

AN ABSTRACT OF THE THESIS OF

Jeffrey John Steiner for the degree of Doctor of Philosophy

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Title: EFFECT OF SHEEP GRAZING ON SUBTERRANEAN CLOVER

(TRIFOLIUM SUBTERRANEUM L.) SEED PRODUCTION

**Redacted for privacy**

Abstract approved: \_\_\_\_\_

Don F. Grabe

Subterranean clover (Trifolium subterraneum L.) seed production practices in Oregon are different from those in Australia. There, seed is produced on fields which are managed primarily for forage. In Oregon, seed fields are managed for seed as the primary product and forage is of secondary concern.

Some sheep grazing is necessary to prevent low seed yields due to excessive forage growth. However, no information is available describing how grazing can be used to optimize seed yields.

The purpose of this study was to determine the effects of defoliation on the seed yield of subclover and to suggest alternative management practices, if necessary, to improve seed yields.

Moderate grazing applied during flowering and continued until early bur fill, increased seed yields 51 and 27% in 1979 and 1980. Grazing treatments shorter in duration increased seed yields, but not as much. A short-duration grazing treatment prior to flowering

reduced seed yields 13% in 1980. Seed yield increases were due to an increase in the number of seeds produced ( $r = 0.99$  in both years).

The effect of grazing was to modify the canopy to allow for an increase in plant growth during the later stages of reproduction. However, the plants needed to be allowed to recover fully from the grazing treatments to allow for adequate seed fill. Plants which were mechanically defoliated did not fully recover and showed seed size reductions.

Defoliation, position of leaves within the canopy, phenological stage of development, and the conditions under which the plants are grown all affect the specific leaf weight of the leaves. This introduces variation which reduces the predictability of leaf area from leaf dry weight. Care should be given to selecting the samples of leaves which are used to make leaf area predictions.

EFFECT OF SHEEP GRAZING ON SUBTERRANEAN CLOVER  
(TRIFOLIUM SUBTERRANEUM L.) SEED PRODUCTION

by

Jeffrey John Steiner

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Typed by Gloria M. Foster for Jeffrey John Steiner

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EFFECT OF SHEEP GRAZING ON SUBTERRANEAN CLOVER  
(TRIFOLIUM SUBTERRANEUM L.) SEED PRODUCTION

INTRODUCTION

Subterranean clover (Trifolium subterraneum L.), or subclover, is a winter annual pasture legume which is adapted to much of the hill-land pasture region west of the Cascades. Subclover is native to the Mediterranean and western European regions. It has been successfully cultivated in Australia since the late 1800's as a pasture crop with seed being produced there commercially since 1906.

Subclover was introduced into the United States from Australia in the 1920's. The first plantings in Oregon were made in 1922. The first commercial seed fields of subclover in Oregon were planted in 1941.

Seed production rose in the late 1940's and early 1950's but declined during the late 1950's and early 1960's due to lower demand for subclover seed. An increase in demand for locally grown seed in Douglas County has brought about an increase in interest in seed production.

Fields which are grown for seed are managed with seed as the primary product. This differs from the Australian system of seed production in which fields are managed primarily for forage and seed as a secondary product.

It is known that some grazing is necessary to increase seed yields. However, very little information is available which

describes how subclover seed fields should be grazed to achieve optimum seed yields. Also, since most subclover seed is presently produced in Australia, little is known about the specific problems involved with growing seed here in Oregon.

These studies were conducted to determine:

1. If sheep grazing does in fact increase seed yields.
2. Which yield components and growth responses are affected by sheep grazing.
3. If mechanical defoliation could substitute for sheep grazing.
4. If alternative management practices are necessary to increase seed yields.

The results are presented in the form of four manuscripts. The first describes the effects of sheep grazing and mechanical defoliation on subclover and its seed yields. The second is concerned with the influence of natural moisture stress variation in the field alone and in combination with sheep grazing on subclover. The third is a brief paper discussing problems involved with the prediction of leaf area from leaf dry weight of subclover. The fourth is a general production guide for the production of subclover seed. This paper contains information gained from these studies as well as observations of various seed growers' methods.

