inermis, Lolium perenne, Alopecuris pratensis, Festuca pratensis and Dactylis glomerata. Malahov (1937) working with Agropyron repens and Weinmann (1940) with Chloris gayana find, similarly, depletion of reserves after and during flowering. They noted also a marked rootward gradient in sugars and hydrolyzable materials as autumn approached.

Intimately related to the vigor of alfalfa, and its storage of reserve food is cutting practice. The works on this, while numerous, (and only a few of the more significant ones can be mentioned here) seldom take the rhizome specifically into account. Miss Holliday (1932) makes the observation that with heavy clipping or cutting rhizomes are not produced in alfalfa predisposed to vigorous rhizome production. It would seem then that severe depletion of food reserves inevitably reduces rhizome production, and vigor in alfalfa. In Kentucky Bluegrass, however, Ahlgren (1938) found that the carbohydrate content of rhizomes was not appreciably affected by cutting or grazing practice and that in <u>Bromus inermis</u> attempts to increase food reserve by applying fertilizer actually reduced the rhizome reserve. Graber and his associates (1927) found also that in alfalfa the frequent cutting of top growth, while ultimately reducing the vigor of the plant, will increase the number of buds, shoots and rhizomes per plant.

Accumulation of reserves and hence cutting practice in alfalfa has an important bearing on the survival of the plant in extremes of heat, drought, and cold. Sylven (1934) found that the later the last cut could be taken, the less would be the winter injury and the greater the yield in the following spring. Late cutting permitted over-wintering buds to remain in a hardened condition, while early cutting forced them into activity and a condition of cold susceptibility. Silkett (1937) expressed this physiologically by stating that shoots of plants low in dry matter and high in percent moisture were most susceptible to winter injury. Plants cut in September drew on their root and rhizome reserves to produce full growth, thereby lowering their resistance. Rather and Dorrance (1938) found that, in similar manner, fall pastured plants suffered more winter injury and developed fewer and less vigorous crown buds. Mark (1936) found a positive correlation between winter injury and root reserves. A single cutting at the end of August lowered the underground reserves of Grimm alfalfa 100% by October 8th. Reference in this connection should also be made to the pertinent work of Barr (1936, 1939 and 1940) on root reserves in Convolvulus arvensis, the Bindweed, and to the work of Wettstein (1938) on the tuberous plant Helianthus tuberosus.

Rhizomes may serve an additional purpose in storing water during periods of drought. Falcata alfalfas apparently have special facilities in this regard. Contrast this with tap roots which store little water but seek the water table. The rhizomes, instead of storing water, too, may be dormant during periods of drought as the work of Almeida (1926) with Nymphaea pubescens Willd., and Bailey (1940) with Agropyron smithii, has shown.

The role of rhizomes in relation to plant competition is not clear. In many plants rhizomes have a definite value in competition as is well exemplified in many of our perennial weeds. Both Dehay (1939) and Woodcock (1932) found Agropyron repens to have very great powers of spreading itself. Dehay found its rhizomes to have penetrated a living root of Althoea officinalis whilst Woodcock showed it to have entered a one year old seedling of Juglans cardiformis. Zade, on the other hand, found the rhizomes of Poa pratensis unable to spread in the presence of Dactylis glomerata.

Several other additional functions associated with rhizomes may be mentioned. In certain cases rhizomes will serve to protect plants from alkali, as Hansen (1909) found some rhizomatous strains of M. falcata growing under alkaline conditions in Siberia. Rhizomes on the other hand will not serve to protect alfalfa against excess acid, as a level of acidity is soon reached where initiation and growth of rhizomes ceases. Haas (1927), Holliday (1932), Watenpaugh (1936) and Oliver (1913) credit rhizomes with leaving a large amount of humus in the soil. Rhizomatous alfalfa, although admitted by Koenekamp and Lehmann (1933) to serve as a valuable pasture plant, may also, they claim, suffer considerably from trampling, as a large part of the tillering zone lies near the surface of the ground.

(d) The Story of Alfalfa

Alfalfa, *Medicago sativa* L., is a native of the temperate regions of Western Asia, notably Media, the countries southeast of the Caucasian Range and the northwestern part of modern Persia (Klinkowski, 1933). It is the oldest forage plant known, being mentioned in the Old Testament. The progress of this "queen of the forage crops" was from East to West, following the path of historic civilizations. It reached Greece in King Darius' time in 470 B. C. and took 300 years to reach Italy. About 150-200 B. C. it obtained a foothold in North Africa where it is still found. Whether it came directly from Asia to North Africa or whether it was brought by the Romans is not clear. In Asia it followed the old caravan routes to China; in Europe it was limited to the two Mediterranean countries; in North Africa it was confined to the oases. The 13th century found its area of cultivation increased and at this time it was found in Southern France. At about the same time it was brought into Spain under the name of "alfalfa" or "best of fodder" by the Moors with their invasion of that country. At the start of the 16th century alfalfa was taken by the Spaniards to Mexico, thence to Peru, Chile, Argentina and Uruguay. Thus in the Western Hemisphere alfalfa was confined at first to Central and South America. In Europe it spread from Spain to France about 1550, reaching Belgium in 1565, and Holland under the name of "Bourgoens hay" at the same time. This "Burgundy hay" reached Hungary around 1587, disappearing shortly. However it was reintroduced at the end of the 18th century. The black soil region of Russia was reached by way of Spain and France, and not from the West Asiatic steppe district. In Germany it was first cultivated in the province of Thuringia in 1710, meeting much opposition at first, but later its real worth was recognized and the rate of expansion of "Old Fraconian hybrid lucerne" was quite extraordinary.

While lucerne cultivation had reached great importance in South America as early as the 18th century, it was unknown in North America until it arrived in the middle of the 19th century. Alfalfa entered North America by two different routes, one from South America, and one from Germany. Gold seekers brought the "Chilean Clover" from South America to California in 1851-54, also a few years later Missionaries brought it from Mexico to Colorado, but the intense cold of northern winters prevented this strain penetrating farther north. The most important of the strains introduced was the German strain brought into Minnesota by Wendelin Grimm in 1857. The seed he brought with him was the old Franconian hybrid lucerne which can be traced back in Germany to 1710, it being introduced into Bavaria at that time by the Cistercian monks of the Ebrach Monastery in Franconia (Klinkowski, 1933). Much of Grimm's first plantings died, owing to winterkilling, but by saving some seed each fall and planting it the next spring, through natural selection his strain became hardy enough to withstand Minnesota winters. From it has sprung the "Grimm" alfalfa which has contributed so immensely to alfalfa production in North America.

Another strain of alfalfa that has proved of especial value to alfalfa growers of Canada and the Middle West is "Ontario variegated". This strain was introduced into the Niagara district in Canada some thirteen to fifteen years after the introduction of the Grimm into Minnesota (Southworth, 1921). The Ontario variegated probably arose from a mixture of two strains with variegated flowers. One strain being introduced in 1871 by a farmer named Bethel, living near St. Catherines, who obtained it from a German shepherd who brought it from his native country. The other strain also came from Germany in 1875 through the agency of Dr. Cellyer, a medical man then living at a port called Welland, near Niagara. Reference will be made later to both Grimm and Ontario variegated in the discussion on the development of rhizomatous strains of alfalfa in Canada.

THE HISTORY OF RHIZOMATOUS ALFALFA

Coming more specifically now to the study of rhizome producing types of alfalfa their history will be traced. The Common alfalfa already studied does not usually spread. However some species do spread, among which are *Medicago falcata* L., *Medicago gaetula* Urb., and some species of *Medicago sativa* L. Of these *M. falcata* is the most prominent and is important economically.

Responsible for the first introduction of these spreading strains to America was Professor N. E. Hansen of South Dakota. The United States Government seeing the value of alfalfa and the need for hardier strains sent him to Siberia to collect these. Hansen made two trips, the first in 1897-1898, bringing back Turkestan Alfalfa, and the second in 1906-1908, returning with *Medicago falcata*. *M. platycarpa* and *M. ruthenica*, he also noted, were of likely agricultural value, but the onset of winter made it impossible to make any collection.

Later a journey was made to Siberia and North Africa to gather more seed of new forage plants, especially alfalfas and clovers in Siberia, and also to determine how people survive in such vast deserts as the Gobi of Mongolia, the Salt Steppes of southwestern Siberia, the Hunger Steppe of Turkestan and the Sahara of North Africa. Of the forage plants found in this area were Medicago falcata and Trifolium lupinaster. By way of emphasizing the extreme hardiness of M. falcata we may cite examples of where it was found growing between Verkyozansk, latitude 68°N. (minimum temperature recorded, - 90.04° F), and Yakutsk, latitude 62° N. (minimum temperature recorded, - 83.92° F.) due north of Lake Baikal, where the subsoil remains frozen all year round. Hansen states that the peasants of Siberia have long ago found the value of these alfalfas, M. falcata, especially being an important factor in making the rich cream and butter for which Siberia is noted.

Medicago falcata (falcata referring to the sickle or falcate shaped pods), is distributed over a wide range of Europe and Asia, but in the milder regions has been largely replaced by Common lucerne. It extends through Western Europe, central and southern Russia, the Crimea, Caucasus, the western two-thirds of Siberia, North China, the Trans-Caspian region, including Turkestan and Persia, through Asia Minor, Afghanistan, and Western India.

M. falcata has proved of outstanding value at the dryland Agricultural Experimental Station at Bensentsug, 30 miles east of Samara in the Volga River region of European Russia. This area is in the heart of a vast steppe region suffering from summer droughts and winter cold. Here recurring crop failures have caused distress among the peasants. M. sativa or French lucerne, winterkills and does not endure pasturing, whilst the native M. falcata is perfectly hardy and endures pasturing for at least ten years, being long-lived with a strong root system. The protein content of its first cutting is two per cent above M. sativa but later cuttings are less. As many as one hundred branches were found by Klingen (Hansen, 1909) from the one plant of M. falcata which when pastured is of strong upright growth, standing fully waist high in the Steppes, but creeping and having only the ends erect when closely pastured.

Recognizing these qualities of M. falcata, Hansen (1909) made many collections of seed of M. falcata. He brought back with him to the United States in 1909, seed from Kharkof Province, southeastern Russia; Omsk, Siberia; Irkutsk, Eastern Siberia; Samara Province in the Volga River region; Don Province of the lower Volga River region, southeastern Russia; and Tomsk, Siberia. These were found to produce fine upright plants, 3-3½ feet high where range was not overcrowded. In the native habitat on the banks of Irtisk River near Semipolatinsk, Hansen, in 1908, found it growing 5' 8" high. He found M. falcata to be relished by stock, to withstand considerable pasturing, to green early in the spring, to endure severe summer droughts, to thrive on dry upland soils underlaid with hardpan and to resist alkali. Working with nature, by making use of her work of countless thousands of years in producing a hardy alfalfa, Hansen felt, is a sounder policy than that advocated by some workers of selecting hardy types from non-hardy strains. It was in accordance with this belief that M. falcata as one of many economic plants was introduced into the United States and Canada. In addition to Professor N. E. Hansen, Mr. Frank N. Meyer, the United States Department of Agriculture's agricultural explorer, has been responsible for introducing seed of the greater number of various forms of the species especially from localities in Asia. Approximately 50 lots were introduced mostly from Russia where Siberia conditions were very similar to those in the north western American plains.

Oliver (1913) utilized all these imported strains of M. falcata in his work to produce a new alfalfa variety for pasture which would be winter hardy and drought resistant. Early in his work Oliver found that these characteristics were associated with the underground development of rhizomes and modified rhizomes. As a result he undertook a very extensive breeding program with the idea in mind of obtaining rhizomatous pasture forms which he hoped would grow in areas in the United States not suited to the standard varieties at the time. He concentrated his efforts, then, upon obtaining plants having good top growth and abundant underground bud potentiality for production of rhizomes, these being formed preferably, well below ground level, with the crown of the plant deeply set in the soil.

While Oliver was the first man to do work consciously on rhizomatous alfalfa production, we have mention of other men who observed rhizomatous growth. Even as early as 1733 Jethro Tull noted "perpendicular roots springing from the very branches of the lucerne crown". M. Lullin de Chateau-vieux (Duhamel du Monceau, 1759) in 1759 may also have observed them as he speaks of the crown extending itself every year. These men, however, seemed to have overlooked its importance, and credit for calling attention to this character and pointing out its value should be given to Thomas Le Blanc (1791), who, in 1783, mentioned its habit of branching below the surface of the ground, it, consequently, not being choked by natural grasses nor injured by sheep grazing and as well, being hardier than other alfalfas in bearing cold. It may be said that he so understood this strain's value that he tried to introduce it into general use. Despite the definiteness with which he wrote little subsequent attention was paid to his work and it was soon lost to sight. Prior to Oliver's work, Blinn (1911) had written an article, "Alfalfa: The relation of type to hardiness". Then followed Oliver's (1913) bulletin, referred to previously, on "Some new alfalfa varieties for pasture", followed by Oakley and

Garver's (1913) work "Two types of proliferation in alfalfa" the same year. The two types of proliferation referred to by these workers are rhizomatous growth or stem proliferation, and root proliferation not previously mentioned in the literature. This article serves to call attention to peculiarities that have been found in alfalfa.

Following this early work, Oakley and Garver (1917) undertook a very thorough investigation of M. falcata, and established an extensive living collection. It is of interest in connection with the breeding work that Fryer (1930) found two types of M. falcata, one with 16 chromosomes, and the other with the normal 32 chromosomes. Other than the report by Southworth (1921) of the history of alfalfa in the United States and Ontario where he mentions three root systems, one of which is a branched tap-root with underground stems, and Garver's (1922) histological studies mentioned later, no further references on rhizomatous alfalfa were found until 1930 when certain European workers became interested in it.

Leschenko (1930) describing lucerne naturally reproduced by means of rhizomes believed he was the first to mention rhizomatous types in the literature. The type that he described was found at Poltava after the severe 1928-29 winter. At that time all nursery stocks of American, West European, Ukrainian, Russian and Turkestan types were killed out. The surviving plant whose rhizomes recovered was a yellow-flowered lucerne native of Krassnokutsk. Close examination showed the young shoots which covered it to be underground shoots. From the crown thick living roots were found, these giving rise to lateral roots from the nodes and extending horizontally 45 cm. before bending and growing downwards. In two years this root system had developed to cover one square meter.

