THE STRUCTURE AND GROWTH HABIT OF WHITE CLOVER IN RELATION TO ITS PERSISTENCE

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THE STRUCTURE AND GROWTH HABIT OF WHITE CLOVER IN RELATION TO ITS PERSISTENCE.

University of New Hampshire, Ph.D., 1966
Agriculture, plant pathology

University Microfilms, Inc., Ann Arbor, Michigan
THE STRUCTURE AND GROWTH HABIT
OF WHITE CLOVER IN RELATION TO ITS PERSISTENCE

BY
HSUEH-SHUN CHOW

A THESIS
Submitted to the University of New Hampshire
In Partial Fulfillment of
The Requirements for the Degree of
Doctor of Philosophy

Graduate School
Department of Botany
May, 1966
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ACKNOWLEDGEMENTS

The author is indebted to Dr. A. E. Rich, Dr. R. A. Kilpatrick, and Dr. G. M. Dunn for their consistent encouragement and advice throughout this work.

The author wishes to express his appreciation to Dr. C. G. Nast, for her valuable suggestions on the morphological and anatomical phases in this work. Also to Dr. B. C. Staugaard, for his assistance with the photographic work.
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INTRODUCTION

As one of the most important pasture plants, white clover provides feed of high nutritive value, and also, it is exceedingly attractive to most animals. In addition, this species consistently benefits the growth of the associated grasses by adding nitrogenous substances to the soil through the nitrogen-fixing bacteria in the nodules of its roots.

White clover has a world wide distribution. It is generally agreed that white clover is indigenous to all the countries of Europe and many parts of Asia and Africa. It was introduced into Australia and New Zealand. There is some disagreement about its status as native or alien in the western hemisphere. However, the cultivated types, at least, were brought to this continent by the early settlers. It is distributed throughout the United States and Canada, and also in some parts of South America.

The cultivated white clover has three different types, the common or Dutch white clover, the intermediate, and the Ladino white clover. All belong to *Trifolium repens* L. The Ladino clover, originated in northern Italy, is a giant mutation from the wild type both in its morphological and anatomical features. Since Ladino clover is the most productive and widely distributed in the United States, it was the source of samplings for this research.

Rapid depletion of white clover stands has posed a great problem for the agronomists. It is generally agreed that white clover is most productive in the year after seeding
and less productive the following year. After the third year it varies considerably, but usually it becomes less and less productive until it disappears from the pasture. It appears that lack of persistence of this plant has become more serious in recent years. The acreage has declined steadily. According to Crowder's* survey, the shipment of seed into the 12 north-eastern states has dropped from 997,000 pounds to 138,000 pounds in the past decade.

Many efforts have been made to improve persistence, but the problem remains. Some basic research must be done before any substantial progress can be made.

The purpose of this research is to study the external and internal structure of the plant and its growth habits, to find out if they have any significant effect on persistence.

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LITERATURE REVIEW

Root and Crown rot

Among the many factors which have been reported to affect the persistence of white clover, root and crown rot have attracted the most attention. Rotting roots and crowns especially, the rot of the taproot, are the first symptoms of a dying plant.

The short life of the taproot was noted during the last century. Hayes (21) reported that the taproot of white clover is relatively small compared to the taproots of other clovers, and it died within one or two years. Westbrook and Tesar (43) reported from Michigan that some taproots died in the first production year, and the percentage of dead roots increased with the age of plants until all taproots died before July of the second production year. In New Hampshire, Kilpatrick and Dunn (26) recorded that the discoloration and rotting of taproots appeared in the fall of the first year and developed rapidly the next year. They found no living taproot 24 to 26 months after seeding.

The causal factors of rotting appear to be extremely complex. Many organisms have been isolated from the decaying tissue. Westbrook and Tesar (43) found that Fusarium oxysporum Schlect, F. solani Mart, and Rhizoctonia solani Kuhn were the most important fungi associated with the decayed tissue, the percentage of recovery being 48%, 6%, and 19%, respectively. Kilpatrick and Dunn (25) isolated some 39 fungi from the aerial and underground decaying parts of the plants. In another report, Kilpatrick and Dunn (26) found that Fusarium oxysporum constitutes 64% of the total fungal isolates. The remaining isolates
were distributed in an extremely wide range.

The general agreement among various workers on the occurrence of the associated fungi, plus pathogenicity tests of seedlings indicate that *Fusarium* spp. are the leading causal organisms of this disease. Recent pathogenicity tests by Moody (28) using older plants, produced similar results. However, as Young (45) pointed out 40 years ago: "Some Fusaria were weak parasites, the infection required cooperation from deleterious agencies to induce fatal results." But for some reason, the search for the "deleterious agencies" did not get under way until the last decade.

The Natural Pests and Their Possible Role as Predisposing Factors to Root Rot

Kilpatrick and Dunn (26), in New Hampshire, found numerous larvae of *Sitona* spp. in soil around the clover roots and suggested that these insects might have some significance in predisposing clovers to the rot-inducing organism. In a later report (13), they did find some improvement in the forage yield and persistence through insect control and soil fumigation, but no significant correlation was found between the insect population, injury and root rot. They suggested that the improvement of yield and persistence were due to the pest control. Graham, et al, (19) also found that insecticides were of prime importance in maintaining the original plants for four seasons. Schillinger and Leffel (34) directed some field research on the nature of persistence and made the following suggestion: "The lack of persistence of Ladino clover is an entomological-pathological complex with particular emphasis on
the entomological aspect." The fact that the insecticides may improve the persistence and increase the forage yield to a certain degree but fail to have significant control over the rotting process seems to indicate that insect injury has a direct effect on plant growth rather than acting as a pre-disposing factor to root and crown rot.