## LITERATURE REVIEW

Subterranean clover, Trifolium subterraneum L., or subclover, is a winter annual legume of Mediterranean origin. It was probably introduced into Australia during the settlement period as a contaminant in ryegrass (Lolium spp.) seed (Gardner and Dunne, 1933). Subclover's value as a forage was not recognized until 1889 by Amos William Howard in South Australia. By 1906, seed of subclover was being commercially marketed in Australia (Hill, 1936).

Subclover was introduced into the United States during the 1920's. Rampton (1945) reported that the first plantings in Oregon were made in 1922 on the Agricultural Experiment Station at Corvallis. One of the earliest commercial fields was planted in 1929 on the Smith farm in Douglas county. That field, though not well managed, is still producing subclover forage from that original seeding (W.D. Mosher, personal communication).

The first commercial plantings for seed in Oregon were made in 1941 (Rampton, 1945). Seed production reached a peak in 1951 with 972 ha being harvested with an average yield of 92 kg/ha. Most of the seed produced was grown in the Willamette Valley, with a small amount produced in the south central valleys. The market price was approximately \$1.21 per kilogram (Oregon State College, 1954). By the early 1960's, the total area of subclover grown for seed had declined to less than 20 ha.

In 1980, the number of hectares of subclover for seed in Oregon had risen to an estimated 123. Most of this area was in Douglas

County with some production in Lane County. An estimated 63.5 metric tons of seed was produced at a value of \$2.75 per kilogram (W.D. Mosher, personal communication). The area of production in 1981 is estimated to be in excess of 200 ha. The total area being grown for seed is far less than the estimated 61,000 ha of pasture in western Oregon.

The best adapted varieties for western Oregon in order of earliness to lateness in maturity are: Woogenellup, Mt. Barker, Nangella, and Tallarook (Rampton, 1945; unpublished field trials).

#### Canopy Development

A detailed account of subclover reproductive development is given by Aitken and Drake (1941). A partial summary of their observations follows: subclover is a prostrate growing annual with a rather indeterminate growth form. Approximately five basal leaves are produced prior to the formation of the winter rosette. Five prostrate runners, each having about three nodes, are produced from the basal nodes. In late winter as flowering is initiated, the distal internodes begin to elongate. In the variety Mt. Barker, each plant has the capacity to produce about 16 basal runners. Each of these runners in turn produces about 20 or more nodes. The first three or four nodes are strictly vegetative in nature and therefore only produce leaves. Nodes four through nine are also vegetative but have the capacity to produce lateral runners. The more distal nodes, ten through twenty +, are reproductive in nature and produce the inflorescences. The first nine of these nodes produce most of

the viable seed. The most distal internodes are often dwarfed and little viable seed is produced. Lateral runners, which are only produced below the first flowering node of the basal runners, are also capable of producing reproductive structures and seed.

Black (1955) found that germination of subclover at 21 C gave optimal hypocotyl extension per unit of cotyledonary material translocated. When seeds were planted 0.5, 1.0, 1.5, and 2.0 inches deep, cotyledons amounted to 61, 54, 48, and 43% of the total seedling dry weight, respectively. Raguse et al. (1970) described the rate of seedling development in a morphological index which showed development at various temperatures to be related to the number of days from germination. Collins and Smith (1974) showed that the temperature to which the mother plant is exposed during seed development affected the rate of development of the plants produced from that seed. The lower the temperature, the faster the rate of plant development.

In respect to seedling growth, Taylor (1972) found that seedlings produced from small seeds had a higher proportionate growth rate than plants developed from large seeds. Large seeds produced seedlings which exhibited more rapid leaf expansion rates. This offset the advantage of higher growth rate in seedlings derived from small seeds. By following the competition of seedlings derived from small and large seed, Black (1958) showed that the plants produced from small seeds were eventually eliminated by plants derived from large seeds due to competition for light. Even though

small-seeded plants made up 25% of the total leaf area of the canopy, only 10% of the incident light was available to these leaves due to the spatial arrangement of the leaves within the canopy.