Klapp and Schubert (1931) presented a very far-reaching analysis of the Thuringian hybrid lucerne in which they showed its superiority over southern strains. Schubert (1931) was aware of the importance of the roots in promoting winter hardiness in northern strains. In 1931 he presented a paper, "Observations on the roots of lucerne of different origins" in which he divided lucerne into 3 groups: (1) those in which the hybrid proportion was high, and have branch roots (evidently Schubert is here referring to rhizomes as branch roots) at right angles to the main root in which were included lucernes of northern origin, old Franconian and varieties from the Eifel, the Palatinate and Northern France; (2) those in which the hybrid proportion was low with branch roots mainly turned down and which possess (a) a single unbranched tap root (b) a single forked root, all of which were obtained from Crescat, Hungary, Turkestan and Cossack; (3) those in which the hybrid proportion was slight or lacking and were mostly of southern origin such as the Italian, Provence, Spanish, Chilean, South African and, Argentine lucernes. No mention is made in the third case of the kind of roots found.

Klinkowski (1932) draws attention to various "discoveries" of lucerne forms which produce rhizomes and in particular to a plant recently found by a roadside near Berlin in which this growth was particularly marked. Immediately upon publication of this "discovery" of Herr Klinkowski, Professor Klapp (1932), under the same heading, states that the formation of rhizomes by hybrid lucerne is no rare thing. Professor Klapp points out for example that production of rhizomes is mentioned in the American literature as characteristic of _Medicago falcata_ and that _M. sativa_ forms have been known to exhibit short rhizomes.

The development of rhizomes under various environmental conditions has been discussed by many European workers. Wick (1932) gives a comparison of the following varieties grown for five years at nine different places in Germany. Old Franconian, Franconian, Thuringian, Cossack, Grimm, Hungarian, Kaiserie, Provence, Italian and South African. He found Franconian and Thuringian strains with Cossack and Kaiserie taking first place, Grimm taking intermediate place, whilst the South African, Provence and to a large extent the Italian varieties cannot compete with the others under German conditions. Professor Klapp (1932) in a comparison between southern strains of lucerne and some German strains developed at Swätzen, Jena, after the hard winter of 1928-29, made the following statement: "Forms distinguished by winter hardiness are also distinguished by a branching of the roots, and in hybrid lucernes the forming of rhizomes is not a rare occurrence." Koenekamp and Lehmann (1933) showed an interest in the "lucerne which sends out rhizomes", remarking on their value as a highly desirable character in a pasture plant but stating that a large part of the tillering zone lies near the surface of the ground, where there is danger of injury due to trampling. Also they claim the preparation of M. falcata in the hybrids is very heavy and that this species is poor as regards second growth. Ufer (1933) studied interspecific hybrids of Medicago gaetula Urb., from Batna, Algeria, and Medicago falcata L., from Müncheberg, Germany, with a view to obtaining strains resistant to cold and drought. The resulting F_1 showed an intermediate growth between the two parents and although a full description of the resultant plant is given no reference to its hardiness is made. They did prove the contention of Trobut, the French scientist, that M. sativa arose from a cross of M. falcata x M. gaetula, was erroneous. That the energies of these various workers were beginning to bear fruit may be gathered with the announcement by Klinkowski (1934) that a new rhizomatous lucerne for Germany, named Eifel, had been produced. This Eifel lucerne is very leafy and much branched. The root system is highly branched with many parts attached at right angles to the main axis. Furthermore there is an abundant formation of fibrous roots. Koenekamp (1934) published results on a comparison of this Eifel rhizomatous hybrid lucerne with ten Argentine varieties. He found the difference in root development, though slight, was sufficient to prove the superiority of the Germany hybrid in this respect.

Following the progress of the work with rhizomatous alfalfa in Europe, attention is directed again to its development in North America. Little information is available of the more recent work. However, a spread of the alfalfa grown has been receiving the attention of all breeders, and Dr's. L. F. Graber and R. A. Brink of Wisconsin, and Dr's. C. H. Myers and D. B. Johnstone-Wallace of Cornell have developed a number of strains of pasture types. Dr. L. E. Kirk, too, now Dean of the Faculty of Agriculture, Saskatoon, states in a letter received from him that while he was at Ottawa, work was being conducted there with rhizomatous strains of *Medicago glutinosa*. Also Mr. W. J. White, Officer in Charge of the Dominion Forage Crops Laboratory, Saskatoon, says that selections of the more widely creeping types of *Medicago glutinosa* have been made at that institution. Work is continuing there both with this species and with *M. falcata* from which a very strongly creeping strain has evolved.

For many years the Agronomy Department of the University of British Columbia has been interested in rhizomatous alfalfa, the "U.B.C. alfalfa" developed here representing the fourth generation of a cross between variegated alfalfas, *Medicago media*, and yellow-flowered

Medicago falcata. Dr. L. S. Klinck, President of the University of British Columbia, brought from MacDonald College, Quebec, several cuttings from one plant of *M. falcata*. Dr. Klinck had obtained the strain from Professor N. E. Hansen of South Dakota, who imported it from Russia, probably, although this is uncertain, under the introduction number S. P. 1. 20725. The cuttings were planted and sixty plants propagated from them.

Professor Boving, formerly head of the Department of Agronomy of the University of British Columbia, since retired, grew several short rows of variegated alfalfa, both Grimm and Ontario variegated, next to the *falcata* type designated, to obtain a cross between these in 1917. Using the Don as a maternal parent seed was obtained and planted. Two hundred and fifty plants were grown and only 6 showed hybrid characters. Dr. G. G. Moe, the present head of the Department of Agronomy (1928) of the University of British Columbia, endeavoured to self these F_1 plants in 1919 by enclosing them in cheesecloth tents and manipulating the flowers. As so few seeds were produced they were left untented in 1920, all other alfalfas being cut back except the six hybrids, and a heavy yield of seed obtained after again manipulating the flowers. In 1921 this seed was set out in rows and the plants permitted to grow several years. In 1924 Dr. Moe planted the seed of these F_2 plants, and in 1925 open fertilized seed was taken from all F_3 plants that produced seed, this being set out in a block to establish the F_4 stand from which the present writer obtained his material.

Most of the plants in this stand are today showing great spread, the one chosen to secure cuttings covering 4 sq. feet. Dr. Moe mentioned the Don parent in this cross as "being characterized by short, semi-erect stems, a thick matted growth and yellow flowers, rhizomes being formed several inches below the ground and spreading varying distances from the plant." Evidently the plant chosen inherited much of its spread from its _falcata_ parent, along with more vigorous growth from its Grimm and variegated ancestry.

III. EXPERIMENTAL WORK

(a) Introduction

When the subject of rhizome growth was first considered as a topic for study, those factors which would likely prove of practical value in the production of rhizomes in alfalfa were given first consideration. The earlier work was laid out on this basis. However, as much of this first work, through unfortunate circumstances, had later to be laid aside, it was found necessary to modify the practical point of view, and lay more emphasis on obtaining definite rhizome growth response, whether this should prove to be of practical value or not.

In the experimental work conducted ~~here~~ rhizomatous strains of University of British Columbia alfalfa were studied. The rhizomatous habit of growth was thought to be most valuable for a pasture strain, although its worth as a hay crop could not be underestimated either. Work has been carried on at the University of British Columbia in both these regards. The most practical way to determine its pasturing value was to actually clip the plots to simulate pasturing, and also cut them twice or three times a year, weighing each cut to get a record of hay yields. Comparisons with a normal strain would have to be made. Fortunately, a series of alfalfa plots had been set out the same summer in the Agronomy Field to determine the yields from Grimm and University of British Columbia strains sown at different rates of seeding. These were kindly turned over by Dr. Moe to the writer to be used in his rhizome studies and were ideal for the purpose in mind.

If fertilizers were important in accentuating rhizome growth this would be of great value. Accordingly, plots were laid out to discover just what effect fertilizers had on either initiating or accentuating rhizome growth. More details of this work will be given later under its appropriate heading.

In conjunction with this field work, studies were begun in the greenhouse where more controlled methods could be applied to the plants and work done which was impossible to accomplish outdoors. As alfalfa is primarily a plant thriving in non-acid soils containing calcium, the effects of acid soils on rhizome growth would be of value and so a series of soils of varying acidity were planned to be used to study this effect. Other factors which would have a bearing on production of rhizomes such as length of day and temperature were also studied and these results will be given later.

(b) Field Studies

1. The Clipping Experiment.

Purpose:

The clipping experiment was undertaken to study the effects of the removal of the top growth of the alfalfa plants on their rhizome development and on the subsequent yield of hay from the plots.

Discussion of the Literature:

(Considerable attention has been paid in the literature to this subject of clipping and its effects on plants. This attention is justified as cutting and its effects on root reserve is important. The time of cutting has already been shown in previous discussions to be a matter of great ? moment to the alfalfa plant, so much so as to condition its chance of subsequent survival. Thornton and Nicol (1934) studied a more general effect of cutting treatments on alfalfa reserves, both below and above ground. The cutting did not alter the number of nodules, their mean size, nor did it alter total nitrogen content of the plants (tops and roots), but it did decrease the nitrogen content of the roots forty per cent, this nitrogen going to the tops and being removed. When clipping weights were taken, the total yield of the tops increased, but that of the roots decreased. Grandfield (1935) studied alfalfa more in relation to the effects of time of cutting on the storage of food, finding eight to ten inches of fall growth must occur after the last cutting to permit maximum storage of root reserves. This maxium storage he found to take place at full bloom. Early and frequent cutting in the bud stage resulted in lower carbohydrate and nitrogen content of the roots. Harrison (1939) clipped alfalfa to one inch, two inches and six inches every week for eight weeks and found the one-inch clipped plants had only fifty per cent of the dry weight in their roots that they had at the start of the experiment, and only fourteen per cent as much as the uncut ones. The two-inch clipped plants had forty per cent as much, and the six-inch approximately equalled the uncut plants. The stored starch in the roots of the one-inch clipped plants was entirely depleted; in the two-inch ones it was partially depleted, and in the six-inch uncut ones an abundant supply occurred.

Hildebrand and Harrison (1939) showed how a borderline effect in an alfalfa clipping experiment may occur. Below this line of cutting, poor growth of both tops and roots results, and above it good growth. This line was found to be between the nine-inch and six-inch levels in an experiment where twelve-inch, nine-inch, six-inch, three-inch and one-inch cuttings were made, was the same for weekly, bi-weekly and monthly intervals of cutting for a period of twelve weeks.

Tysdal and Kiesselbach (1939) found a differential response of varieties to time of cutting. The Ladak variety could be made to outyield

Grimm by varying the time when it was cut. Nebraska Common, Hardistan and Grimm, as well as Ladak, all showed this effect of different time of cutting.

The investigations undertaken by Holliday (1932), Silkett (1937) and Rather and Dorrance (1938) on the effects of cutting alfalfa have already been discussed in the general review of literature. They showed that time and frequency of cutting is very important for the laying down of adequate food stores in the underground parts of the plant.

Proceeding from the study of alfalfa to that of grasses, their manner of growth is found to be affected in very nearly an identical manner to that of alfalfa. Harrison (1931) studying the effects of cutting and fertilizers on grass development, found the shorter the grass was cut the smaller was the quantity of roots produced. He showed Fescue tillered more when cut short than when cut long, whilst Kentucky Bluegrass tillered less under similar treatment. Mineral fertilizers did not compensate for lack of top growth in production of roots. The lack of removal of the buds
root growth was due to a gradual carbohydrate starvation and not to the buds being cut off. Nitrogen increased the top growth but the weight of the roots was unaffected by its application.

Graber (1933) also studied the effect of cutting grass at two levels. He used Poa pratensis, cutting it "close" and "tall" and determined the yields from each for two years. The first year the oven dried grass from the short cuttings gave the higher yield, making most of this gain in the first cutting May 10th. The following year, with three cuttings being taken, the "close" cut field was found to give the lower yield with weeds five to seven times more abundant than on the "tall" cut field. Graber found that when high potentially productive bluegrass was stimulated by N_2 and moisture supply, the delays in recovery after cutting and removing a large quantity of top growth, may greatly limit subsequent yields. Kuhn and Kemp (1939) found similar effects when cutting a tall and a short-growing strain of the same grass. An increase in the severity of defoliation was found to cause a highly significant decrease in the production of roots, rhizomes and tops. The short-growing strain withstood the clipping better than the tall-growing strain when both of these grasses were cut at a one-inch or one and one-half-inch level. Graber and Ream (1931) used Kentucky Bluegrass, but fertilized one series of plots with nitrogen, the other series remaining unfertilized. Each of these series was divided into two portions, one receiving one defoliation and the other eight defoliations. After one hundred and twenty-three days of growth, an accumulation of organic foods resulting in increased root and rhizome growth occurred in the fertilized and unfertilized plots of the one defoliated series, as compared to those defoliated eight times.

Cutting treatments have been conducted with a variety of other grasses. Wilsie (1940) and co-workers used Napier Grass (*Pennisetum purpureum*) under Hawaiian conditions and obtained similar results to those conducted with bluegrass. An increase in the total yields was obtained as the intervals between the cuttings were raised from six, eight, ten, twelve to fourteen weeks. As palatability forage, protein and ash content decrease with increase in the age of the plants and as a six-week interval caused poor recovery, depleted stand and greater weed competition, an eight-week interval between cuttings was recommended for Hawaiian conditions. Sturkie (1930), working with Johnson Grass (*Sorghum halepense*) found any cutting treatment reduces rootstalk development, and the more frequently the cutting is made the greater is this reduction. Hanson and

Stoddart (1940) investigated the effects of grazing on bunch wheat grass (Agropyron inerme). They found heavy grazing decreased root weight in the soil, decreased basal area, height and number of seed stalks of above ground parts, decreased germination and number of seeds, and decreased carbohydrates in stem bases and roots, as compared to ungrazed stands. This caused, as would be expected, a general adverse effect on the grass. Sprague (1933), studying Colonial Bent and Kentucky Bluegrass, showed one-half the root system is newly generated each spring. Hence the soil and cultural conditions have more effect in the spring than at other times. As compared to uncut grasses, a level cutting of seven-eighths of an inch with Kentucky Bluegrass showed no change in root development, a seven-eighths of an inch cutting with Red Top had a reduced effect, and three Bent species even tolerated a one-quarter inch cutting. Graber (1931) in a general summary of the results of cutting on Bluegrass, Red Top, Fescue and Timothy concludes that not only did the amount of root and rhizome growth and total top growth vary inversely as the frequency of defoliation, but reduced growth sometimes occurred for several months following excessive defoliations. The productive capacity of grasses, he claims, is not alone dependent upon adequate supplies of available nutrients. Moisture, combined with favorable light and temperature, as well as the food reserves of the plant, will also condition this productive capacity. The removal of succulent top growth caused a heavy drain on available nitrogen. This was the first important factor in growth limitation, but when nitrogen was supplied abundantly, a severe drain was caused on the carbohydrate reserve. With slight opportunity being available for this carbohydrate reserve to be replenished, a limitation in the growth of the grasses naturally resulted.