Baxter and Gibson (2) found that root-knot nematodes markedly reduced the persistence of Ladino clover. Halpin (20) found that when root-knot nematodes were combined with Rhizoctonia solani, Fusarium roseum Link, F. oxysporum or Microspora sp., the resulting pathogenic effect was greater than for any of the constituents alone.

Chapman (10) found that the cyst nematodes (Heterodera trifolii Goffart) reduced the dry weight of the white clover in his experiment 25-50% of the control. However, he made no effort to relate his results with root and crown rot.

Probably viral infection is still the most important disease on the above-ground portion of the plant. Kreitlow (27) found the incidence of viral infection increased progressively from 0 to an estimated 95% over a 3-year period. The yield declined 40-60% during the second and third year.

A very interesting virus-fungal interrelationship in red clover was found by Watson and Guthrie (40). The severe root rot developed only when the plants were infected with clover yellow mosaic virus (CYMV). The parasitic fungi isolated from the rotting roots were F. oxysporum, F. roseum and Tetracoccosporium paxianum Szabo. They thus suggested that these fungi were weak parasites; their virulence depends heavily upon
the condition of the host; i.e., the clover was weakened by viral infection and became susceptible to this group of weak parasites.

Besides root rot, some other disease may also play a role in affecting persistence. Albrecht (1) reported that diseases were responsible for most failures of clovers during the summer in Alabama. Some 10 diseases were recognized, with summer blight, whose causal organism is Sclerotium rolfsii Sacc., the most destructive.

The Effect of Field Practice

As a pasture crop, white clover is usually grown in a mixture with grasses; and sometimes with weeds. A severe competition among the components is going on constantly. Any field practices which encourage the clover and/or inhibit the grasses and weeds have often been employed to improve or maintain the clover stand.

Brown and Munsel (7) suggested that mowing the forage 2 inches above the ground resulted in better stands and a larger yield of dry matter and protein than cutting at a height of 4 or more inches. Tesar and Ahlgren (39) preferred cutting the plants to a height of 3½ inches four times a year. They claimed that "This treatment appeared to simulate the type of grazing management necessary to maintain a high production of Ladino clover over a period of years." However, Robinson et al (31), Gervais (15), and Jeffers (22) all favored the lower cutting. It has become a general practice in management of clover pastures to cut as close as 1-1½ inches from the ground. It
gives a higher fraction and total yield of clover and maintains a desirable balance between the grasses and clover. In contrast the higher cutting encourages the grasses and thus inhibits clover indirectly. Cutting closer than 1 inch may still increase the clover yield but it seems too drastic for maintaining a solid sod.

Fertilization is another field practice which has often been employed to maintain a good stand of clover. It is generally agreed that annual application of nitrogen has a detrimental effect on clovers. (30, 31, 36, 37) However, an annual application of potash appeared to be the most effective practice for maintaining a good stand of white clover (6, 30, 37, 42).

Stewart and Bear (37) explained that "Because of its shallow root system, Ladino needs a readily available supply of potash in the plow depth of soil. Heavy initial applications are likely to become leached beyond the reach of the roots." According to Blaser and Brady (4) the rapid plant succession in the pasture was due to nutrient competition. They suggested that a nitrogenous fertilizer increased the growth of grasses, and thus increased the amount of potash absorbed by grasses. Therefore, it resulted in deficiency of potash in legumes, and eventually decreased the growth of the leguminous plants in a mixed association.

Reports on the effect of irrigation on the persistence of white clover are inconsistent. Tesar et al (40) found that irrigation helped to maintain a better stand; and Robinson et al (31) suggested that irrigation was only a minor factor. Apparently, the results have been modified by other factors:
the associated grasses, the pathogenic organisms, etc. In
general, clover has a relatively smaller root system than
grasses; thus high soil moisture usually favors the growth of
clover.

The Significance of Its Branching and Rooting System

Though white clover is one of the most important pasture
crops, very little work has been done on the morphological as-
ppect of this plant. Erith (14) appears to be the only one who
has ever made a comprehensive investigation. Although she made
no attempt to approach the problem of persistence, some points
she mentioned seem to have great significance in understanding
this problem.

On the root system, she indicated: "It (the adventitious
roots) appears to be in direct connection with the axillary
branch and probably functions more particularly for this rather
than for the main stolon." On the stem system, she noted: "In
plants that are two or more years old, the connection between
some of the primary stolons and the parent plant is severed by
the rotting of the basal portions of the former. These stolons,
which bear adventitious roots at their distal nodes then lead an
independent existence."

Plant breeders have long tried to find some morphological
characteristics as indicators for persistence. Knight (24) found
that persistent clones and their progenies produced a large num-
ber of stolons. In fact, the density of stolons has been used by
most workers as an indicator for measuring the persistence of
white clover. Dunn, et al (12) also found highly significant
correlation coefficients between recovery and density of stolons in the fall.

Gibson, et al. (17) and Beinhart, et al. (3) found two morphologically contrasting types of white clover, designated as viney and non-viney. The non-viney plants which branched more frequently than those of the viney plants were found to be superior in both persistence and forage production. They suggested that the branching frequency of a clone may be a useful predictor of its persistence potential. Since high frequency of branching will result in high density of stolons, the two characteristics may mean the same thing or are highly correlated.