Black (1958) also gave a good description of the canopy of subclover as related to leaf area at 2 cm increments in height in relationship to time of development. A characteristic pattern developed which showed the total leaf area rising in height with time as the lower regions near the ground were increasingly shaded. Stern (1965) measured the light profiles at 2 cm height levels through the canopy in relationship to time. These data showed that the lack of leaf area in the lower regions of the canopy was probably due to the inability of light to penetrate the canopy and reach these lower regions.

Donald (1951) had concluded that light was the factor which determined dry matter yield in plant communities. He showed that with increasing densities of plants, a maximum level of dry matter production is reached. Donald (1954), to explain the effects of plant density on seed yield of subclover, showed maximum seed production occurred at densities of about 10.9 plants per 20 dm<sup>2</sup>. Densities greater than this reduced seed yield but not as greatly as lesser densities.

Davidson and Donald (1958) showed that a leaf area index of 4 to 5 yielded the greatest growth rate as the dry matter production of subclover rose during later development. Growth rate decreased

about 30% with a LAI of 8.7. The leaf component of total dry matter production was stopped at this high LAI. The optimum LAI could be increased if the light intensity levels were increased (Stern and Donald, 1962). Black (1963) confirmed these results and mathematically quantified them. He further concluded, as had Donald in 1951, that light, and not temperature, was the limiting factor for growth in subclover. He used his findings to show that at low levels of radiation, growth rate fell to zero with high leaf LAI's.

King and Evans (1967) measured the net photosynthesis of subclover and confirmed Davidson and Donald's (1958) finding that a LAI of 4.5 resulted in a maximum rate of dry matter production. The rate of CO<sub>2</sub> fixation at an LAI of 5 with 3300 foot-candles of light was 31.5 mg CO<sub>2</sub>/dm<sup>2</sup> surface area/day. King and Evans also showed that evapotranspiration increased with increasing LAI up to a level of 3.0 and only slightly increased at higher LAI levels.

Cocks (1973) found that temperature influenced the development of subclover at various LAI's. At a low LAI of 0.2, increasing temperature to a maximum of 22 C day/17 C night resulted in an increased growth rate. At a LAI of 3.0, growth rate was unaffected by these treatments. However, at a LAI of 5.5, growth rate was decreased with increasing temperatures. The increased rate of growth with increasing temperature at the low LAI may have been due to the beneficial effects of the warmer temperatures on the root system. This confirmed the findings of Sumner et al. (1972) who showed root ambient temperature rather than shoot ambient temperature determined early plant growth rate.

### Response to Defoliation

The response of subclover to defoliation has been shown to be quite variable. Collins and Aitken (1970) contend that leaf removal delays flowering in the variety Mt. Barker. Hagon (1973), using the variety Woogenellup, showed results similar to those of Collins and Aitken. Both studies suggested a stimulus was produced by the leaf which induced flowering. Plants which were defoliated initiated flowers at a later node due to the removal of the earlier leaves. However, much of the effect of delayed flowering in defoliated plants may also have occurred due to a competition between reproductive and vegetative development for available carbohydrate.

The competition between foliage and reproductive development as proposed by Collins and Aitken (1970) and Hagon (1973) can be considered from the view of Loomis (1932) who described a growth-differentiation balance in which differentiation is dependent on available carbohydrate. If a plant is in rapid growth, as is the case in recovery after defoliation, carbohydrate is not available for differentiation. On the other hand, if a rapidly growing plant is checked in its growth by gradual reduction of nutrients or moisture, carbohydrate used in growth is then accumulated and used for differentiation of flower structures. The work of Clarkson and Russell (1976) with several species of Medicago confirms this notion since the application of moisture stress accelerated flowering if the stress was applied after the initiation of flowering.