Plan of Procedure:

The interest in this cutting experiment centered mainly on the response of the rhizomes of the alfalfa to the various clipping treatments given. Other results such as hay yields were taken to supplement this work. To determine such a response each individual plant would have to be dug up and the rhizomes counted, measured and weighed and comparisons between the different treatments made. This was impossible to do in the plots of alfalfa, so individual plants (16 for each cutting treatment) were set out, to be treated identically to the plots, and later, at the conclusion of the experiment, would be dug up, and the measurements made. In the seeded blocks any effect the clipping would have on the rhizomes would show up indirectly through the weight of tops removed. It was logical to believe that if clipping were tending to initiate new underground growth in the form of rhizomes, these, when they come to the surface of the soil, would cause the weight of clippings to increase, or if clipping proved deleterious, as it was likely to do, a falling off in yields of clippings would mean less food was available for top growth and so in all probability less for rhizome growth as well. The actual effect on the rhizome growth would be obtainable from the separate experiment already mentioned. In order to complete the picture of changes taking place in the seeded plots, the plots were charted. The actual number of plants appearing in one square metre from the centre of each plot was determined. Supplementing this count the basal cover was estimated as the per cent of bare ground per square metre. The area was also drawn to show the appearance of the square metre when the recordings were made at the start of the experiment. At the end of two summers' treatment the plots were to be charted again and any changes noted. Each plot was divided into three portions, one on which no cuttings would be made (simulating seed production), one on which two or three cuttings per summer would be made (to simulate conditions where crop was cut for hay), and one clipped every two weeks (to simulate heavy pasturing). Accordingly, every plot had these areas marked off and in each four stakes (12 stakes per plot) were placed to record the exact position of each square metre for first and final charting. Altogether some thirty plots were treated in this manner, and twenty-five more had just the one square metre charted in each.

An oak quadrat frame was made, dove-tailed at the corners with cross lines of medium copper wire placed every ten cm. both ways across it, thus forming one hundred small squares in each of which it was possible to count the plants and estimate the bare ground. The work of charting was completed by the second week of May, 1940, and arrangements made to begin clipping the plots at once. A lawn mower was to have been used for this work. Growth on the plots seemed very poor so clipping was postponed until more improvement would be shown, which improvement, however, never came, and in July all thought of proceeding with the original plans had to be abandoned. On June 6th the plots were cut as originally intended, and one portion of each was kept cut with a sickle to simulate pasturing conditions.

The following photograph in Figure 1, taken on June 6th, 1940, will give some idea of the conditions as they were at that time before the plots were cut, Figure 2 giving a close-up of the same.

Figure 1.

Photograph taken June 6th, 1940.
Note thin, stunted and generally
exhausted appearance of plants.

Figure 2

Close-up of Figure 1, to show dwarfed plants and lack of vigor.

Figure 3

General view of Figures 1 and 2 taken July 8th, 1940 after cutting treatments to show poor comeback. The "seed" portion of plot uncut is at extreme left. The "hay" plot is next, after growing 21 days and "pasture" plot to the right having just been cut.

Figure 4

Photographed July 8th, 1940, and shows average heights of "seed" and "hay" plots 21 days after "hay" plot was cut.

The general appearance of all the plots gave every indication of a mineral deficiency existing, despite the fact that basic slag and lime had been applied in April, 1940. The field had lain bare for the previous three summers and extensive leaching might be responsible for the poor growths obtained. The pronounced yellowing of the foliage seemed to point to a boron or potassium deficiency. One particularly interesting point, however, was a narrow strip two feet wide and five feet long, extending through one plot, in which growth was normal, being two feet high and of a rich green coloration. Examination of the roots of these plants showed heavy nodulation, whereas stunted plants merely showed an occasional nodule or usually none. Thus the belief was held that the seat of the trouble lay in lack of nodule bacteria, even though inoculation with suitable earth culture had been made before seeding down the blocks. As the season was now approaching July, and being very hot and dry, difficulty was expected in introducing the cultures of *Rhizobium meliloti* at hand, into the soil. This was done, however, on July 4th at night by spraying on the cultures followed by a liberal watering. Fearing that the following very hot and dry weather had killed the bacteria, as no great improvement was shown, the plots were reinoculated with heavier cultures during a rain storm on July 18th. In addition, boron at the rate of fifteen pounds per acre, potassium at one hundred pounds per acre, and during the last application of bacteria, nitrogen at the rate of one hundred pounds per acre were applied. Results were not expected by this time to be shown during the current season but it was hoped the plants would recover for the next season's growth.

As the principal aim of the writer was to study rhizome growth, no exact experiments were undertaken to ascertain the reason for this abnormal behavior, but it would be interesting to see if mineral or bacterial deficiency were the cause. On July 3rd, therefore, twelve large pots of earth from land similarly treated to seeded blocks, were brought into the greenhouse. "Sick" plants from the field were placed in them and four were given boron plus fertilizer, four bacteria plus fertilizer, and four fertilizer treatments alone, to see if the plants would grow normally with the treatment given in the field (i. e. fertilizer only), or if boron and bacteria had any beneficial effect upon them. Later, on July 8th, eight more pots were included, these being treated, four with potassium plus fertilizer, and four with no treatment whatsoever. On July 19th nodule bacteria obtained from the roots of the strip of normal alfalfa previously mentioned were placed in four pots in similar soil to the above and inoculated as well onto some more "sick" plants' roots. The pots were brought into the greenhouse. On July 23rd four pots, each containing nodule bacteria, potassium and phosphorus, were brought in. On July 26th eight more, four of which contained nodule bacteria, lime (two tons per acre), and fertilizer, and four contained lime (two tons per acre), and fertilizer, were also brought into the greenhouse.

Whilst it would have been best to have these all brought into the greenhouse at once, this was impossible, so the results obtained are unreliable as far as comparisons between treatments are concerned. The results did serve the purpose in mind, however, which was to see if normal growth could be obtained from the "sick" plants in the field. Upon observing the growth of these plants in the greenhouse, only the lime fertilizer pots showed any tendency whatsoever towards abnormal behaviour, these being very stunted and sickly, the others growing normally and showing a luscious green foliage.

Observation of Results:

The following results were obtained on February 3rd, 1941, from three of the pots of each set of four in which the roots had been washed out, and set in a warm place to dry on January 19th, 1941. The top growth at this time was not taken, being too matured. The remaining pot in each treatment was left to continue growth.

Table I

Weight of Dried Roots of Alfalfa Having Had Different Fertilizers or Bacteria Added.
Weights Taken February 3rd, 1941.
(Roots From Three Plants in Each Result)

Date Placed in Greenhouse	Treatment	Weight (Grams)
July 3rd, 1940.	Bo - with fertilizer (3 - 10 - 8)	34.1
" 3rd, 1940.	Check - with fertilizer (3 - 10 - 8)	21.3
" 8th, 1940.	Check - no fertilizer	19.1
" 8th, 1940.	K - with fertilizer (3 - 10 - 8)	24.2
" 26th, 1940.	Lime - with fertilizer (3 - 10 - 8)	8.5
" 3rd, 1940.	Bacteria - (Rhizobium meliloti) with fertilizer (3 - 10 - 8)	27.7
" 19th, 1940.	Bacteria (from Alfalfa nodules) with fertilizer (3 - 10 - 8)	13.5
" 23rd, 1940.	Bacteria (from Alfalfa nodules) + K + P	28.4
" 26th, 1940.	Bacteria (from Alfalfa nodules) + lime + fertilizer (3 - 10 - 8)	18.6

The photograph in Figure 5, taken April 3rd, 1941, shows the growth as it occurred at that time, also showing the quadrat frame used and the stakes set in each plot. Indications seem to be that the plots may have a better growth during 1941 than 1940, but still will not be normal as the borders of the plots have growth three to four inches above the inner portion of the plots. This inner portion is not growing as rapidly as it should be, and seems to resemble the condition somewhat as they were in the spring of 1940.

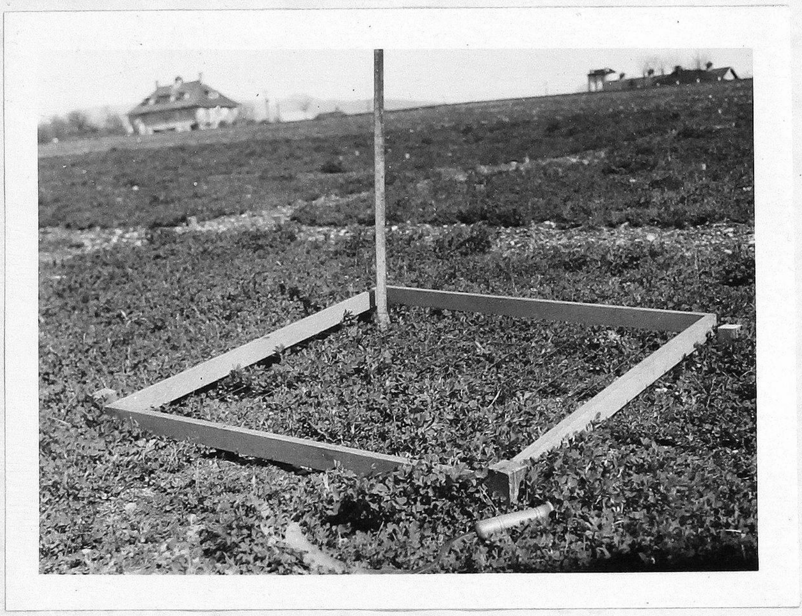


Figure 5.

Photograph of the same plot as in Figure 1 and centre Figure 3, taken April 3rd, 1941.

Conclusion:

For more significant results a more exact and extensive experiment is required, but the results obtained serve to show that nothing is fundamentally wrong with the land, for when the plants from it were transferred, using the same soil, to pots in the greenhouse they grew normally. Suspicion is still directed at the bacteria as being the cause of the trouble, as examination in the spring of 1941 showed nodulation on the plants two to three inches below the ground, but where the nodules should be - at the ends of the roots - they were lacking. The results with application of two tons of lime per acre in the greenhouse are also of interest. In both cases when the lime was added, the weight of the roots was less than where no lime was used. At present nothing can be put forward in the way of a definite cause for the poor growth in the field until more work is done on the problem, or until another season's results are known. Certainly there is no lack of Bo, N, K, and P in the soil. Whether the plants will grow better and grow normally during 1941 remains to be seen.

From the discussion of the literature given on the effects of cutting the top growth from alfalfa and forage grasses given by other men, we may assume the results that would have been obtained here would have been similar. That is the "pasture" portions, being cut too frequently and at too low a level, would have had their reserves depleted, no new rhizomes would have been produced, the resulting growth would have been poor and many plants would in all probability have died. In the "hay" plots little change other than normal change would have occurred, the plants just increasing in basal area and quantity of roots. If new rhizome growth were to be initiated we would have found it in the "seed" plots where reserves would have been laid up, strong root growth maintained and the yield of hay from this plot being greater than from the "pasture" or "hay" portions. Unfortunately, this was not able to be proved here.

2. <u>The Fertilizer Experiment.</u>

Purpose:

The purpose of this experiment was to determine which fertilizer, if any, gave the most pronounced effect, both in initiation and maintenance of rhizome growth in alfalfa. Hay yields were also to be taken to see if any correlation existed between yield of tops and yields of rhizomes.

Plan of Procedure:

The alfalfa plants used in this study were to be grown individually, four plants constituting a plot, and each plot to receive the various fertilizers. Top growth yields were to be taken twice or thrice a summer, and at the end of the second summer each plant was to be dug up and a record secured of the weight of the roots, and number, length and weight of rhizomes produced. Accordingly, a site was selected upon which no fertilizer had been used for several years previously, which at the time was in grass. This was ploughed. Inadvertently, barnyard manure had been spread over it and the adjoining field before the grass was turned under, which may have affected the first year's response. The field was then made ready for transplanting the alfalfa.

The treatments to be given the plants were to include nitrogen, phosphorus, potassium, sulphur, nitrogen and potassium, complete fertilizer, copper and boron, to find out any fertilizer effects. In addition, two different lime treatments were to be given, a "moist", "dry", and the "pasture", "hay" and "seed" treatments already referred to when we were discussing cutting effects. Thus, sixteen treatments were to be used, each treatment replicated four times, using one plot for each treatment with four plants to a plot. Thus, sixteen plants would be necessary for each treatment, making a total of two hundred and fifty-six plants in the experiment. If it had been possible, these two hundred and fifty-six cuttings would have been taken from one parent plant to avoid strain differences as much as possible, but one parent would not supply so large a number, so sixteen parents had to be used. These were selected for their great spread from a block of F_4 plants of the University of British Columbia rhizomatous type alfalfa. Sixteen cuttings were obtained from each of these, having a bit of root and top growth attached and transplanted to the field March 30, 1940. Figure 6 is a photograph of the plan of the field layout. The rows A, B, C, D, run lengthwise, each containing the sixteen treatments randomized, as do the plots 1, 2, 3, 4, and 5, 6, 7, 8, and 9, 10, 11, 12, and 13, 14, 15, 16 of all rows. Unfortunately, the plants themselves were not randomized, but were transplanted systematically. An error was thus introduced which made statistical analysis of the results impossible.

When the results from the clipping experiment were seen to be unattainable for the current summer, yet hoping they would be obtainable the following summer, the "pasture", "hay", and "seed" plots in the fertilizer experiment were accorded the same treatment as the clipping experiment plots, in order to keep the treatments in both experiments identical. That is, when the clipping experiment plots received their first cut the "pasture", "hay" and "seed" plots also were cut, and later the "pasture" only in both cases. Thus, instead of the "pasture" plants in the fertilizer experiment being cut every two weeks, they were cut only twice during the summer. The following table gives the kind and rate of fertilizers used

Element Wanted	Fertilizer Used	Rate of Application
N	Sodium nitrate	100 lbs. / acre
P	Triple-superphosphate	113 " "
K	Muriate of potash	100 " "
S	Gypsum	300 " "
PK	Triple-super + muriate of potash	213 " "

Element Wanted	Fertilizer Used	Rate of Application
NPK	Complete	313 lbs. / acre
Cu	Copper sulphate	60 " "
Bo	Boric acid	15 " "
Lime	Slaked Lime - $Ca(OH)_2$	$1\frac{1}{2}$ tons / acre
Lime	" " "	3 " "

The different plants used in the various treatments are designated by the location of their parent in the F_4 alfalfa block.

Plant or Strain	Location of Plant	Plant or Strain	Location of Mother Plant
1	6A - 32 - 2	9	6A - 48 - 1
2	6A - 32 - 6	10	6A - 51 - 1
3	6A - 32 - 21	11	6A - 51 - 2
4	6A - 32 - 24	12	6A - 51 - 4
5	6A - 32 - 27	13	6A - 52 - 7
6	6A - 33 - 18	14	6A - 56 - 11
7	6A - 36 - 1	15	6A - 72 - 14
8	6A - 46 - 1	16	6A - 73 - 17

Figure 6

A photograph of the field plan for the fertilizer experiment, showing the treatments accorded each plot.