There is an interesting relationship between the tap-root and the adventitious roots. Although Stewart and Bear (37) found that the minerals taken up by the adventitious roots can be translocated to both basal and terminal portions of the plant, virtually all of them were translocated to the terminal portion. They also stated that when the adventitious roots were prevented from growing on stolons, a large taproot system was formed. The taproot system tended to deteriorate in direct proportion to the adventitious roots which were allowed to grow. This concept was confirmed later by Nelson and Brady (29). They supplied potash to the basal part of the plant and the potash was found to be effective in maintaining growth in both the basal and terminal part of the plant. When they supplied potash only to the terminal part of the plant it maintained only the terminal part of the plant. They thus concluded that potash was not readily translocated from the terminal portion to the basal portion through the stolon.
The Effect of Flowering

Gibson (16) studied the effect of flowering on the persistence of white clover and found that under normal daylight, the non-flowering plants persisted longer than the profusely flowering plants. However, this did not hold true under extended light periods. Calder (8) found that the growth of stolons was most rapid on sterile plants, less rapid on intermediate flowering plants, and slowest on profusely flowering plants. Brigham and Wilsie (5) found a $r=+29$ between seed setting and forage yield and suggested that high yield of both forage and seed are not incompatible. Dunn, et al (12) found a negative correlation between numbers of flowers and recovery. However, they still questioned whether low flowering lines of Ladino clover would significantly improve persistence.

The Effect of Winter Injury

According to Ruelke and Smith (33), the species resistant to lower temperatures were able to maintain a higher level of total sugar content. Wood and Sprague (44) found that hardier varieties of Ladino clover were higher in most carbohydrates than were the non-hardy varieties. It is interesting that Westbrook and Tesar (43) also found a striking contrast in total available carbohydrates between the first and second winter. According to Ronningen (32) there was a highly significant negative correlation between winter injury and plant density.
MATERIALS AND METHODS

The Greenhouse Culture of Sampling Plants

Certified Pillgram Ladino white clover (F. C. 34628) was used for this study. Seeds were planted in a 4 x 12 ft. bench, spaced six inches apart, November 20, 1964. The soil was fully cultivated and mixed with lime and 5-10-10 fertilizer at the rates of 2,000 lbs/acre and 1,500 lbs/acre, respectively. The temperature in the greenhouse was approximately 75°F. Watering and weeding were done whenever necessary.

For the investigation of internal breakdown of white clover, the samples were collected from a pasture at the corner of U. S. Rte. 4 and 155A. The clover was seeded 1½ years previously and the stand was moderately good.

The Preparation of Permanent Slides

The procedure of making permanent slides basically followed Johansen's (23) paraffin method. However, due to the size of the sample and presence of fiber in the roots, some modifications were made. Bandel and Presger's personal suggestion of softening the embedded tissue before cutting was very helpful.

Collection of Sample: After the samples had been harvested, they were cleaned thoroughly with plenty of water. The size of sample was 1-2 cm long; some of the crowns and taproots were split into halves.

Fixation: The samples were fixed in Graf III for at least 48 hours. This fixative consists of two solutions: A and B
which are mixed immediately before use. Solution A contains 3 parts of 1% aqueous chromic acid and 2 parts of 10% aqueous acetic acid. Part B consists of formaldehyde and distilled water in the ratio of 1 to 4.

**Dehydration:** After washing with 50% alcohol three times, the samples were dehydrated in the series of tertiary butyl alcohol (T. B. A.) as shown below:

<table>
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<th>Components</th>
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<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
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<tr>
<td>T. B. A.</td>
<td>10%</td>
<td>20%</td>
<td>35%</td>
<td>55%</td>
<td>75%</td>
<td>100%</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>40%</td>
<td>50%</td>
<td>50%</td>
<td>45%</td>
<td>25%</td>
<td>0</td>
</tr>
<tr>
<td>Water</td>
<td>50%</td>
<td>30%</td>
<td>15%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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Four to 12 hours were allowed for each change. Finally, three changes of 100% T. B. A. completed the dehydration process.

**Infiltration and Embedding:** Material was infiltrated with 50-52 C melting-point paraffin, slowly and in a low temperature oven (40 C). It was then transferred to a higher melting-point paraffin (56-58 C) and placed in an oven kept at 60 C. This paraffin was replaced with Tissue Mat and then embedded.

**Softening:** Because fibers caused difficulty in sectioning the tissue, it was necessary to soften the embedded material by exposing one surface to a softening solution. The solution found most effective was a mixture of 10 ml glycerol, 1 g of Drift detergent, and 90 ml of water. The blocks needed to be soaked in this solution for approximately two weeks. The time for softening was often reduced to 4-5 days by placing the vial with the wax blocks and softening solution on top of the paraffin oven.

**Sectioning:** All the material was sectioned on a rotary
microtome at 15 µ thickness except the root segments which were often cut at 25 µ. The stainless steel blades were found more satisfactory than the ordinary ones.

**Mounting:** Haupt's adhesive was used for mounting the sections on the slide. Four percent formalin solution was used to flood and straighten the ribbons. The slides were placed on the warming plate (38-40°C) overnight.

**Staining:** The material was stained with safranin and fast-green. After the slides were brought down to 50% alcohol, they were stained with 1% safranin in 50% alcohol for at least two hours or overnight, washed with water, and dehydrated with 95% alcohol. They were counter-stained with a few drops of fast-green for about 10 sec., rinsed with absolute alcohol, and washed twice with pure xylene, then mounted in balsam.
The Response of Stolon Growth to The Removal of Taproot or Adventitious Root

Selection of plants: Twelve plants from the sampling bench in the greenhouse were selected and labeled. They had well developed main stolons bearing at least one branch stolon. This investigation was centered on the weekly growth of the main stolon and one of its branch stolons. The recording of the amount of growth started on April 3, 1965, three weeks before treatment. The pretreatment growth rates were measured and recorded to determine the normal growth rate and to aid in selection of uniform plants for each treatment.

Treatments: There were three treatments for these plants. Four plants in treatment A were control plants. They received no treatment. The four plants of treatment B were chosen for detecting the effect of the taproot on growth of primary and secondary stolons. The adventitious roots were cut off from the node. The plants of treatment C were used for detecting the effect of adventitious roots. The main stolons were cut off just below the node. Treatments were performed on April 24, 1965.