May and Davidson (1958) questioned whether there is any role of

reserve carbohydrate in the regeneration of defoliated subclover. They felt that any reserve carbohydrate was lost to respiration during senescence and plays no role in regeneration. No mention is given to the possible role of these stored carbohydrates in maintaining reproductive differentiation under defoliated conditions.

Some early carbohydrate analysis of subclover by Hardwick (1954) showed a drop-off in free reducing sugars and total reducing sugars at the time of floral initiation. This was followed by a rapid increase in these two components with further development. This would tend to indicate that there is a high demand for these products at reproductive differentiation and seems to verify Loomis' (1932) hypothesis that increased amounts of carbohydrate are required at this time. This finding also tends to support the contention of Collins and Aitken (1970) and Hagon (1973) that there is a competition between newly developed leaves which replace defoliated ones and the initiation of flowers. This was shown by the delay in induction of flowers in the defoliated treatments.

Defoliation greatly affects the structure of the plant canopy. Black (1963) showed the vertical arrangement of the leaves was modified by defoliation. This allowed the plant to produce more leaves than if it had not been defoliated and at a higher LAI.

Warren Wilson (1960) carried this concept even further by suggesting that the spatial arrangement of the leaves in the canopy provides the key to maximum development. Other factors such as leaf inclination, height distribution, and horizontal dispersion of the

foliage area were all considered in relationship to the gathering of incident light. Since defoliation greatly affects the structure of the entire canopy, it seems that such treatments would affect the light relationships within the canopy on an individual leaf basis.

The work of Belikov (1955a, 1955b), Prokofyev et al. (1957), Linck and Sudia (1962), and Flinn and Pate (1970) showed the importance of the individual subtending leaf to its dependent inflorescence in several crops. The degree to which seed is filled is dependent on the ability of the subtending leaf to provide photosynthate. Removal of the subtending leaf greatly reduces seed fill. The organization of vascular connections limit the ability of neighboring leaves to substitute for defoliated subtending leaves. A detailed account of the primary vascular connections in Trifolium is given by Devadas and Beck (1972).

The timing of the defoliation appears to be most critical in affecting reproductive development. If applied after the start of floral initiation, defoliation may act as a stress similar to the moisture stress effect shown by Clarkson and Russell (1977) which accelerated flower production. Active vegetative development may thus be checked, which would divert sugars produced by the plant to the differentiation of floral structures from use in the synthesis of proteins and for maintenance requirements (Loomis, 1932).

Application of a stress prior to the initiation period may be detrimental to floral production as seen in the work of Clarkson and Russell (1977) and of Davidson and Donald (1958). Defoliation

late in development may not affect differentiation, but may reduce seed yield by reducing seed fill (Rossiter, 1961). The final seed weight of Vicia faba was also reduced when plants were defoliated (Hodgson and Blackman, 1957).

### Reproductive Development

The inflorescences consist of three to five perfect florets attached to a peduncle. The florets are self-pollinated with fertilization occurring when the end of the corolla has elongated to a point which is equal with the tips of the calyx lobes. The characteristic bur of subclover is formed from sterile and partially developed flowers which encase the fertile florets after fertilization. The bur contains two to four pods which usually contain dark purple seed.

Flowering begins in late winter along with a slight elongation of the distal internodes of the basal runners. Lateral runners also begin to develop at this time (Aitken and Drake, 1941). Aitken (1955) showed that late maturing varieties do not flower without vernalization and the cold period must be of a certain duration in order for flowering to occur. Medium maturing varieties are not dependent on vernalization but flowering is hastened after vernalization. Early maturing varieties do not require as cold a treatment as later maturing varieties nor does the treatment have to be as long. Collins and Smith (1975) found that vernalization only hastened flowering and was not required in the late maturing variety Tallarook. Middle maturing varieties such as Mt. Barker and Yarloop

were in fact delayed in flowering when given a cold treatment prior to flowering. These findings support the results of Morley and Davern (1956) which also showed a great amount of interaction between genotype and environment in respect to flower formation.