Figure 7

Photo taken June 17th, 1940, $2\frac{1}{2}$ months after the cuttings were transplanted.

Figure 8

A close-up taken of one of "phosphorus" plots, secont plot from the right in front row of Figure 7. Photo taken June 17, 1940.

Figure 9

Photograph taken April 18th, 1941, from the same spot as in Figure 8 to show the great growth made by the cuttings in their first season. They were cut twice during the summer of 1940, the growth shown coming on mainly since the last week in March.

Observation of Results:

Two cuttings were made the summer of 1940, that is, on June 18th and September 9th. The weight of top growth obtained from each plant was recorded. From the results obtained the nitrogen plots appeared to yield lower than the phosphorus, potassium, N and K, sulphur, and NPK plots, the latter two giving greatest returns. However, as previously mentioned, the significance of these, although determined and a significance found, was unreliable. The three ton per acre application of lime appeared detrimental under field observations. Agreement was found, too, statistically with these observations, which though cannot be proved. On April 3rd, 1941, the total basal coverage or spread of all plants was made, the "seed" plots being outstandingly greater in their spread than the others, but outside of this one treatment no other differences were possible to ascertain.

Conclusion:

It is hoped with the material at hand that the experiment may be laid out again with the plants randomized, and so enable differences to be reliably determined, as sufficient evidence presents itself from this work to show such differences to be present. The lime plots were decidedly inferior in growth to the other plots, and as already stated, the nitrogen ones appeared to yield lower than the phosphorus, potassium, N and K, sulphur and NPK plots. The sulphur and NPK plots seemed to outyield all others.

(c) Greenhouse Studies.

1. The pH Experiment.

Purpose:

The purpose of this experiment was to ascertain the effect of acid and alkaline soil on the growth of rhizomes in alfalfa.

Discussion of Literature:

Alfalfa being a lime-loving plant, growing best in conditions approaching neutrality, it is reasonable to suppose a highly acid or alkaline s would not only discourage the growth of alfalfa, but would discourage rhizome growth on that alfalfa as well. Such a retardation in rhizome-producing alfalfa has been shown by Holliday (1932) where conditions of high acidity prevail in a soil. She finds that rhizomes favor slight alkalinity over slight acidity for their optimum growth. The plants showing greatest rhizome growth in her work were those in a moist soil, high in humus and of a slightly alkaline reaction. In other words, soils of a generally good condition for alfalfa growth will produce alfalfa having the greatest rhizome formation.

Other works have shown that excess acidity is unfavorable for alfalfa growth, and so we must assume, for rhizome production in this alfalfa as well. Joffe (1920) grew alfalfa in pots of soil adjusted by means of H_2SO_4 and $CaCO_3$ to a range of pH 3 to pH 7.1. Germination he found the same from pH 4.5 - 7.0, but greatly reduced below 4 and 5; the yield of tops increased as pH increased from 3.8 - 7.0 and once established showing normal green color and high vigor; nodule formation, as would be expected, he found to decrease as pH value decreased; root development was shown to be poorer at lower than higher pH; and nitrogen content increased with increase in pH. Haas (1937) conducted similar work to that of Joffe only he adjusted the pH with different reagents. Instead of growing the alfalfa in pots of soil, he grew seedlings in modified Haagland's solution, adjusting the pH with NaOH, $Ca(OH)_2$ and KOH. Neither approximate neutrality nor moderate alkalinity appeared to be unfavorable to growth, the average growth being least at pH 5 and greatest at pH 8. The best growth was made in the order, NaOH, KOH, $Ca(OH)_2$, which shows the value of calcium for the best growth of alfalfa. Later in 1936, Watenpaugh showed alfalfa root growth to be meagre in a soil layer under pH 5. Upon addition of lime, however, bringing the pH up to 5.5 - 6.2, good root growth and then good top growth resulted. Poor growth under pH 5 he attributes to deficient nitrogen supply, aluminum toxicity or high H^+ ion concentration. The root development correlated definitely with the pH and with the replaceable calcium. Growth was checked entirely at pH's of 4.8 whilst those above pH 5 proved satisfactory for good growth.

Plan of Procedure:

Facilities were not available for the more exacting experiments conducted by these previous workers. However, it was thought that soil could be brought to pH's of 6, 7 and 8. Boxes of soil were to be used having these pH's established by the use of lime. Cuttings from a pronounced rhizomatous plant in the field were to be placed in them and the boxes brought into the greenhouse.

With this plan in mind, then, some two hundred cuttings were obtained on November 24th, 1939, in the field from plant 29, row 19, in the composite block of alfalfa, this plant having the greatest spread of any seen in the block. The cuttings taken were about three inches long. Rhizome tips were preferred, but shoots with green growth already started were also used. These were placed two inches apart and three inches deep in flats of sand.

As the plants had by January 12th attained the proper growth for transplanting, work was immediately begun to prepare soil of pH's 6, 7 and 8. By reference to the literature, Morgan (1930) and Pettinger's (---) work seemed most satisfactory for the purpose in mind and Morgan's was chosen.

His method was to add lime to the soil to obtain the pH wanted according to the following formula:

$$\left(\begin{array}{l} CaCO_3 \text{ required in tons} \\ \text{for } 2,000,000 \text{ lbs. soil} \\ \text{(1 acre foot)} \end{array}\right) = \left(\begin{array}{l} 0.8 \times CaCO_3 \text{ absorption factor} \\ \times \text{ (pH wanted - pH found)} \end{array}\right)$$

The absorption factor was obtained from a table listing different classes of soil.

Accordingly 15 cubic feet of soil were brought into the greenhouse on February 3rd, 1940. The plants were to be placed in one cubic foot capacity boxes to give ample room for rhizome growth. The pH of this soil was determined by the colorimetric method and found to be approximately 6.0. The earth was divided into three piles and hydrated lime added according to Morgan's formula. The hydrated lime had a $CaCO_3$ equivalent of 135 per cent, so less was needed. This earth was left for one week. The results were entirely erroneous and was found to be air-slaked, having been improperly stored. Rock lime, having a $CaCO_3$ equivalent of 178 per cent, was then used in new soil and after six more trials, when the work was checked and rechecked, with far more lime being used than Morgan specified, only the pH 7 figure could be obtained by March 16th. (The soil was considered to be sandy loam, 2.5 - 5 per cent O.M. and at pH 6, for using Morgan's tables). As it was getting late in the year, the "cuttings" were set in boxes in this soil at pH 6 and 7, with the hope that the pH 8 figure could soon be obtained. By April 16th the pH 8 result was still not obtained, so the effort was abandoned until a series of soil plus lime pots could be set up. Five boxes were used with soil at pH 6 and five at pH 7, these being set on a bench at the East side of the greenhouse until December 10th, 1940, when they were removed and washed clean.

As so much time and effort had been spent in an endeavour to bring the third pile of soil up to pH 8, a series of soil plus lime mixtures were set up to find exactly how much was needed, in case it would be possible to repeat the experiment. Accordingly, 42 "tin" tightly sealing flour cans were obtained, in each of which one pound of sandy-loam was placed, obtained from the same field as greenhouse soil (Peas, Oats, Vetch, mixture sown there in summer of 1940). Freshly slaked rock lime in the form of $Ca(OH)_2$ was added at various rates and the cans put away July 29, 1940.

As previously mentioned, the cuttings were put in the boxes March 16th, 1940, five being placed in soil to which no lime had been added, and five placed in soil to which $Ca(OH)_2$ had been added at an equivalent rate of 4,000 lbs. per acre $CaCO_3$, bringing the pH to approximately 7. These remained on the bench in the east side of the greenhouse until December 10th, 1940, when the soil was washed from the roots, typical plants were photographed, and the plants set in paper bags in the laboratory to dry.

Observation of Results:

The appearance of the rhizomes originally set out as cuttings in the boxes of sane corresponded with Oliver's (1913) description. Each had short internodes, and was marked at intervals by "rudiments of leaves and very large stem-clasping stipular growths at each internode covering a dormant bud." Thes stipules gradually turned reddish in color as occurs naturally when they push to the surface of the ground. The cuttings set in the flats of sand were again observed on November 27th, the reddish coloration of the stipules was still seen and new green leaves appeared on all the cuttings. On examining the underground portions, slight swellings were noticed around the cut edges. By December 1st the new green growth was more pronounced and the underground swellings had increased. Upon examination again on December 6th these swellings had assumed the shape of definite nodules which on some appeared to be developing into minute roots. By January 3rd the tops had grown three inches, and by January 12th this top growth had become very pronounced; the roots had attained a length of two inches, but no rhizome growth was being initiated.

The plants remained in these boxes of sand until March 16th, when they were transferred to boxes of soil which were at pH's of 6 and 7. Boxes of soil at pH 8 were not obtained, so the final results given on rhizome growth are only those from the soil at pH 6 and 7. Later on figures were obtained which, if time would have permitted, would have enabled soil at the original ranges of pH 6, 7 and 8 to have been used. This information was obtainable from the samples of soil plus lime already mentioned as having been set aside July 29, 1940. These were taken out on October 25, 1940, and the pH's determined. A sample of soil one-half an inch by two and one-quarter inches long was taken from each can in order to do this, and the pH found by means of a hydrogen quinhydrone potentiometer. The results that were obtained are given below in Table II. It is seen that to obtain a pH of around 8, 12,220 - 25,550 pounds per acre of lime as $CaCO_3$, must be added per acre. For a similar soil, Morgan claimed only 4,000 pounds per acre of lime as $CaCO_3$ need be added.

Table II

Can No.	$Ca(OH)_2$ Added	Equivalent $CaCO_3$ Added in lbs./acre	pH	Can No.	$Ca(OH)_2$ Added	Equivalent $CaCO_3$ Added in lbs./acre	pH
1	0.00 gms.	0	5.7	22	4.75 gms.	28,610	8.3
2	0.00 "	0	6.0	23	5.00 "	30,570	8.4
3	0.00 "	0	5.9	24	5.25 "	32,100	7.9
4	0.25 "	1,530	6.2	25	5.50 "	33,630	8.4
5	0.50 "	3,060	6.5	26	5.75 "	35,160	8.4
6	0.75 "	4,590	6.5	27	6.00 "	36,660	7.9
7	1.00 gms.	6,120	7.1	28	6.25 "	38,190	8.0
8	1.25 "	7,650	7.3	29	6.50 "	39,720	8.5
9	1.50 "	9,280	7.9	30	6.75 "	40,350	8.1
10	1.75 "	10,810	7.6	31	7.00 "	42,770	8.5
11	2.00 "	12,220	8.0	32	7.25 "	44,300	8.6
12	2.25 "	13,750	8.0	33	7.50 "	45,830	8.5
13	2.50 "	15,280	7.9	34	7.75 "	47,360	8.5
14	2.75 "	16,810	8.0	35	8.00 "	48,880	8.5
15	3.00 "	18,340	7.7	36	8.25 "	50,430	8.4
16	3.25 "	19,870	7.9	37	8.50 "	51,960	8.7
17	3.50 "	21,400	7.9	38	8.75 "	53,490	8.6
18	3.75 "	22,930	8.2	39	9.00 "	54,990	8.7
19	4.00 "	24,460	7.9	40	9.25 "	56,420	9.3
20	4.25 "	25,500	8.1	41	9.50 "	57,950	9.3
21	4.50 "	27,080	8.5	42	9.75 "	59,480	9.1

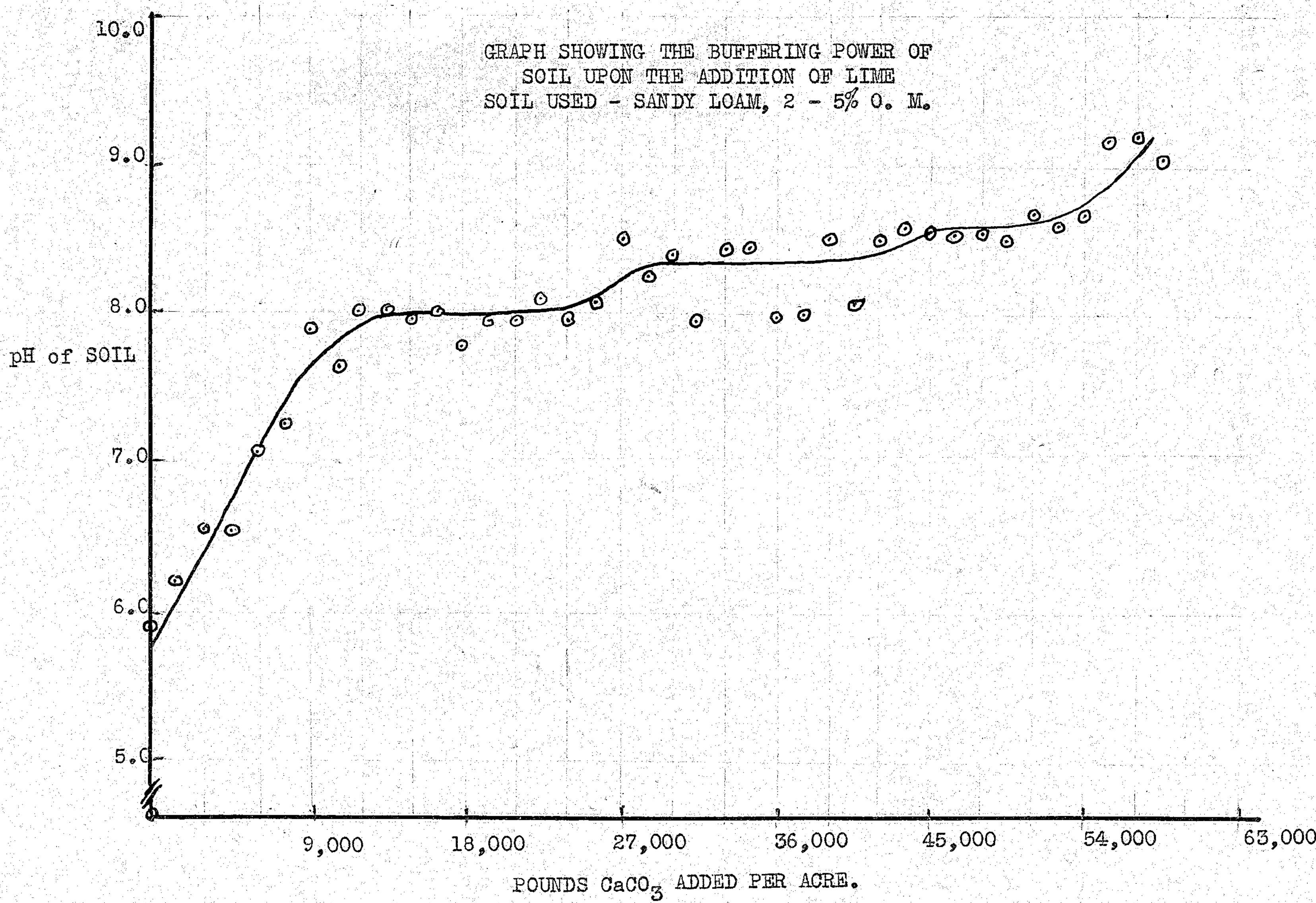
With our first set of soil using hydrated lime that had air slaked, we applied 26,000 pounds of $CaCO_3$ per acre to get a pH of 7.1, showing the slow reaction of $CaCO_3$. Later, after several trials using CaO on fresh soil, 4,000 pounds per acre $CaCO_3$ gave a pH of 7.1, which serves to illustrate the faster reaction of CaO. When this was doubled in an effort to bring pH up to 8.0, an actual decrease to pH 6.8 was noticed, which was checked, and showed either an error in the first reading of pH 7.1, or in the colorimetric determinations, or variations in the soil. As this is 4,000 pounds per acre short of the first figure given in the above pH 8.0 range, it is seen why we could not get our greenhouse soil up to pH 8.0.