Method of recording: The weekly growth was recorded for eight weeks, three weeks before treatment and five weeks after treatment. Tooth picks, used as markers, were placed in the ground each week, adjacent to the tips of the stolons. By the end of eight weeks all the distances between tooth picks were measured and recorded.
The Intervention of Adventitious Roots on Petal-basal Carbohydrate Translocation

Experimental plants and location: In order to reduce the genetic heterogeneity of plant materials, the seeds of a single cross between a solid green plant # 24849 and a filled-V plant were used. They were seeded on March 30, 1959, in the greenhouse, and later transplanted to the field near the Agronomy Field Station, Durham, N. H., in July, 1959.

There were 64 plants arranged in four rows with a distance of four feet between rows and three feet between plants. The area was limed at the rate of about 1500 lb. per acre prior to transplanting and top-dressed once in August with 0-15-30 fertilizer about one tablespoonful for each plant.

Treatment: There were four treatments in this experiment (Fig. 1).

A. Inserting aluminum foil between the stolons and the ground to prevent all the adventitious roots from developing.
B. Same as A but only in the central area about 8 inches in diameter and allowing the remaining stolons to root.
C. Cutting off all stolons from the crown.
D. Control.

The treatments were completely randomized. There were 16 plants for each treatment. Six plants from each treatment were dug in November, 1959, and the stolons were cut off from the crown as closely as possible. The crown and taproots were cleanly washed and allowed to air dry about 10 minutes. After the fresh weight and visual rating for root rot were taken for each plant, these center portions of plants, including crown
and taproot system were used for carbohydrate analysis.

**Carbohydrate Analysis**

**Extraction:** A 6.5 g sample of fresh root and crown was cut into small pieces and extracted in boiling 95% alcohol according to the Waring blender method of Sullivan \(^38\), Section The alcohol extract was diluted to 100 ml. Ten ml were used to determine dry weight and the rest was used to determine free sugars and sucrose. The alcohol insoluble portion was used for starch determination.

**Clarification and sugar determination:** The alcohol extract was clarified according to the same guide of Sullivan \(^38\), Section B. Ten ml of this clarified solution were used for total sugar determination. The Shaffer and Hartmann \(^35\) procedure was followed. Another 10 ml of this solution were used for free sugar determination. The same procedure \(^35\) was followed except incubating with invertase.

**Starch determination:** Sullivan's guide \(^38\), Section E was closely followed except the final volume of the solution was 1000 ml instead 100 ml.
Fig. 1 A sketch of the treatments for carbohydrate translocation experiment.

A. Formation of all the adventitious roots was prevented by aluminum foil.

B. The central area of eight inches in diameter had no adventitious roots.

C. All the stolons were cut from the taproot.

D. Control.
RESULTS

The white clover plant has been divided into two systems. The primary system includes the taproot, the crown and the main stolons. The secondary system includes the adventitious roots, the secondary stolons and the nodes from which the secondary stolons and the adventitious roots arise.

Primary System

The taproot: The taproot system is developed from the primary root of a seedling. The primary root is bounded externally by a piliferous layer and internally by a relatively wide cortex and a well defined triarch stele. During the subsequent growth of the seedling, the primary root increases considerably in length, and numerous branches are developed, thus forming a typical taproot system. From the standpoint of persistence, the primary root of a seedling may not be of any significance, because the root rot or deterioration usually occurs after the secondary growth has taken place.

Compared to other clovers, white clover has a rather small taproot, rarely reaching a foot in length. They bear several primary branches and numerous secondary branches.

As a result of the secondary growth in thickness, the primary cortex becomes stretched, ruptured, and finally sloughed off and replaced with periderm.

The secondary tissue, (Fig. 2) is well developed. The only unusual feature about the internal structure of the taproot is the abundance of fibers.

From the studies of the rotted taproots (Figs. 3,4), the
Fig. 2 A transverse section of a young white clover taproot.
Fig. 3 The transverse section of a slightly rotted tap-root. Notice the deteriorating parenchymatous cells beneath the periderm.
Fig. 4 A transverse section of a severely rotted taproot. Notice the loss of parenchymatous cells and rays inside the periderm and destroyed rays.
parenchymatous cells are most susceptible to destruction. Actually, only the parenchyma tissue has readily available foods for the rot-inducing organism. But this should not lead one to assume that plants having more parenchymatous cells would be more susceptible to rot. In fact, the young plants have a much higher proportion of parenchyma than the older ones; but, under ordinary conditions, the young plants are highly resistant to the rot-inducing organisms. Apparently, the physiological activity of the plant tissue seems to play a more important role than the structure of the tissue.

The investigation of the internal breakdown of the taproot was limited by the available material. Out of 58 taproots found in the middle of October, 1964, only six plants had visible internal breakdown. However, by June, 1965, only a few taproots were found, none of which had any visible sign of internal breakdown.

The anatomical studies on breakdown of the tissue seem to confirm the previous assumption (11) that the internal breakdown was more a physiological problem rather than a pathological one which involved living organisms. The breakdown of tissue in the center of root (Fig. 5), started just below the crown, developed downward a few inches but never extended into branch roots nor reached the end of the main root. The development of internal breakdown seems to parallel the age of the tissue. The failure to find any external origin seems to provide additional support for the physiological concept.

The crown: Morphologically, the crown of white clover is the lower half of a condensed main stem from which the tap-
Fig. 5 A transverse section of a taproot showing the internal breakdown in the center.
root penetrates downward into the soil, and stolons grow out in all directions. The upper half of the main stem is bent almost at right angles and gives an appearance of another stolon (Fig. 6). It produces a relatively large number of leaves arranged alternately and in two ranks. The stolons are not arranged in two opposite rows; instead, they arise from the leaf axils at various angles, thus resulting in a radiating growth from the crown. From this structure, the crown would be able to produce an indefinite number of stolons, but usually not more than ten are produced. Apparently the limiting factor is environment. When the old stolons are cut off, the new stolons will bud and replace them.