Flowering in subclover is usually terminated with the onset of summer drought (Collins and Aitken, 1970). Prolonged moisture stress during development may greatly reduce seed yields. Short-term interruption of growth by drought may affect seed yield, depending on the time of the interruption and the variety (Andrews et al., 1977).

If water is adequately supplied through the growing season, flowering may be encouraged. However, seed yield may not be optimized due to microorganisms attacking the seed which is not allowed to dry (Collins and Quinlivan, 1980).

Calcium in the form of calcium sulfate was found to greatly increase seed number per bur, seed weight, and number of burs produced on soils which were lacking in calcium (Ozanne and Howes, 1974). The same results were found in a greenhouse study by these authors (Ozanne and Howes, 1973). It was also discovered that the forage yield was not affected by the calcium fertility levels.

Though data are not given for seed yield responses, it would be expected that proper fertility levels must be maintained to insure good seed yields. Phosphorus (Barrow, 1975; Jones and Ruckman, 1969a; Jones et al., 1970b; Jones et al., 1972; Jones et al., 1977), sulfur (Jones and Ruckman, 1969b; Jones et al., 1970a; Jones et al.,

1970b; Jones and Ruckman, 1973; Jones et al., 1977), and molybdenum (Jones and Ruckman, 1973) were all shown to give a response in forage yield. On serpentine soils in California, Jones et al. (1977) were able to show a forage response to calcium when sulfur and phosphorus were not limiting.

### Seed Production

Forage Management. In Australia, seed is produced on fields which are managed primarily for forage. Few fields are managed strictly for seed as a specialty crop. Seed is therefore a secondary product of subclover forage production. In Oregon, fields which are grown for seed are managed with seed as the primary product. Forage utilization is of secondary concern.

The importance of vegetation management to increase seed yield was reported as early as 1940 (Duggan, 1940). It was stated, though, that no definite grazing treatment could be recommended, only that some grazing was required to prevent excessive topgrowth production at the expense of seed production.

Many experiments have been done to investigate the effects of defoliation on reproduction of subclover. These have included mechanical defoliation experiments (Collins, 1978; Hagon, 1973; Collins and Aitken, 1970; Rossiter, 1972; Walton, 1975) and one experiment using sheep and mechanical defoliation (Rossiter, 1961).

Explanations as to why defoliation increased seed yield in subclover were generally not given other than a description of the

yield components affected. Since all of the experiments were performed under different conditions and with numerous varieties, it is difficult to compare the results of these different experiments.

Hagon (1973), using the variety Woogenellup, found that seed yield as well as the number of flowers, number of burs, seed size, and seed number were not significantly affected by any of the defoliation treatments. Walton (1975), using several varieties, also showed that seed yield was not affected by defoliation; however, seed bur burial was increased by defoliation. Both of these experiments were done in the field and were given supplemental irrigation.

The effects of defoliation on flowering were investigated by Collins and Aitken (1970) under field conditions and by Rossiter (1972) under greenhouse conditions. Collins and Aitken, using Mt. Barker variety, found that flowering was delayed by defoliation and that the total number of inflorescences was reduced in the defoliated treatments. Rossiter, however, found that the number of inflorescences produced was increased in Dwalganup if defoliation was applied prior to or during early flowering. Single plants which were defoliated had fewer flowers than the undefoliated controls. No mention was given of final seed yield in either of these experiments.

Collins (1978) showed the number of inflorescences produced in the varieties Seaton Park, Yarloop, and Midland B was not greatly affected by defoliation. However, the rate of inflorescence production and final seed yield of Yarloop and Midland B were increased

when the defoliation was discontinued prior to flowering. Seed yields in Seaton Park were not reduced when defoliation continued into mid-flowering. Defoliation later than mid-flowering reduced seed yields in all three of these varieties.