The following graph, making use of the figures in Table II, shows the buffering power of the lower (West) portion of Agronomy Field when lime is added. This graph would seem to indicate that little difficulty would be obtained in securing soil at pH's of approximately 7.9, 8.3 and 8.6. These are where the curve flattens out. (See P. 53a)

The boxes of soil at pH's of 6 and 7, containing the alfalfa plants, were removed from the bench in the east side of the greenhouse December 10, 1940. The soil was washed from the roots, and after photographing the plants, they were dried in the laboratory. The dry weights of these plants were taken February 28, 1941, and are given in Table III.

Table III

5 Plants in Soil at pH 6			5 Plants in Soil at pH 7		
Top Growth	Roots	Rhizomes	Top Growth	Roots	Rhizomes
17.5 gms.	53.8 gms.	2.2 gms.	53.0 gms.	173.5 gms.	16.3 gms.


GRAPH SHOWING THE BUFFERING POWER OF
SOIL UPON THE ADDITION OF LIME
SOIL USED - SANDY LOAM, 2 - 5% O. M.
pH of SOIL
10.0
9.0
8.0
7.0
6.0
5.0
0
9,000
18,000
27,000
36,000
45,000
54,000
63,000
POUNDS $CaCO_3$ ADDED PER ACRE.

These results show that the five plants at pH 7 had top growth and root growth three times the weight of those five plants at pH 6, whilst the rhizome growth was nearly eight times that at the power pH.

To give a better idea of how remarkably different was the rhizome development in the two cases, the following pictures were taken. In these plants at pH 6 no rhizome growth was evident, but where it did occur in other plants the growth was very stunted and thin. In the plant at pH 7, on the other hand, a great outward projection of the rhizomes took place, these being very sturdy and thick, of white appearance and tending to brach frequently. Note, too, the much better and heavier growth of tops and roots in the latter case.

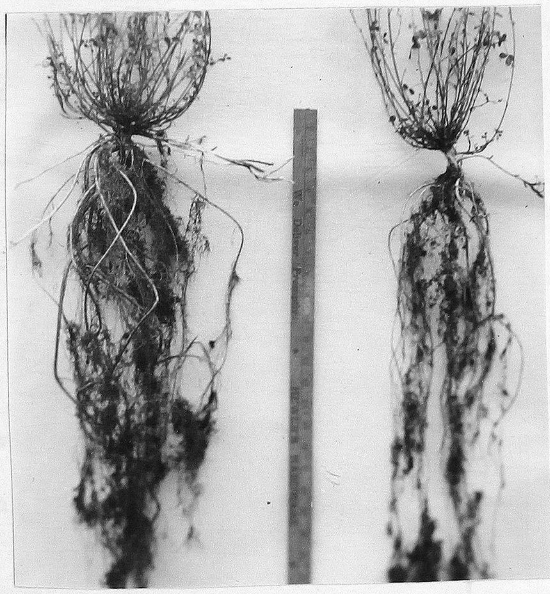


Figure 10

Plant from pH 7 soil to the left, and from pH 6 soil to the right. Note lack of rhizome growth and generally poorer growth in latter case.

Figure 11

A close-up of rhizomes from Figure 10 to show more clearly their growth, origin and manner of dividing.

Conclusion:

From the results of this experiment we may conclude that there is a critical pH below which rhizome development will not occur. Holliday (1932) showed that conditions favoring growth of alfalfa will favor an abundant rhizome growth, if genetical factors for such growth are present. Excess acidity certainly does not favor alfalfa growth. In this experiment in the soil at pH 7 conditions were very suitable for alfalfa, as the soil was nearly neutral and abundant lime was present. Just how much this lime contributed to the effects seen could only be proved by reducing acidity by other means than lime and comparing the results with those obtained here. It is reasonable to suppose, however, that rhizomes will not form below a critical pH. Above this they will develop, lime accentuating their growth by supplying more ideal conditions for the plant, enabling more vigorous growth to be made, more photosynthesis to be carried on, and hence more food to be available for underground storage.

2. The Photoperiodism Experiment~~s~~.

Purpose:

The purpose of this experiment was to observe any effects which short and long day-length would have on the rhizome development in alfalfa.

Discussion of Literature:

From the voluminous literature available on the effects of photoperiods on plants, it has been found that critical light periods occur in the normal development of every plant. Attention has been confined more to the response of the leaves, stems, and flowers to changes in day length, than to the response of the underground portions of plants. Plants requiring a long day for normal growth and reproduction, will, under a short day fail to produce flowers. The reverse holds true for normal short day plants, these under a long day failing to flower, their growth remaining vegetative throughout the treatment with long days. This effect of alternation of normal day length was found by Chailakhian and Aleksandrovskaia (1935) to be carried over by plants from their seedling stage to their time of flowering and maturity, even when the day length was only altered for a short time in the seedling stage. The length of time the effects of this abnormal day length may be held may be exemplified by the work of Stoughton and Hole (1937) on the short day plant Tithonia speciosa. Here the short day treatments were given at the time of planting the seeds, remained for a certain period and then normal day length was restored. The effect of this treatment was seen 70 days later at the time of initiation of flowering. This was observed to be 9 days earlier than plants not given so many short day treatments when young. Rasumov (1931) concludes "that experimental evidence is presented which suggests that a true photoperiodic after-effect is seen only in the acceleration of reproductive processes and development of the organism, a short day after-effect thus being possible only for short day plants and a long day after-effect for long day plants".

There appear to be critical photoperiods, too, in the formation of underground parts. Evans and Watkins (1939) found Kentucky Blue Grass needed long days to produce rhizomes whilst Canada Blue Grass needed short days. Under long days Kentucky Blue Grass produced upright shoots, elongated internodes, and same inflorescences. The rhizome growth was shown to be greater under the long days of late spring and early summer than the short ones of fall and early spring. Canada Blue Grass gave quite the opposite response, under the long day its shoots were decumbent, its stems were not elongated, no inflorescences were formed and rhizome growth was greatest in late fall and early spring.

The effects of photoperiod on rhizome growth should be compared with its effects on tuber formation as these two structures are somewhat similar in function. Rasumov (1931) found a short photoperiod favored maximum tuber formation, a long photoperiod giving a limited production of tubers but extensive top development in the tuberous plants he studied. Combining these two light periods, Rasumov found that with the same plants long photoperiods affording extensive vegetative growth, which, followed by short photoperiods gave a maximum yield of tubers. Here the abundant top growth established by the long days, provided a great area for the carrying on of photosynthesis, the foods formed being then carried to and stored in the tubers initiated when short day lengths were begun. Bates (1935) and Werner (1934) found environmental factors to be important in regulating the size of the potato tuber, Werner obtaining maxium tuberization with a low temperature, abundant nitrogen and a short day, which he modified in 1935 to a low fluctuating temperature, restricted nitrogen supply and a short day. With Cyperus esculentus L. Karisnev (1937) found somewhat different effects of the photoperiod on tuber formation. The short day in this case merely retarded the time of formation of the tubers, long day giving bigger, heavier and more numerous tubers. Contradiction or [illegible]

Plan of Procedure:

To conduct an experiment of this nature, the work would have to be done indoors under controlled conditions. Hence, arrangements had to be made in the greenhouse to subject boxes of earth to different day lengths. A five hundred watt bulb and reflector were to be used for lengthening the day, but as no facilities were available for shortening the day, the plants would have to receive just normal day and night conditions. In the section of the greenhouse, too, where the plants were to be placed, the temperature was uncontrolled, so some fluctuations in it would be encountered.

On March 29th, 1940, twenty boxes similar to those used in the pH experiments, were filled with soil obtained from a near-by compact heap, high in organic matter, and brought into the greenhouse. In each was placed one of the cuttings that had been obtained November 24th, 1939, which were still in the flats of sand. Sixteen of these boxes were placed on the floor in one corner (southwest) of the greenhouse, the other four acting as checks being placed on the east bench along with the pH boxes. Above the sixteen boxes on the floor, four feet from them, was hung the five hundred watt bulb and reflector. The light was turned on every night starting April 16th, 1940, from 5 p.m. till 8 a.m., thus giving the plants continuous illumination. Those on the bench were protected from the lamp by a screen hung between and allowed to receive the normal day length. The temperature in the greenhouse was the same as that outdoors, no heat being in the section and the ventilators in the roof being always open.

As the results of this work indicated it would be worth while to give the plants a definite long and short day illumination, hours of sixteen and eight daily light period were chosen as suitable for this. A special cage with dark blinds on three sides and the top was built so that the short photoperiod could be properly regulated. The other side of the cage was built solid, with a shuttered opening placed in it to allow a fan to pull fresh air in, and so keep the temperature inside and outside the cage constant. The sixteen hour day was obtained by utilizing the full normal day to within one-half hour of sunset with extra light provided in the morning to complete the sixteen hour illumination. This extra light was the same five hundred watt bulb and reflector previously used, but now was connected to a time clock to insure it coming on and off at the correct time. As the days lengthened less artificial light was needed so the time of coming on and off was adjusted each week. Thus the 16-hour day was secured by using the artificial light until one-half hour after sunrise, plus the normal day length. The eight hour day was secured by lifting the blinds on the cage every day at 8:30 a.m. and lowering them every day at 4:30 p.m.

As it took considerable time for the necessary material to be gathered and the experiment set up, the alfalfa plants under continuous illumination remained under this day length until they could be incorporated into the new experiment. This meant they were under a full twenty-four hour daylight treatment from April 16th, 1940, until the new experiment began on January 13th, 1941. At the end of December, however, all the plants were cut back to a height of eight inches. Thus a considerable photoperiodic after effect would be found in the plants which were placed under the short day. This may have helped to explain the results that were obtained.

As exact times of illumination were to be run in this experiment other strains and species of rhizomatous types of alfalfa were secured and some grasses also used, in order to give a more complete picture of day length and rhizome growth.

The following strains and species, therefore, were procured and included in the work:

<u>Medicago glutinosa</u> - from an increase block of seed kindly sent by Dr. W. J. White, Officer-in-Charge of Dominion Forage Crops Laboratory, Saskatoon, Sask. Dr. White mentions these plants in this group to consist of a great variety of different types, a

considerable proportion being rhizomatous, some very strongly creeping. Dr. Brink of Madison, Wisconsin, from whom the seed was originally obtained, says that this alfalfa was not rhizomatous under Wisconsin conditions, the crown being much spread out and broad. It was introduced into Wisonsin from the N. Caucausus region of U. S. S. R.

Medicago falcata - under the strain number S--33--1, also obtained from Dr. White of Saskatoon, this strain of M. falcata was noted to be quite strongly creeping, particularly when kept clipped or mowed. The parent plant of this strain was found growing in a Kentucky Bluegrass lawn on the University of Saskatchewan plots and had spread over an area of four feet in diameter.

M. falcata - S. P. I. 75,745 obtained through the courtesy of Dr. H. L. Westover, Senior Agronomist, Division of Forage Crops and Diseases in the Bureau of Plant Industry, U. S. Dept. of Agriculture. In his letter, Dr. Westover mentions this and the next two strains, S. P. I. 35,087 and 42,715 represent early introductions of pure M. falcata, the germination of the seed sent being extremely low. The seed itself was 60 per cent of a brown coloration and of small size.

M. falcata - S. P. I. 35,087 obtained from Dr. Westover and is pure falcata. It also is small seeded with 70 per cent brown coloration.

M. falcata - S. P. I. 42,715 obtained from Dr. Westover and is falcata also with small seed, 80 per cent brown.

M. falcata - S. P. I. 111,614 from Dr. Westover who mentions it is a more recent introduction but may show considerable crossing with M. sativa. This seed sent was yellow with 60 per cent brown.

M. falcata - S. P. I. 115,364 from Dr. Westover who mentions it too to be a later introduction and likely to have crossed with M. sativa. It has very small seed, 60 per cent of which is brown.

M. gaetula - S. P. I. 26,590 from Dr. Westover who states it is from Algeria, Northern Africa, but he is uncertain as to its trueness to type. The seed is plump, medium to large, and yellow-brown in color. Oliver (1913) on p. 27 of his bulletin mentions this same strain number where he was using it in his crosses, claiming it is quite hardy, has a vigorous lot of rhizomes deep in the soil, these being developed early in autumn.

M. gaetula - S. P. I. 7292 from Dr. Westover and introduced from Algeria, but doubt as to its trueness to type again mentioned. This seed was not threshed, the pods being spirally coiled, wound two to four times with an open centre and scattered pubescence over the reticulum. Three to four well developed seeds were found per pod, the seeds being well filled, yellow and a few brown.

M. gaetula - S. P. I. 88,930 from Dr. Westover and originally from Africa with doubt, too, as to type adherence. Here again the seed was unthreshed, the pods being fattened, coiled two to three times with a fine, sparse, spiny pubescence on them. The seed was large, well-filled and had five per pod.

Agropyron repens - (Couch grass) obtained in the form of a four inch tall three-bladed young plant and transplanted into pots in the greenhouse.

Poa pratensis - (Kentucky Bluegrass) - obtained as seed by Dr. V. C. Brink from Kamloops, B. C., where he noted its abundant rhizome growth when growing there.

The U. B. C. alfalfa already mentioned as obtained from cuttings November 24th, 1939, all belonging to one mother plant and having been under continuous illumination since April 16th, 1940.