The crown (Fig. 7) closely resembles the stolons except for the very short internodes and the relatively large diameter of the pith. The parenchymatous pith functions presumably for storage.

The development of crown rot closely follows the rot of the taproot, but at a much more rapid pace. The possibility exists that direct infection of the crown is through the heat injury by the midsummer sun. It seems to prevail especially in the greenhouse. However, the rotting usually starts from the root, proceeds into the crown toward the base of the primary stolon, and produces a bare space in the center of the plant (Fig. 8).

The Main Stolon: The stolons have a characteristic monopodial growth. They creep along the ground with alternating leaves in two ranks.

The stolons have relatively long internodes and slightly
Fig. 6 The main stem of a young plant. Notice the new stolons are budding from the upper portion of the main stem after the old stolons are cut off. (N.S. = new stolons; E.S. = excised primary stolons).
Fig. 7  A longitudinal section of a crown showing developing stolons.
Fig. 3 A. The deterioration of a white clover plant.

Fig. 8 B. The same plant with center recovering after all its stolons have been cut off.
swollen nodes. The number of nodes and the length of stolons vary considerably. The age of the stolon, the available space, the moisture and fertility of the soil are the major reasons for this variability.

The internal structure of the main stolon is more complicated than the root. It includes: an epidermis, a cortex of assimilating parenchyma, a medulla of parenchyma, and a vascular system of a variable number of bundles (Fig. 9). Later a periderm and secondary vascular tissue develop.

The density of living stolon population has often been used as an index for measuring the stand of this plant. Actually, the factors which contribute to the density of live stolons are: (a) the number and length of primary stolons; (b) the number and length of secondary and tertiary stolons; and (c) the rate of rotting. In other words, the density of stolons is decided by the balance of the new growth and the rotting. Any means which may encourage the new growth or slow down the rotting process would help to maintain a better stand. However, the control of root or crown rot has not yet given us any promise of success. Arising from the nodes of a primary stolon, the secondary stolons may establish an elaborate secondary system, which may survive independently after the primary system has disappeared. It seems to have the greatest potential to solve the persistence problem of white clover.

The Secondary System

The secondary system is initiated at the nodes of the primary stolon. The node behaves like a crown but it produces
Fig. 9 Transverse section of a stolon of white clover.
only one secondary stolon and one adventitious root. A comparison between the primary system and secondary system follows:

<table>
<thead>
<tr>
<th>Primary System</th>
<th>Secondary System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stems</td>
<td>Crown or main stem has many nodes condensed</td>
</tr>
<tr>
<td>Stolons</td>
<td>Stolons with long internodes</td>
</tr>
<tr>
<td>Roots</td>
<td>Taproot</td>
</tr>
<tr>
<td></td>
<td>Adventitious roots</td>
</tr>
</tbody>
</table>

Since the secondary system originated at a single node of the primary stolon, it never attains the size of the primary system. The significance of the secondary system is its capability of leading an independent existence after the primary system has rotted and died out.

The adventitious root is located at the node of the main stolon and lateral to the secondary branch (Fig. 10). The anatomic studies of the initiation of adventitious root indicates that it originates from parenchymatous tissue between two vascular bundles of the stolon. One of the vascular bundles is a bud trace and the other is a cauline bundle of the stolon (Fig. 11). The parenchymatous tissue on each side of the central leaf trace is able to initiate adventitious roots, and although usually only the one on the lower side does so. However, occasionally each area produces a root, thus two roots are formed at a node (Fig. 12).

In order to establish the independent nature of the secondary system, an investigation was conducted to detect the response of stolon growth by severing the connections with the primary system or the adventitious roots. The results are shown in Fig. 13.
Fig. 10 The adventitious root and the secondary stolon arise at the node of a primary stolon. Notice that the adventitious root is connected mostly on the side of secondary stolon.

(P.S.=Primary stolon, P.=Petioles, S. S.=Secondary stolon, A. R.=Adventitious root)
Fig. 11 The relative position of an adventitious root of white clover to the median leaf trace (M LT) and bud trace (BT).
Fig. 12 Both sides of the branch stolon give rise to an adventitious root.
Fig. 13 The response of stolon growth to the removal of their primary or adventitious roots. Treatment A—Control, Treatment B—The primary stolons were cut off from the primary system, Treatment C—The adventitious roots removed from the node.
When the primary stolons were cut off just below the node, the growth of the tip portion of the primary stolon slowed down sharply in the first and second week, and ceased entirely after the third week. On the other hand, the growth of the branch stolon, supported by the adventitious root, slowed down only slightly. This indicated that the branch stolon, supported by its adventitious root, constituted a somewhat independent system. When the adventitious roots were removed from the nodes, the original taproot gave its support to both main and branch stolons, but the growth of branch stolons, lacking the support from the adventitious roots, was much less than the main stolon. This is also another indication of the independent nature of the secondary system.

However, not every node can produce a secondary system. Some axillary buds of the nodes give rise only to inflorescences. Since each node has only one axillary bud, the formation of an inflorescence is actually at the expense of a branch stolon. Consequently, the profusely flowering plants result in a few primary stolons with very few secondary stolons, while the nonflowering or sparsely flowering plants have many secondary and tertiary stolons (Fig. 14). Furthermore, the growth of inflorescence seems so dominant that the main stolons lag behind, with the young inflorescences leading the growth. (Fig. 15). Apparently the vegetative growth is markedly reduced.
Fig. 14  A comparison between the primary stolons of a profusely-flowering plant (above) and a sparsely flowering plant (below). The foliage has been removed to show the branching and rooting. Inflorescence stems (IS); Petioles (P); secondary stolons (SS); adventitious roots (AR).
Fig. 15 A transverse section of an inflorescence bud enclosed by its subtending leaf.
The Intervention of Adventitious Roots
In The Translocation of Organic Foods

The above studies have clarified the fact that the adventitious root gives its major support to its respective branch stolon. On the other hand, it is also possible that the adventitious root takes over the lion's share of the organic foods which are manufactured in the leaves and normally would be translocated to the taproots. Since the taproots are rotted first, it is quite logical to assume that the rotting of the primary system is caused by lack of food. The purpose of this part of the study is to evaluate this concept.