Rossiter (1961) also studied the effects of mechanical defoliation on seed yield of Dwalganup and Yarloop, and investigated sheep grazing effects on seed yield of Dwalganup, Yarloop, and Baccus Marsh. The results of the mechanical defoliation experiment were similar to those of Collins (1978) -- early defoliation increased seed yield and later defoliations reduced seed yield. The number of inflorescences were also increased with early defoliation as were the number of seeds per bur. All of the clipping treatments reduced the amount of total dry matter present at harvest, with the amount of reduction depending on the severity of the defoliation. The sheep-grazed plots showed an increase in seed yield in all three varieties when compared to the ungrazed control. It was also shown that the number of seeds produced was significantly increased in the grazed treatment. No results were given for the effect of sheep grazing on seed size or number of inflorescences produced.

Rossiter's experiment was the only study of those mentioned above which did not use supplemental irrigation in field studies to insure adequate moisture throughout the growing season.

Under field conditions in which subclover is growing with grasses, defoliation has been shown to shift the competitive balance between the clover and the grasses toward the clover and

thus increase seed production (Rossiter, 1961; Collins, 1978; Cameron and Cannon, 1970). The balance of varieties planted in mixed stands may also be affected by the stocking rate and time and duration of defoliation by sheep. Much of this is due to differences in the phenological development of the varieties at the time of defoliation (Rossiter and Pack, 1972).

Harvest. The prostrate growth form of subclover, with the seed burs being produced near the surface of the soil, make it a difficult crop to harvest. Hill (1936) gave an account of the earliest attempts to harvest the seed in Australia. This involved shoveling and raking the burs by hand and then cleaning the seed with a special huller and a thresher. Implementation of horse-drawn rakes, road graders, and revolving road brooms greatly improved the collection of burs which could then be easily gathered by hand.

The bur-gathering process was further advanced by the introduction of sheepskin-covered rollers. These were pulled over the field after the topgrowth had been removed and the burs dislodged by scarification of the soil surface with a harrow or other similar implement (Ballard, 1956; Duggan, 1940; Meadly, 1945). The burs were picked up on the fleece covering while roller brushes mounted on the sheepskin rollers then brushed the burs from the wool. The burs were collected in a box which was emptied at the stationary huller and thresher near the field. This method greatly reduced the amount of labor required to collect the burs.

An alternative method which was used in the 1940's was that of direct heading. This was described by Rampton (1945) in Oregon and by Wilkie (1946) and Thaine (1949) in Australia. The crop was harvested in this manner when the foliage was completely dried. Cutter bars were often fitted with lifters to raise the foliage well above the sickle bar to reduce the number of burs which were not collected due to mowing too high above the ground. Yields were reported to be as high as 1412 kg/ha by this method (Thaine, 1949).

A lesser known practice was to use a vacuum to gather the burs from a field after as much of the topgrowth was removed as possible (Rampton, 1945). This method was used in Oregon by LaVern Murphy of Douglas County until the introduction of vacuum harvesters into Oregon in 1977. Again, the seed was threshed with a stationary thresher. This method was the predecessor of the vacuum harvesters which did not only vacuum up the burs but also thresh the seed (Quinlivan, 1969). This method has one major drawback in that harvest may take up to 2 hours per acre to complete. Poor preparation of the field may also greatly reduce the amount of seed which is collected.

All of the seed in Douglas County was harvested with a vacuum harvester in 1980. The seed in Lane County was harvested in a manner similar to the direct heading method. When the crop was completely dry, the field was windrowed with a conventional grass seed windrower with a standard sickle bar angled slightly downward. The seed was then harvested out of the windrow with a combine.

Afterward, the field was gone over with a conventional combine fitted with a Murphy flail-type pickup to pick up any burs which were left by the first process.

Seed Quality. The two seed dormancy mechanisms which subclover exhibits are an impermeable seed coat and embryo dormancy. These two mechanisms may exist separately or in combination. Loftus Hills (1944c) demonstrated that embryo dormancy was inherited in subclover. Loftus Hills (1944d) also showed that the embryo dormancy was not affected by the level of hardseededness present in the seed coat. Embryo dormancy behaved the same whether the seed was naturally softened or scarified. Morley (1958) performed a series of crosses of subclover lines which exhibited different levels of dormancy and found that embryo dormancy was highly inherited. Grant Lipp and Ballard (1964), however, showed that within varieties, large seeds were less dormant than small seeds and that soft seeds had less embryo dormancy than hard seeds.