All plants except U. B. C. alfalfa and Agropyron repens were grown from seed. This seed was germinated in petri-plates in the laboratory the latter part of December, 1940. The seedlings were then transferred to flats of sand in the greenhouse. On January 30th, 1941, when the first trifoliate leaves had appeared, the plants were transplanted to pots of earth obtained from the same pile as that in the boxes of alfalfa. As already mentioned, the U. B. C. alfalfa began the light treatments January 13th, eight boxes being placed in the short day conditions and eight in the long day.

On January 22nd the Couch Grass from the field was brought in, three plants being put under the long day and three under the short day. On February 2nd, two of each type of the seedling alfalfa were put under the long day and two under the short day. The Kentucky Bluegrass was included at this time as well.

Observation of Results:

The first observations of the results of the exposure of the alfalfa plants to varying light conditions were made on the normal and twenty-four hour day plants. On June 6th, those plants under the twenty-four hours daily light exposure were seen to be starting to bloom. These plants were three feet tall, whilst the controls under the normal day length had only made a growth of one foot. On June 17th plants under the light were cut back to a height of four inches. About July 18th the plants again came into flower, the checks making very little growth and showing no signs of flowering. By July 27th the illuminated plants were in one-quarter inch bloom, having made a growth of about two and one-half feet, but producing very slender stems and rather sparse foliage. The check plants still showed no sign of flowering, were only seven inches high, and the growth, though a more luscious green than the continually illuminated plants, was not very heavy. By August 15th the illuminated plants were well past full flowering.

On October 15th the plants from the two light treatments were examined to see what effects the treatments were having on rhizome formation. A striking difference was observed. On November 20th photographs were made of some of the roots which had been washed clean. The plants under continual illumination showed absolutely no sign of rhizome initiation, whilst those under the normal day length gave very pronounced growth. On some plants the rhizomes had grown out ten inches and were very numerous, whilst on others they were short and few.

The top growth in the twenty-four day plants was much taller and heavier than in the normal day ones.

The following photographs show these effects very clearly:

Figure 12

Photograph to show effect of photoperiod on top growth. Normal day plants to the left twenty-four hour day plants to the right.

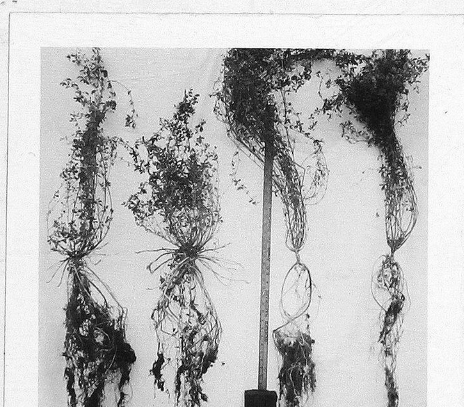


Figure 13

Photograph to show effect of photoperiod on rhizome and root production. Normal day length to the left, twenty-four hour day to the right. Note heavier and thicker roots in short day plant with abundant rhizome growth.

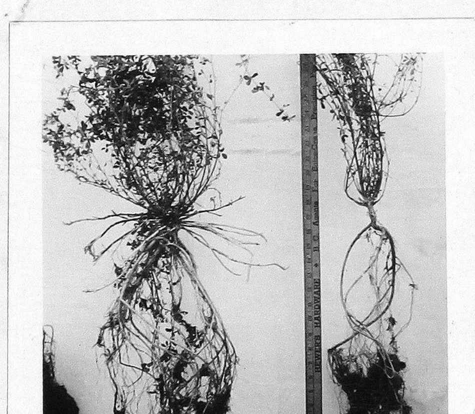


Figure 14

Photograph of centre plants in Figure 13 taken closer to give details of rhizome growth more clearly. The more branched and fibrous roots of short day, and slender, thinner tops of long day plants are well seen.

On November 20th, 1940, the weight of the top growth of the plants under the two light periods was taken. The results, which are given in Table IV, emphasize the differences already observed in the photographs.

Table IV

Weight of Top Growth of Alfalfa used in the Summer's Photoperiodism Experiment
Weights (Green) taken November 20, 1940.

Plants under Continuous Illumination				Plants under Normal Day Length	
Plant	Weight	Plant	Weight	Plant	Weight
1	24.6	9	51.7	1	30.5
2	58.6	10	63.6	2	33.1
3	46.3	11	156.1	3	16.3
4	107.7	12	56.6	4	23.2
5	142.1	13	36.2		
6	31.4	14	-		
7	41.5	15	31.9		
8	36.1	16	29.1		

The effect of the light treatment of eight hours and sixteen hours per day was seen to be very similar as regards top growth to that previously observed of the normal and twenty-four hour light exposures. However, no rhizome growth was noticed on the plants at any time. Several times before the end of the experiment the plants had been examined for rhizome development but no evidence for rhizome initiation was found. The long day plants grew much more quickly in all cases than the short day, the Couch Grass in particular making very rapid growth.

On February 26th a spike was seen appearing on the Couch Grass under the sixteen hour day which, by February 28th, had emerged two inches, with one on another plant also showing. It took this first spike until March 16th to begin flowering, and by March 21st all the spikes were in full flower. The Kentucky Bluegrass did not grow as tall as the Couch Grass and showed no sign of flowering during this time, under either sixteen or eight hour daily light treatment. March 4th was the earliest time of flowering of the alfalfa, one flower opening under the sixteen hour day on plant 14. This plant had six flowers March 12th. Plant 9 began flowering on the same date. March 19th showed a third alfalfa to be flowering, with the first plant having four racemes open and the second, three. By March 27th five plants were seen to be flowering, the first group being in half-bloom.

None of the plants included in the eight hour day showed any indication of flower buds beginning to appear during this same period, nor up to the end of the experiment.

The time it took the various plants to flower can be readily seen from Table V.

Table V

Time Required for the Different Plants to Flower Under Sixteen Hour Day

Plant	Light Treatment Began	First Day of Flowering	Days to Flower
U. B. C. Alfalfa	January 13th) Grown) from	March 4	50
Couch Grass	" 22nd) Cuttings	March 16	53
Kentucky Bluegrass	February 2nd)	--	--
Medicago gaetula (3 strains)	" ")	--	--
Medicago falcata (6 strains)	" ")	--	--
Medicago glutinosa (1 strain)	" ")	--	--

Thus it is seen that the Couch Grass and alfalfa took about the same period of time to flower, the other plants showing no indication whatever of flowering.

On April 6th the plants were photographed, two plants from each type, one long day, one short day, being used. On April 10th the roots of each of these were washed clean to show any rhizome growth appearing. In only two cases did they appear, in both cases these being the grasses and under the sixteen hour day. The rhizomes of the Couch Grass were about six inches and twelve inches long, that of the Kentucky Bluegrass eight inches. The roots of the U. B. C. alfalfa under the 8-hour day, although showing no rhizome response, were much heavier and more branched than those under the sixteen hour day, indicating less drain on their reserves.

The following photographs illustrate some of the results that were attained.

Figure 16

The two alfalfas obtained from Saskatchewan M. glutinosa were considered as having a considerable portion rhizomatous. The strain of falcata shown was noticed as quite strongly creeping. The falcata under the 8-hr. day was eaten off by sowbugs.

Figure 17

The _M. gaetula_ on the left is one of the original strains Oliver mentions as coming from Algeria, that on the right a _falcata_ Westover mentions as perhaps crossed with _sativa_.

Figure 18

Two of Dr. Westover's _falcatas_. That on the left a pure strain of early introduction, that on the right a _falcata_ perhaps crossed with _sativa_.

Figure 19

Two more of Dr. Westover's plants. On the left another pure *falcata*, on the right *M. gaetula* introduced from Algeria and true to type. Eight hour plant eaten by sowbugs.

Figure 20

On the left another pure *falcata* from Dr. Westover and a *M. gaetula* of true type from Africa. Eight hour plant eaten by sowbugs.

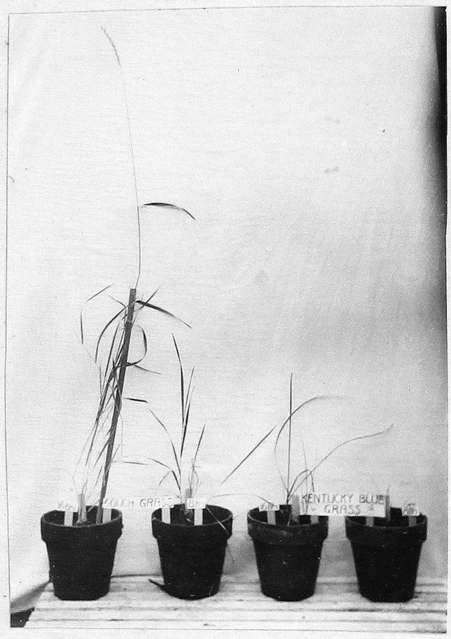


Figure 21

Note the great variation in response to long day and short day in the two grasses.

Figure 22

Note difference in type of growth in this U.B.C. alfalfa. That on the left is the 16 hr. day and is in flower, on the right is the 8 hr. day plant with no sign of flowering.

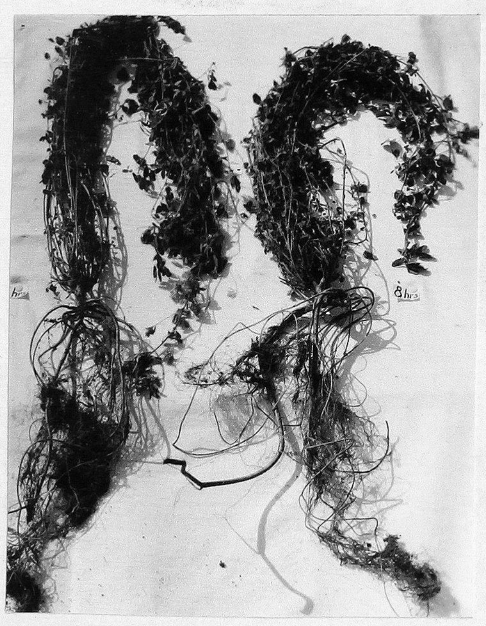


Figure 23

Showing the root growth of the U.B.C. alfalfa under 16 hr. and 8 hr. day. The 8 hr. day plant had heavier and more abundant growth.

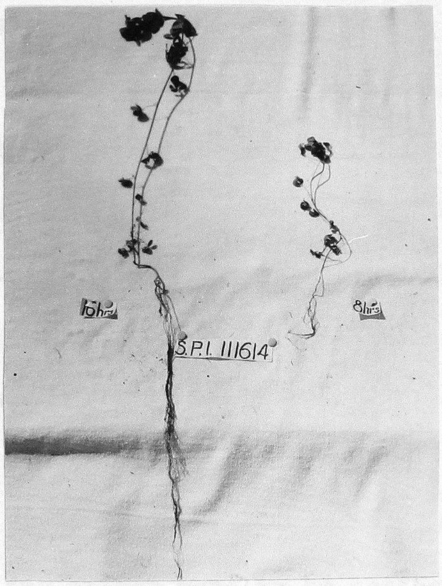


Figure 24

Illustrating the entire lack of rhizome growth in both the seedlings. A much more vigorous plant was formed under the sixteen hour day. This is a strain of Dr. Westover's impure Medicago falcata.

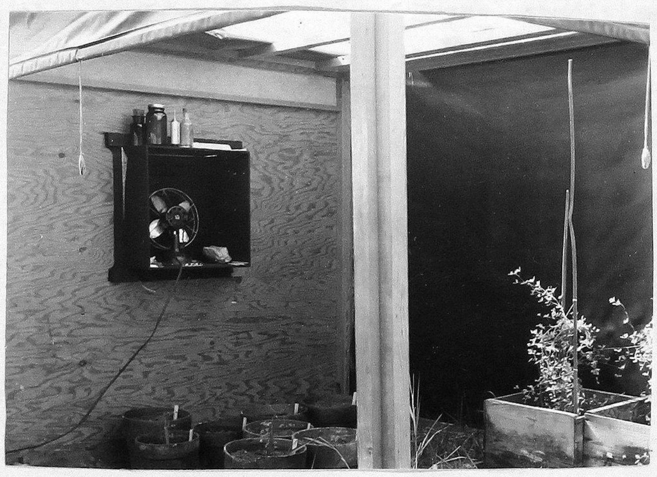


Figure 25

A view of the "cage" used in obtaining an eight hour day. The blinds shown were lifted at 8:30 a. m. and lowered at 4:30 p.m. each day.

Conclusion:

The lack of rhizome production in the alfalfa plants exposed for eight hours was rather surprising . The gradual change of day length to which they were exposed before in the normal day length, in contrast to the limited eight hour day in the final experiment, certainly cannot have been the cause of the rhizome growth. As alfalfa is a long day plant the extreme period of time when continuous light was given to the plants conceivably may have carried over and stopped initiation of rhizome growth. From the discussion of the literature already given, this after-effect of the long day's exposure must be discounted. The results given there would seem to indicate that if any effect were to be noticed, it would only be a delaying or hurrying-up process of the rhizome initiation and not a cessation of their growth altogether. Season, conceivably may have had an effect on their lack of initiation, alfalfa being known to begin rhizome growth in the fall. However, if conditions in the greenhouse had been suitable for their growth, they would have formed. Hence, conditions must not have been suitable.

The only remaining logical reason why the rhizomes were not produced was that there was a difference in the temperature in the two sections in which the plants were grown. Otherwise, conditions were very similar. As already mentioned, the first experiment was carried on in a section where a continuous exchange of air within and without the greenhouse could take place. The temperature thus was about 50° - 60° F. The later experiment was conducted in a different, heated section. Here the temperature was between 70° - 80° F. This never varied very much between these figures, perhaps going higher but certainly not lower than this. The first section both rose and fell from the 50° - 60°F. temperatures mentioned.

Thus, it must be concluded that the failure of the U. B. C. alfalfa to produce rhizomes was that the temperature was above the optimum for their growth.

Other observations have been made of similar effects. Dr. Moe (1928) found, upon transferring this U. B. C. alfalfa to Ithaca, New York, that a decided drop in rhizome production occurred. He concluded the environment was responsible for this effect. From the results obtained here we may conclude day length and temperature were mainly responsible. New York has warmer and longer days than Vancouver during the time that rhizomes should be forming, which must have been above the limits necessary for rhizome growth. It was amply demonstrated from the work just conducted that alfalfa needs cool, short days in a soil above pH 6 for production of rhizomes. A further example of this limitation of rhizome growth in alfalfa will be given by reference to growth noticed in the Agronomy plots at the University of British Columbia. Here, upon examination of the F_4 progeny of the U. B. C. strain of alfalfa quite a striking effect in the manner of spread of each individual plant was noticed. The side of the plant (i.e. the north side) away from the sun was found to have spread eight to ten inches from the main root system, whilst that on the sunny side (south side) had no spread whatsoever. Thus, even at Vancouver the effect of the increased warmth from the sun's rays striking the crown of the plant more directly, was sufficient to prohibit rhizome growth on that side, normal spread occurring where the warmth was less, from the rays striking more obliquely. Purer (1936), it will be remembered, found the rhizomes of Convolvulus soldanella L. responding very markedly, too, to a change in temperature. A rise in it causing the rhizomes to grow upwards, and a decrease in it causing them to grow downwards. Sentence Structure?