Among the four treatments listed on P. 15, the plants of treatment A had a much larger crown and taproots than plants of other treatments (Table 1). The differences between those of treatment A and those of other treatments are highly significant in both fresh and dry weight. This provides evidence that the adventitious root did interfere with food translocation. Since no adventitious roots were allowed to develop in treatment A, the foods synthesized in leaves were thought to be readily transferred and thus resulted in a much larger crown and taproot.

The treatment C, with all the stolons cut off, had relatively smaller crowns and taproots. It was very interesting to find that some new stolons and leaves were growing from the old deteriorating crown (Fig. 8). This seems to imply that the old primary system has been revitalized. The small size of the crown and taproot in treatment C might be due to this new growth.

There was no significant difference in percentage of the carbohydrate reserves between any treatment. However, as with
Table 1  Effect of treatments on fresh and dry weight, and the visual ratings of plant stand and root rot.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ratings* of Root dis-</th>
<th>Ratings** of plant stand</th>
<th>Fresh Weight of taproot</th>
<th>Percentage of dry weight</th>
<th>Total weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>3</td>
<td>3</td>
<td>17.2 g</td>
<td>34.1%</td>
<td>5.8</td>
</tr>
<tr>
<td>foil</td>
<td>3</td>
<td>4</td>
<td>21.7</td>
<td>32.2%</td>
<td>6.5</td>
</tr>
<tr>
<td>A. under</td>
<td>3</td>
<td>4</td>
<td>45.0</td>
<td>34.7%</td>
<td>15.6%</td>
</tr>
<tr>
<td>all stolons</td>
<td>3</td>
<td>3</td>
<td>20.9</td>
<td>37.3%</td>
<td>7.6%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>35.3</td>
<td>28.0%</td>
<td>9.8%</td>
</tr>
<tr>
<td>Mean</td>
<td>2.8</td>
<td>3.3</td>
<td>29.1</td>
<td>33.5%</td>
<td>9.6</td>
</tr>
<tr>
<td>Eight inches</td>
<td>3</td>
<td>4</td>
<td>15.0</td>
<td>39.3%</td>
<td>5.5</td>
</tr>
<tr>
<td>aluminum</td>
<td>2</td>
<td>4</td>
<td>19.0</td>
<td>34.4%</td>
<td>6.6</td>
</tr>
<tr>
<td>foil</td>
<td>3</td>
<td>3</td>
<td>8.8</td>
<td>31.0%</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>26.4</td>
<td>38.3%</td>
<td>10.1%</td>
</tr>
<tr>
<td>Mean</td>
<td>2.7</td>
<td>3.7</td>
<td>17.1</td>
<td>34.2%</td>
<td>5.9</td>
</tr>
<tr>
<td>C. stolons</td>
<td>3</td>
<td>3</td>
<td>13.0</td>
<td>24.8%</td>
<td>3.2</td>
</tr>
<tr>
<td>cut</td>
<td>3</td>
<td>3</td>
<td>8.0</td>
<td>32.8%</td>
<td>2.5%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>9.7</td>
<td>36.7%</td>
<td>3.4%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>19.4</td>
<td>38.3%</td>
<td>7.5%</td>
</tr>
<tr>
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<td>3</td>
<td>3</td>
<td>15.4</td>
<td>33.2%</td>
<td>5.1%</td>
</tr>
<tr>
<td>Mean</td>
<td>3.2</td>
<td>2.8</td>
<td>13.7</td>
<td>34.1%</td>
<td>4.7</td>
</tr>
<tr>
<td>D. Control</td>
<td>3</td>
<td>3</td>
<td>8.0</td>
<td>35.1%</td>
<td>2.8%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>25.7</td>
<td>32.6%</td>
<td>8.3%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>15.0</td>
<td>33.8%</td>
<td>5.0%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>17.2</td>
<td>31.2%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Mean</td>
<td>3.2</td>
<td>3.2</td>
<td>16.4</td>
<td>33.2%</td>
<td>5.3</td>
</tr>
</tbody>
</table>