From the results obtained here it is seen that in order to produce rhizomes, different plants certainly need widely varying conditions. Alfalfa requires cool, short days in order to initiate and form extensive rhizome growth. The grasses studied here, that is, Couch Grass and Kentucky Bluegrass, both need long days. The temperature levels for these grasses were not obtained in this experiment. The other alfalfas studied produced no rhizomes under either the short or long day because they were too immature.

IV. CONCLUSION

The results obtained in this study have helped considerably to limit the conditions under which (must occur before) rhizomes will form in alfalfa. High temperature is a very important factor limiting their growth. Soils which are too highly acid will also prevent their occurrence. From the results of other workers, it has been found that too severe and too frequent removal of the top growth will also cause cessation of rhizome formation. For abundant growth of rhizomes in alfalfa, then, the plants need to be left uncut in soil well above pH 6, and grown in a climate where cool, short days occur.

From the field studies, no conclusive results could be drawn. The effects of cutting practices on rhizome formation was obtained from previous workers. The effects of fertilizers on rhizome production was not found. The hay yields from the variously treated plots were obtained, but no reliance could be placed in any significant differences obtained. However, differences were noticed. The complete fertilizer, phosphorus, potassium, and sulphur appear to be beneficial, at least to the top growth and nitrogen and excess lime may be detrimental.

V. SUMMARY

1. Rhizomatous strains of alfalfa require rather exacting environmental conditions for optimal rhizome growth, their genetical makeup alone not being sufficient to assure this.

2. A pH of 6 is very detrimental to rhizome growth in alfalfa in some cases prohibiting it. A pH brought up to 7 by the use of lime was beneficial, abundant rhizome growth occurring.

3. For optimum rhizome production in the U. B. C. strain of alfalfa, cool, short days are essential, the plants being grown in soil above pH 6.

4. Maximum spread of alfalfa in the field was observed in the unclipped plants.

5. Long, warm days gave maximum top growth in U. B. C. alfalfa, producing taller, finer and heavier foliage with less root weight than the short, warm, day plants which had a shorter, thicker top growth, and a coarser, heavier and more fibrous root system.

6. Long days initiated flower production in U. B. C. alfalfa in fifty days, Couch Grass in fifty-three days, the Kentucky Bluegrass and seedling alfalfa strains showing no indication of flowering during this time under short or long days.

BIBLIOGRAPHY

AHLGREN , H. L., 1938, The effect of fertilization, cutting treatments, and irrigation on yield of forage and chemical composition of the rhizomes of Kentucky Bluegrass (Poa pratensis). Jour. Am. Soc. Agr., 30 (8), 683-691.

ALMEIDA, J. F. R. d'., 1926, Some notes on the structure and life history of Nymphaea pubescens Willd. Jour. Indian Bot. Soc., 5 (2), 62-71, 8 pl.

ALMEIDA, J. F. R., d'., 1928, On the shoot morphology of Limnanthemum. Jour. Indian Bot. Soc., 7(1), 1-11, 6 fig.

ANGST, C., 1927, Observations on Pteris aquilina. Publ. Puget Sound Biol. Sta., 5, 261-265, 1 pl.

ARTSCHWAGER, E., 1927, Wound periderm formation in the potato as affected by temperature and humidity. Jour. Agric. Res., 35(11), 995-1000, 4 fig.

BAILEY, L. F., 1940, Some water relations of three Western Grasses. I Drought resistance. II Root developments. Am. Jr. Bot., 27(3), 129-135, 1 fig.

BARR, C. G., 1936, Preliminary studies on the carbohydrates in the roots of bindweed. bibliog., tabs., Jour. Am. Soc. Agr., 28, 787-98.

BARR, C. G., 1939, Organic reserves in field bindweed as affected by cultivation. Jour. Colorado - Wyoming Acad. Sci., (2(5), 31.

BARR, C. G., 1940, Organic reserves in the roots of bindweed. Jour. Ag. Research, 60, 391-413 tabs., bibliog.

BATES, G. H., 1935, Study of the factors influencing size of potato tubers. Jour. Ag. Sci., 25, 297-313, bibliog., tabs.

BLINN, P. K., 1911, Alfalfa: The relation of type to bardiness. Colorado Agric. Exp., Sta., Bull. 181, 16 p. illus.

BOWER, F. O., 1935, Primitive land plants - also known as Archegoniatae. XIV, 658 p., 449 fig., MacMillan Co., New York.

BROWNE, I. M. P., 1933, A fifth contribution to our knowledge of the anatomy of the cone and fertile stem of Equisetum. Ann. Bot. 47(187), 459-475, 8 fig.

BURKHART, B. A., 1936, Hemicellulose constituents in alfalfa roots. Plant phys., 11, 421-8, tabs., bibliog.

BUXBAUM, F., 1927, Nachträge zur vergleichenden Anatomie der Melanthioideae. (Supplements to the comparative anatomy of the Melanthioideae). Beih. Bot. Centralbl. 1 Abt., 44(3), 255-263, 4 fig.

CARLSON, M. C., 1938, Origin and development of shoots from the tips of roots of Pogonia ophioglossoides. Bot. Gaz., 100(1), 215-225, 13 fig.

CARLSON, M. E., 1927, Origin of the adventitious roots of Coleus. Bot. Gaz., 87, 119-127.

CHAILAKHIAN, M. K. H., I ALEKSANDROVSKAIA, V. A., 1935, (Nature of the photoperiodic after-effect (induction) and the length of day length on the activity of oxidizing enzymes. (In Russian and English) Doklady Skademii Vauk., S. S. S. R., (Compt. Rend. Acad. Sci., U. R. S. S.) 2(2), 161-166, 1 fig.

CHAKRAVERTI, D. N., 1939, The occurrence of fugacious cambium in the rhizome of Curcuna longa. L. Philippine Jour. Sci., 69(2), 191-195, 2 pl.

CHANG, C. Y., 1927, Origin and development of tissues in the rhizome of Pteris aquilina. Bot. Gaz., 83(3), 288-306, 18 fig.

CHOUARD, P., 1934, Un cas singulier de transformation de bulbes en rhizomes drageonnantes. Compt. Rend. Acad. Sci., (Paris), 199(2), 163-165, 2 fig.

COOPER, W. S., and ABRAHAM, D. S., 1931, The subterranean organs of Helianthus scaberrimus. Bull. Torrey Bot. Club., 58(2), 67-72, 2 fig.

CROSS, G. L., 1931, Meristem in Osmunda cinnamomea. Bot. Gaz., 91(1), 65-76, 15 fig.

CULPEPPER, C. W., and Moon, H. H., 1938, Composition of the rhizome, stem, and leaf of some horticultural forms of canna in relation to their possible use. U. S. Dept. Agric. Circ., 497, 1-21.

DEHAY, C., 1939, Performation of living roots by the rhizome of Agropyron repens. Bull. Soc. Bot. Fr., 85, 403-6, pl.

DUHAMEL du Monceau, H. L. 1759, A practical treatise of Husbandry. P. 358, London.

ENNVERE, A., 1937, Päris - orascheina, Agropyron repens L. P. B. bioloogiast. (On the biology of Agropyron repens L. P. B.) Agronoomia, 17, 553-67. (German summary 614) Exp. Sta. Univ. Tartu, Esthonia.

EVANS, M. W. and ELY, J. E., 1935, Rhizomes of certain species of grasses. Jour. Am. Soc. Agron., 27, 791-97, bibliog., tabs.

EVANS, M. W., and WATKINS, J. M., 1939, The growth of Kentucky Bluegrass and of Canada Bluegrass in late spring and in autumn as affected by the length of day. Jour. Am. Soc. Agron., 31, 767-74.

FROESE, H., 1938, Das Verhalten der Rhizome der Wiesenplatterbse (Lathyrus pratensis) unter wechselnden Versuchsbedingungen. (Behaviour of the rhizomes of Lathyrus pratensis under varying experimental conditions). Futterb. GarfBereitg., 337-50. (Tech. Hachsch. Danzig).

FRYER, J. R., 1930, Cytological Studies in Medicago, Melilotus, and Trigonella. Can. Jour. Res., 3, 3-50.

FUCHS, A., and ZIEGENSPECK, 1926, Entwicklungsgeschichte der Axen der einheimischen Orchideen und ihre Physiologie und Biologie. I Teil. Cypripedium, Helleborine, Limodorum, Cephalanthera. (Development of the axis of native orchids and their physiology and biology. Part I. C. H. L. C.). Bot. Archiv. (Konigsberg), 14 (3/4), 165-260, 19 pl., 5 fig.

GARVER, 1922, Alfalfa root studies. U. S. Ag. Bull. 1087, 1-28, bibliog., il.

GOETTE, W., 1931, Untersuchungen über die Beeinflussung des anatomischen Baues einiger Liliaceen durch Standortsfaktoren under experimentelle Eingriffe "Blausäure - Begasung." Beitr. Biol. Pflangen, (19(1), 35-66.

GRABER, L. F., NELSON, N. T., LUEKEL, W. A., and ALBERT, W. B., 1927. Organic food reserves in relation to the growth of alfalfa and other perennial herbacious plants. Univ. of Wisconsin Agric. Exp. Sta. Res. Bull., 80, 3-128, 35 fig.

GRABER, L. F., and REAM, H. W., 1931, Growth of bluegrass with various defoliations and abundant nitrogen supply. Jour. Am. Soc. Agron., 23, 938-44.

GRABER, L. F., 1931, Food reserves in relation to other factors in the growth of grasses. Plant Phys., 6, 43-71, il, bibliog.

GRABER, L. F., 1933, Competitive efficiency and productivity of bluegrass (Poa pratensis L.) with partial defoliations at two levels of cutting. Jour. Am. Soc. Agron., 25(5), 328-333.

GRANDFIELD, C. O., 1935, The trend of organic food reserves in alfalfa roots as affected by cutting practices. Jour. Agric. Res., 50, 697-709.

GRAY, A., 1908, New manual of botany. Seventh edition. P. 882.

HAAS, A. R. C., 1927, Effect of reaction of solution on growth of alfalfa. Bot. Gaz., 83, 207-211.

HANSEN, N. E., 1909, The wild alfalfas and clovers of Siberia, with a perspective view of the alfalfas of the world. U. S. D. A. Bull. 150.

HANSON, W. R., and STODDART, L. A., 1940, Effects of grazing upon bunch wheat grass. Jour. Am. Soc. Agron., 32, 278-289, bibliog. tabs., 4 fig.

HARRISON, C. M., 1931, The Effect of cutting and fertilizer applications on grass development. Plant Phys., 6.

HARRISON, C. M., 1939, Greenhouse studies of the effect of clipping the tops of alfalfa at various heights on the production of roots, reserve carbohydrates, and top growth. Plant. Phys., 14, 505-16, il., tabs.

HAYATA, B., 1929, A new theory on the construction of polycyclic steles. (In Japanese). Bot. Mag., (Tokyo), 43(511), 340-354, 52 fig.

HAYWARD, H. E., 1938, The structure of economic plants. The MacMillan Co., New York.

HEATH and LUCKWILL,

HILDEBRAND, S. C., and HARRISON, C. M., 1939, Effect of height and frequency of cutting upon consequent top growth and root development. Jour. Am. Soc. Agron., 31, 790-9, bibliog., il., tabs.

HOLLIDAY, E. M., 1932, Morphological, physiological, and cytological studies of alfalfa (Medicago). Thesis for the degree of Master of Arts in the Dept. of Botany, U. B. C.

HOLMS, T., 1925, On the development of buds on roots and leaves. Ann. Bot., 39, 867-881.

HOLMS, T., 1929, The application of the term "rhizome". Rhodora, 31, 6-17, 2 pl.

HOWE, M. D., 1931, Origin of leaf and adventitious and secondary roots of Gratopteris. Bot. Gaz., 92, 326-329.

JACKSON, B. D., 1916, A glossary of botanical terms with their derivation and accent. Duckworth and Co., London.

JOFFE, J. S., 1920, The influence of soil reaction in the growth of alfalfa. Soil Science, 10, 301-307.

JOHANSSON, K., 1929, Dubbla årsskott hos plataner (Adventitious shoots in Platanus). Svensk. Bot. Tidskr, 23(1), 145-148, 2 fig.

JONES, F. R., 1928, Winter injury in alfalfa. Jour. Ag. Res., 37, 189.

JURICA, H. S., 1924, A study in the grouping of plants. (Contribution from Hull. Bot. Lab. 326 with 14 fig.) Ann. Bot., 78, 326.

KALMYKOVA, A. A., 1935, Ob obl̄igatno - kornev̄ischchn̄ykh ī fakul'tatw̄no - kornevischchnykh raste - nuakh. (Plants with obligatory and facultative rhizomes) (In Russian with German Summary) Botanicheskīi Zhurnal S. S. S. R. (Bot. Jour. U. S. S. R.), 20(4), 411-413, 1 fig.

KANNENBERG, 1931a Ueber die Kraft verschiedener Gräser and Züchtungsformen von Grasern Zum Treiben von Auslaufern (On the capacity of different grasses and bred strains of grasses to produce rhizomes and stolens.) Pflangenbau, 8, 17-18.

KANNENBERG, 1931 b, Praktisch wichtige Eigenschaften der Wiesenrispe. (Valuable characters of Poa pratensis.) Grünl. u. Landesk, 8, Vumberg, 1.

KARRISNEV, R. V., 1937, (The effect of day length on development and tuber formation in Cyperus esculentus L.) Sovet. Bot. No. 2, 109-13 (U. S. S. R. Inst. of Special Crops, Moscow.)

KLAPP, E., and SCHUBART, E., 1931, Ueber Thüringer Luzerne. (On Thuringian lucerne). Pflanzenbau, 7, 353-65.

KLAPP, E., 1932 a, Einiges über Wüchsigkeit und Bevurzelung der geprüften Luzerne - Herkünfte in Jena - Swätzen. (Some observations on the growing capacity and root systems of the lucerne varieties tested at Zwätpen Jena). Mitt deuts. Landw Ges. 47, 208-9. (Anst. f. Pflanzenbau u Pflanzensucht. Jena).

KLAPP, E., 1932 b, Pflanz-und Wurzelsprossemluzerne (Tillering lucerne and lucerne which sends out rhizomes). Züchter, 4, 78. (Anstalt f. Pflanzenbau u. Pflanzenzucht. Jena).

KLINKOWSKI, M., 1932, Pflanz-und Wurzelsprossenluzerne. (Tillering lucerne and lucerne which sends out rhizomes). Züchter, 4, 35-9.. (Biolog. Reichsanstalt f. Land -u. Forstwirt-schoft. Berlin - Dahlem).

KLINKOWSKI, M., 1933, Lucerne: its ecological position and distribution throughout the world. Aberystwyth, Imp. Bureau of Plant Genetics, Herbage Plants. Bull. 12, pp. 65.

KLINKOWSKI, M., 1934, Die Eifelluzerne. Ein Beitrag Zur Kenntnis der deutschen Bastardluzernen. (The Eifel Lucerne. A contribution to the knowledge of the German hybrid lucernes). Pflanzenbau, 11, 172-9.

KOENEKAMP, A., and LEHMANN, U., 1933, 1st die "Wurzelsprossluzerne" eine Weidepflanze? (Is the lucerne which sends out rhizomes a pasture plant?). Züchter, 5, 28-30. (Inst. f. Grünlandw, Preuss. Landw. Versuchs - u. Farschungsanst. Landsberg (Warthe)).

KOENEKAMP, A., 1934, Beitrag zur Kenntnis des Wurzelwachstums einiger Klee- und Graserarten. (Root growth in some legume and grass species). Landw. Jb, 80, 571-89. (Director, Inst. f. Grunlandwirtschoft d. Landw. Versuchs - u. Forschungsanst. Landsberg (Warthe)).

KUHN, A. O., and KEMP, W. B., 1939, Effect of removing different proportions of foliage on contrasting strains of Kentucky bluegrass, Poa pratensis. Jour. Am. Soc. Agron., 31(10), 892-895, 1 fig.

LAUDER-THOMSON, I., 1933, The nodal anatomy of Spartina Townsendii. Trans. Proc. Bot. Soc. Edingb., 31, 262-8.

LEANDRI, J., 1928, Structure particuliere du rhizome d'un Daphne. (Structure of rhizome of Daphne). Bull. Soc. Bot., France, 75(3/4), 243-248, 6 fig.

LEANDRI, J., 1931, Observations sur les rhizomes a structure anormales de quelques especes africaines de Gnidia. Bull. Soc. Bot. France, 78 (5/6), 307-312, 6 fig.

LE BLANC, T., 1791, Experiments on the variegated Medick. Annals of Agriculture, 15, 279.

LE CLURG, E. L., and DURRELL, L. W., 1928, Vascular structure and plugging of alfalfa roots. Col. Exp. Sta., Bull., 339.

LESCHENKO, P. I., 1930, Lucerne naturally produced by means of rhizomes. Poltava Agr. Exp. Station, Plant Breeding Dept. Publication, 105. Visnik Prikladnoy Botanika, 2, 47-52.

MALAHOV, I. I., 1937, On the content of carbohydrates in roots of couch grass (Agropyron repens). (Contained in symposium). The problem of wheat - couch grass hybrids. Edited by N. V. Cicin, p. 205-7.

MARK, J. J., 1936, Relation of reserves to cold resistance in alfalfa. Iowa Ag. Exp. Sta. Res. Bull., 208, 304-35, il., tab., bibliog. (p. 333-5).

MAYER, M., 1939, Ökologisch - pflanzensoziologische Studien über die Filipendula Ulmaria - Geranium palustre Assoziation. Beitr. geobot. Landesauf. Schweiz., 23, 1-64, 2 pl.

McCANN, C., 1935, Some observations on Nymphaea pubescens. Willd. Jour. Bombay Nat. Hist. Soc., 37(4), 895-901, 2 pl., 1 fig.

McCORMICK, F. A., 1916, Notes on the anatomy of the young tuber of Ipomaea batatas Lam. Bot. Gaz., 61, 388-98, bibliog., il.

MIKI, S., 1927, Über den Verzweigungsmodus und die Blattanordnung des Rhizoms von Nelumbo nucifera Gartn. (Mode of branching and leaf arrangement of rhizomes of N. nucifera) Japanese with German summary). Bot. Mag. (Tokyo), 41, 522-526, 4 fig.

MOE, G. G., 1928, Alfalfa Studies: A preliminary study of the inheritance of certain morphological characters. Doctorate thesis.

MORGAN, M. F., 1930, Factors affecting the estimation of lime requirement from pt 1 values. Soil Science, 29.

MOROZOV, A. S., 1939, Storing of carbohydrates by forage grasses. C. R. (Doklady) Acad. Sci. U. R. S. S., 24, 407-9, (Inst. of Forage Crops).

OAKLEY, R. A., and GARVER, S., 1913, Types of proliferation in alfalfa. U. S. D. A., Circ., 115.

OAKLEY, R. A., and GARVER, S., 1917, Medicago folcata, a yellow flowered alfalfa. U. S. D. A., Bureau of Plant Industry, Bull., 428.

OLIVER, G. W., 1913, Some new alfalfa varieties for pastures. U. S. D. A., Bureau of Plant Industry, Bull., 258.

OSBORN, T. G. B., 1919, Some observations on the tuber of Phylloglossum drummondii. Ann. Bot., 33, 485, with plate XXVIII, and 43 fig. in the text.

PACZOSKIJ, J., 1917, Development of the root system of Cirsium arvense and Medicago sativa with reference to their vegetative reproduction, observations carried out in Russia. F. Berg. Int. R. Sci. and Prac. Ag., 8, No. 4, 563-5.

PETTINGER, N. A., Soil reaction and plant food availability. Fertilizer Review, Vol. 10, No. 5.

PRIESTLEY, J. H., and SWINGLE, C. F., 1929, Vegetative propagation from the standpoint of plant anatomy. U. S. Dept. Agric. Tech. Bull., 151, 98 p., 24 pl., 1 fig.

PURER, E. A., 1936, Growth behaviour in Convolvulus soldanella L. Ecology, 17(4), 541-550, 1 fig.

RASUMOV, V., 1931, Influence of alternate day length on tuber formation. Bull. Appl. Bot. Gen. and Plant Breeding, 27, 3-46.

RATHER, H. C., and DORRANCE, A. B., 1938, Study of the time of pasturing alfalfa. Jour. Am. Soc. Agron., 30, 130-4, tab.

RATHER, H. C., and HARRISON, C. M., 1939, Starch reserves in the roots of pastured alfalfa when grown alone and in mixtures. Mich. Ag. Exp. Quar. B., 21, 281-91, bibliog., il pls., tab.

SCAVONE, G., 1939, Possibilita e convenienza della coltivazione della ramia. (Opportunity and requirements for the growing of ramie). Italia Agric., 76(5), 327-336.

SCHUBART, E., 1931, Beobachtungen an Luzernewurzeln verschiedener Herfunfte. (Observations on the roots of lucerne of different origins). Pflanzenbau, 8, 137-41.

SILKETT, V. W., 1937, Effect of late summer and early fall cutting on crown bud formation and winter hardiness of alfalfa. Jour. Am. Soc. Agron., 29, 53-62, tab.

SIMONDS, A. O., 1935, Histological studies of the development of the root and crown of alfalfa. Iowa St. Coll. J. Sci., 9, 641-59.

SMELOV, S. P., and MOROZOV, A. S., 1939 a, Dynamics of reserve carbohydrates in the chief perennial grasses. Sovet. Bot., 5, 18-30. (U. S. S. R. Inst. Fodders, Lugovaja).

SMELOV, S. P., and MOROZOV, A. S., 1939 b, On the localization of reserve plastic substances in perennial grasses. Bot Z, 24, 157-65 (English summary 165). (U. S. S. R. Inst. Fodders, Lugovaja).

SMITH, C. M., 1931, Development of Dionaea muscipula. Germination of seed and development of seedling to maturity. Bot. Gaz., 91(4), 377-394, 36 fig.

SOUTHWORTH, W., 1921, A study of the influence of the root system in promoting hardiness in alfalfa. Sci. Agric., 1, 5-9.

SPRAGUE, H. B., 1933, Root development of perennial grasses and its relation to soil conditions. Soil Science, 36, 189-209.

STOUGHTON, R. H., and HOLE, D. R., 1937, Photoperiodic after-effect. Nature, 140, 808.

STURKIE, D. G., 1930, The influence of various top cutting treatments on rootstalks of Johnson grass (Sorghum halepense). Jour. Am. Soc. Agron., 22(1), 82-93.

SUMPSTINE, W. J., 1931, Origin and development of the tissues in the rhizome of Onoclea sensibilis. Proc. W. Virg. Acad. Sci., 5(2), 37-42.

SYLVEN, N., 1934, Der Einfluss de Schnittzeiten auf die Leistungsfähigkeit und Wintorfestigkeit der Luzerne. (The effect of the time of cutting upon productivity and winter hardiness in lucerne). Rep. 3rd Grassl. Conf. Zürich, 237-49, (English summary 247-9), Svalof, Sweden.

TAYLOR, G., 1926, The origin of adventitious growths in Acanthus montanus. Trans. and Proc. Bot. Soc., Edinburgh, 29(3), 291-296, 2 pl.

THODAY, D., 1926, The contractile roots of Oxalis incarnata. Ann. Bot., 40, 571-584.

THODAY, D., 1931, The mechanism of root contraction. Plant Phys., 6, 721-725.

THODAY, D., and WOODHEAD, N., 1932, Studies in growth and differentiation. II A preliminary survey of the morphology and anatomy of Kleinia articulata Haw. Ann. Bot., 46 (183), 671-682, 1 pl., 4 fig.

THORNTON, H. G., and NICOL, H., 1934, Some effects of clipping the tops upon the root development of lucerne (Medicago sativa L.). Jour. Ag. Sci., 24, 532-9, bibliog., tab.

TUCKEY, I. H., 1940, Seasonal development of roots of several grass species pasture. Am. Jour. Bot., 27, 195.

TULL, J., 1733, Horse hoeing husbandry. London, p. 92-101.

TYSDAL, H. M., and KIESSELBACH, T. A., 1939, Differential response of alfalfa varieties to time of cutting. Jour. Am. Soc. Agron., 31, 513-19, tab.

TYSDAL, H. M., and WESTOVER, H. L., 1937, Alfalfa improvement. Yearbook U. S. Dept. of Agric., pp. 1122-53.

UFER, M., 1933, Beobachtungen über Bastarde Zwischen Medicago gaetula Urb., und M. falcata L. (Observations on hybrids of M. gaetula Urb., and M. falcata L.). Züchter, 5, 49-53. (Kaiser Wilhelm Inst. f. Züchtungsforsch. Müncheberg i. M.).

VARGA, L., 1933, Sonderbare Ringbildungen von Potamogeton pectinatus L. im Ferto (Veusiedlersee). Internat. Rev. Ges. Hydrobiol. u. Hydrograph, 28($\frac{3}{4}$), 285-294, 3 fig.

VLADIMIROV, I. F., 1937, (Falcaria vulgaris Bernh. a rhizomatous weed). Sovet. Bot., 1,150-4. (Agric. Exp. Sta. of Agronomy, Bezenchuk).

VYSOTSKII, G. N., 1928, Sympodial rhizome-farming patch growths. Mem. Soc. Nat. d'Odessa, 44, 37-54, 16 fig.

WATENPAUGH, H. N., 1936, The influence of the reaction of soil strata upon the root development of alfalfa. Soil Science, 41(6), 449-462, 3 pl.

WATKINS, J. M., 1940, Growth habits and chemical composition of bromegrass (Bromus ivermis Leyss.) as affected by different environmental conditions. Jour. Am. Soc. Agron., 32, 527-38, bibliog., tabs.

WEAVER, J. E., 1930, Underground plant development in its relation to grazing. Ecology, 11,543-557.

WEINMANN, H., 1940, Storage of root reserves in Rhodes Grass. Plant Phys. 15, 467-84, bibliog., il., tabs., graphs.

WERNER, H. O., 1934 a, Effect of temperature, photoperiod, and nitrogen level upon tuberinzation in the potato. Am. Soc. Hort. Proc., 32, 507.

WERNER, H. O., 1934 b, The effect of a controlled nitrogen supply with different temperatures and photoperiods upon the development of the potato plant. Nebraska Agr. Exp. Sta. Res. Bull., 75, 132 p.

WETTSTEIN, W. VON, 1938, Über die Züchtung von Helianthus tuberosus (Tapinombur). (On the breeding of Helianthus tuberosus). Zücher, 10, 9-14. (Kaiser Wilhelm-Inst. f. Züchtungsforsch, Müncheberg, Mark.).

WICK, H., 1932, Ueber Lunzerneanbau unter besonderer Berücksichtigung der Sortenfrage. (On the growing of luzerne, with special reference to the question of variety). Mitt. dents. Landw Ges, 47, 205-8, (Berlin).

WILLARD, C. J., 1930, Root reserves of alfalfa with special reference to time of cutting and yield. Jour. Am. Soc. Agron., 22(7), 595-602.

WILLE, F., 1926, Beiträge Zur Anatomie des Cyperaceenrhizoms. (Anatomy of the rhizomes of sedges). Beih. Bot. Centralbl. 1 Abt., 43(3), 267-309, 4 pl.

WILSIE, C. P., AKAMINE, E. K., and TAKAHASHI, M., 1940, Effect of frequency of cutting on the growth, yield, and composition of Napier grass (Pennisetum purpureum). Jour. Am. Soc. Agron., 32(4), 266-273.

WILSON, C. L., 1927, Adventitious roots and shoots in an introduced weed. Bull. Torrey Bot. Club, 54(1), 35-38, 2 fig.

WILSON, O. T., 1913, Studies on the anatomy of alfalfa. Kansas Univ. Sci. Bull., 7, 291-299.

WINTER, C. W., 1932, Vascular system of young plants of Medicago sativa. Bot. Gaz., 94, 152-67.

WOODCOCK, E. F., 1932, The penetrating power of the rhizome of quack grass, Agropyron repens (L.) Beauv. Papers Mich. Acad. Sci. Arts and Letters, 15, 179-182, 2 pl.

ZADE, A., Experiments with grasses and legumes conducted at the Institute for crop production and plant breeding of the University of Leipzig. Supplement Herbage Abstract.

ZIEGENSPECK, H., 1927, Des anomale Dickenwachstum des Rhizomes von _Hedysarum comosum_ als Ursache eiver auffälligen Kontraktilität. (Anomalous thickening of the rhizome of _H. comosum_ as the cause of exceptional contractility). Bot. Arch., 19(5/6), 344-348, 2 fig.

ZIMMERMAN, P. W., and HITCHCOCK, A. E., 1938, Modified storage organs in _Helianthus tuberosus_. Contrib. Boyce Thompson Inst., 10(1), 1-3, 1 fig.