L. S. D. of mean: 1% (insign.)  (insign.  12.4  (insign.)  2.4
  9.1  1.7

* 1=healthy, 2=slight, 3=moderate, 4=severe, 5=very s
** 1=very good, 2=good, 3=moderate, 4=poor, 5=very poo
or died.
Table 2 The Effect of treatments on carbohydrate reserves of taproots of Ladino clover. (In percentage of dry weight)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Free Sugars</th>
<th>Sucrose*</th>
<th>Total Sugars*</th>
<th>Starch*</th>
<th>Total Carbohydrate Reserves*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum foil</td>
<td>0.56</td>
<td>1.40</td>
<td>1.96</td>
<td>20.83</td>
<td>22.79</td>
</tr>
<tr>
<td>A. Aluminum</td>
<td>0.20</td>
<td>1.77</td>
<td>1.97</td>
<td>16.70</td>
<td>18.67</td>
</tr>
<tr>
<td>under A.</td>
<td>0.37</td>
<td>1.59</td>
<td>1.96</td>
<td>17.05</td>
<td>19.01</td>
</tr>
<tr>
<td>all A.</td>
<td>0.51</td>
<td>1.46</td>
<td>1.77</td>
<td>17.89</td>
<td>19.66</td>
</tr>
<tr>
<td>stolons A.</td>
<td>0.87</td>
<td>1.42</td>
<td>2.29</td>
<td>19.18</td>
<td>21.47</td>
</tr>
<tr>
<td>0.97</td>
<td>0.98</td>
<td>1.95</td>
<td>20.22</td>
<td>22.17</td>
<td></td>
</tr>
<tr>
<td>Mean A.</td>
<td>0.55</td>
<td>1.44</td>
<td>1.98</td>
<td>18.65</td>
<td>20.63</td>
</tr>
<tr>
<td>Eight inches</td>
<td>0.38</td>
<td>1.14</td>
<td>1.52</td>
<td>15.26</td>
<td>16.78</td>
</tr>
<tr>
<td>B. aluminum foil</td>
<td>0.47</td>
<td>1.54</td>
<td>2.01</td>
<td>15.75</td>
<td>17.76</td>
</tr>
<tr>
<td>0.51</td>
<td>1.67</td>
<td>2.18</td>
<td>17.59</td>
<td>19.77</td>
<td></td>
</tr>
<tr>
<td>0.82</td>
<td>0.82</td>
<td>1.64</td>
<td>11.65</td>
<td>13.29</td>
<td></td>
</tr>
<tr>
<td>0.46</td>
<td>1.69</td>
<td>2.15</td>
<td>15.34</td>
<td>17.49</td>
<td></td>
</tr>
<tr>
<td>0.57</td>
<td>1.54</td>
<td>2.11</td>
<td>14.98</td>
<td>17.09</td>
<td></td>
</tr>
<tr>
<td>Mean B.</td>
<td>0.54</td>
<td>1.40</td>
<td>1.94</td>
<td>15.10</td>
<td>17.03</td>
</tr>
<tr>
<td>All C. Stolon</td>
<td>0.69</td>
<td>1.90</td>
<td>2.59</td>
<td>14.87</td>
<td>17.46</td>
</tr>
<tr>
<td>Cut C.</td>
<td>0.89</td>
<td>1.07</td>
<td>1.96</td>
<td>16.12</td>
<td>18.08</td>
</tr>
<tr>
<td>0.69</td>
<td>1.29</td>
<td>1.99</td>
<td>17.48</td>
<td>19.46</td>
<td></td>
</tr>
<tr>
<td>0.66</td>
<td>1.10</td>
<td>1.76</td>
<td>15.40</td>
<td>17.16</td>
<td></td>
</tr>
<tr>
<td>0.68</td>
<td>1.46</td>
<td>2.14</td>
<td>18.47</td>
<td>21.61</td>
<td></td>
</tr>
<tr>
<td>0.48</td>
<td>1.11</td>
<td>1.59</td>
<td>15.40</td>
<td>16.99</td>
<td></td>
</tr>
<tr>
<td>Mean C.</td>
<td>0.68</td>
<td>1.32</td>
<td>2.00</td>
<td>16.29</td>
<td>18.46</td>
</tr>
<tr>
<td>D. Control</td>
<td>0.69</td>
<td>1.00</td>
<td>1.69</td>
<td>18.93</td>
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</tr>
<tr>
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<td>2.09</td>
<td>18.18</td>
<td>20.27</td>
<td></td>
</tr>
<tr>
<td>0.87</td>
<td>0.87</td>
<td>1.74</td>
<td>15.55</td>
<td>17.29</td>
<td></td>
</tr>
<tr>
<td>1.18</td>
<td>1.04</td>
<td>2.22</td>
<td>17.64</td>
<td>19.86</td>
<td></td>
</tr>
<tr>
<td>0.60</td>
<td>1.32</td>
<td>1.92</td>
<td>11.98</td>
<td>13.81</td>
<td></td>
</tr>
<tr>
<td>0.69</td>
<td>1.22</td>
<td>1.91</td>
<td>15.02</td>
<td>16.93</td>
<td></td>
</tr>
<tr>
<td>Mean D.</td>
<td>0.79</td>
<td>1.14</td>
<td>1.93</td>
<td>16.20</td>
<td>18.13</td>
</tr>
<tr>
<td>L. S. D.</td>
<td>1% Insignificant</td>
<td>3.18</td>
<td>3.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of mean</td>
<td>5%</td>
<td>2.33</td>
<td>2.52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Expressed in terms of glucose.
the size of taproots, treatment A also had higher content of starch or total carbohydrate reserves than the other treatments (Table 2). Since the difference is significant, it seems reasonable to assume that the adventitious roots or the secondary system might affect the life span of the primary system; but the ratings of the plant stand and root rot in this experiment (Table 1) failed to confirm this. Probably the effect of treatments on root rot would not show up in such a short time.

Furthermore, to increase the size or even prolong the life span of the primary system at the expense of the secondary system may not have any significance in overall persistence in the pasture.
DISCUSSION

After a long search for the causal organisms, and also for the possible predisposing factors, research workers have come to only one conclusion—that the cause of rotting or deterioration of white clover roots and crowns is extremely complex.

The most unusual factor concerning this disease is the extraordinary importance of the predisposing factors. The rotting organisms seem to play only a minor role. One or more organisms may be involved in the rotting of some plants, but only saprophytes are involved in others. However, they all produce the same type of symptoms, and the pace of development is similar.

The predisposing factors may be divided into two groups: one is environmental; the other is the physiological condition of the plants. A great deal of work has been done on the effect of environmental factors. Each has some degree of influence, but none of them appears to be dominant. Apparently, the condition of the plants plays a much more important role than any environmental factor. A healthy plant can lead a normal growth in an infested area without any sign of contamination.

Unfortunately, very little work has been done on the plant itself. In this research, some features of the morphology, anatomy, and growth habits were studied, and the emphasis was placed on their relationship to persistence. However, the lack of a persistent type for comparison has caused a great deal of difficulty.
The survival of the independent secondary system is thought to be the favorable characteristic for persistence. The recent work by Gibson and Trautner (18) has also proved that the adventitious roots can support the growth of the top as well as the primary roots can. However, on the other hand, the establishment of the secondary system seems to hasten the rotting of the primary system because the adventitious roots have been found to interrupt the downward translocation of food materials. Although the abundance of food materials is not necessary to resist the rotting process, constant starvation will certainly result in final collapse. This relationship is apparently unfavorable to the primary system. Disrupting this relationship by cutting off the stolons has produced some revitalization of the crown. However, this practice may not be practical under field conditions. If the clover is seeded in rows, after the stolons have fully grown both sides of the row could be cut a few inches from the center of the row. According to the results in Fig. 13, the growth of the main stolon may slow down and stop, but it seems to have no significant effect on the secondary stolons, and possibly it assists in the development of adventitious roots.

Probably this is the hard way to save the primary system. The easiest way should be reseeding. Since white clover flowers almost throughout the growing season, under the normal cutting practice enough reseeding should take place to replace the old plants. The general lack of persistence has already indicated the failure of natural reseeding, although Graham et al (19) found that natural reseeding helped to maintain a better stand.
than where reseeding was prevented by removing all the flowering heads whenever they appeared. However, the tendency to decline was not checked and the improvement of stand was limited. This only proves that the persistence will be still poorer if natural reseeding is prevented. In fact, there is not much chance for a young seedling of white clover to survive the severe competition from the mature grasses and weeds which usually form a solid sod in the pasture land.

The new growth of stolons, supported by an already established root system, seems to stand a much better chance than the reseeding. Nevertheless, it is true only in the first or second year. Although the primary stolon continues to give new growth, the majority of new growth is produced by secondary stolons. As mentioned before, not every bud can give rise to a secondary stolon; some will form inflorescences. The formation of inflorescences is not only at the expense of secondary stolons; but it also drains out the food reserves from the mother stolon. There is no doubt that the profusely flowering plant will result in low rate of new growth, low density of stolons, and less foliage. However, it also provides more possibility for reseed; thus the overall effect of flowering does not stand out as much it should. Also, some special plants such as polyploids produce few stolons, either primary or secondary, and they persist poor; even though they do not flower much.

Probably the life span of white clover is determined primarily by nothing but its own aging processes. Since the aging process is still a mystery, probably flowering serves as the best indicator.
As perennial plants, some wild white clovers flower sparsely, set very few seeds, and depend mainly on vegetative propagation for survival. The flowering and seed setting serve only as agents for wide range distribution and for surviving natural disasters. When this plant is raised as a crop, seeds become the only propagating agent. The seed setting ability has been promoted during every generation. The following equation can be used for predicting the fraction of a given component in a given generation:

\[ F_j = \frac{A_j^n}{\sum_{i=1}^{m} A_i^n} \]

- \( F \) ...... Fraction of a given component.
- \( j \) ...... The \( j \)th component plant under consideration.
- \( A \) ...... The number of viable seeds of given component plant.
- \( n \) ...... The number of generations.
- \( i \) ...... Running index.
- \( m \) ...... Total number of component plants.

For example: Starting with 3 plants, plant no. 1 yields 1,000 seeds, representing a profusely flowering plant. Plant no. 2 yields 500 seeds, representing a moderately flowering plant. Plant no. 3 yields 100 seeds, representing a sparsely flowering plant. The fraction of each component plant during the next four generations is as follows:
<table>
<thead>
<tr>
<th>Component</th>
<th>Parent</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
</tr>
</thead>
<tbody>
<tr>
<td>plant no. 1</td>
<td>1/3</td>
<td>10/16</td>
<td>100/126</td>
<td>1,000/1126</td>
<td>10,000/10,626</td>
</tr>
<tr>
<td>plant no. 2</td>
<td>1/3</td>
<td>5/16</td>
<td>25/126</td>
<td>125/1126</td>
<td>625/10,626</td>
</tr>
<tr>
<td>plant no. 3</td>
<td>1/3</td>
<td>1/16</td>
<td>1/126</td>
<td>1/1126</td>
<td>1/10,626</td>
</tr>
</tbody>
</table>

After four generations, the entire population is composed of practically nothing but profusely flowering plants. However, the fraction of the profusely-flowering plants would increase at a much lower rate than calculated because of natural selection.
SUMMARY AND CONCLUSIONS

1. Based on the structure and growth habit, white clover has been divided into two systems. The primary system includes the taproot, the crown and the main stolons. The secondary system includes adventitious roots and secondary stolons which arise from the nodes of the stolons.

2. When the plant gets older, the parenchymatous tissue appears to be the most susceptible to decay.

3. The development of internal breakdown appears to increase with the age of the tissue, and the failure to find any external origin seems to confirm the physiological concept.

4. The crown of white clover is the lower portion of a condensed main stem. It can produce an indefinite number of primary stolons. The center of the crown contains a large parenchymatous pith which presumably functions as storage.

5. The stolons have relatively long internodes and slightly swollen nodes from which the leaves, inflorescences, adventitious roots, and the secondary or tertiary stolons arise.

6. The node of a stolon seemingly acts as a crown but produces only one secondary stolon, one leaf, and one adventitious root. The adventitious roots function mainly to support the growth of the respective secondary stolon. When the primary system becomes rotted, the secondary system can lead an independent existence.

7. The adventitious roots were found to divert the downward translocation of foods manufactured in the leaves. Thus they may augment the deterioration of the primary system.
8. When all the stolons were cut off, the crown demonstrated some sort of revitalization by giving rise to new stolons.

9. As an event of the aging process, the effect of flowering on the persistence was emphasized. An equation is proposed to predict the increasing population of the profusely flowering plants, as the generations advance.
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