A detailed account of the development of hard seed in subclover is given by Aitken (1939). She showed hardseededness to be greatly influenced by the environment under which the seed was produced. She set forth the theory that seed lots produced under optimum growing conditions are higher in hard seed content. Aitken also showed that more extreme drying after seed formation resulted in more hard seed being produced than under less extreme drying conditions. Seeds that were forced to dry before reaching maximum size

were thought to be lower in hard seed content. Quinlivan (1965) found that the longer the growing period in spring, the greater the amount of hard seed produced. This was believed to be the critical factor in determining impermeable seed coat dormancy. The findings of Taylor and Palmer (1979) are contrary to those mentioned above. These researchers found that when moisture stress was applied during the development of Daliak seed, hardseededness was not decreased.

Loftus Hills (1944d) concluded that hardseededness was not genetically controlled, or if it were, it was at such a low level that its effects were masked by the environment. The findings of Donald (1959) showed that hardseededness was indeed an inherited varietal character. Evidence shown in a recent review of impermeable seed coat dormancy (Rolston, 1978) indicates that environment is not the only force regulating hard seed formation.

Hard seed coat and embryo dormancies insure the survival of the species from the time the seed is produced in early summer until it germinates in the fall under suitable conditions to allow establishment of the seedlings. Rainfall during the summer may germinate non-dormant subclover seeds. These seedlings may then die before regular fall rains start.

Quinlivan (1971) showed that subclover seed was unable to survive until fall if the seed was protected only by embryo dormancy. This dormancy was at a maximum at maturity in spring and was broken down by the elevated temperatures of summer (Ballard, 1958; Quinlivan

and Nicol, 1971). This dormancy may be effective in preventing the seeds from sprouting during seed development (Quinlivan and Nicol, 1971).

Embryo dormancy is highly effective in preventing germination if imbibed seeds are exposed to elevated temperatures during the summer months (Toole and Hollowell, 1939; Loftus Hills, 1944b). The dormancy may be overcome if the imbibed seeds are first exposed to low temperature (Woodforde, 1935) or if exposed to increased carbon dioxide levels (Ballard, 1958). When seed with embryo dormancy is stored under controlled conditions of moderate temperatures, more than 12 months may be required for the dormancy to be alleviated (Loftus Hills, 1944a).

Hardseededness may last indefinitely if the seeds are stored under moderate conditions of temperature and moisture. Hardseededness may be induced in subclover seeds which are soft at a moisture content of 10% by subjecting them to further drying (Aitken, 1939). Under natural conditions, hard seed of subclover is broken down by exposure to alternating summer temperatures. Much of this alternation is dependent on the amount of dry topgrowth which is left from the previous growing season. Removal of the aftermath results in a faster breakdown of the hard seed coat (Quinlivan, 1965).

Hard seed production has been shown to be affected by defoliation. Collins et al. (1976) and Collins (1978) showed that with most of the varieties tested, hardseededness was increased with

defoliation. This increase in the level of hard seed was attributed to an increase in the number of burs which were buried in the defoliated treatments. Aitken (1939) first showed that buried burs contained more hard seed than the seed produced in burs above the ground. The reasoning behind this was that the seeds developed under more favorable conditions beneath the soil surface and therefore exhibited a greater level of hardseededness. Kitchner and Andrew (1971), however, found that unburied burs of Medicago truncatula contained seeds higher in hardseededness than burs produced under the soil surface.

MANUSCRIPT I

Sheep Grazing Effects on Subterranean Clover  
and Its Seed Production

## ABSTRACT

Subterranean clover (Trifolium subterraneum L.) seed fields in Oregon are managed as a speciality seed crop for optimum seed yield. This differs from the Australian system where seed production is of secondary concern and forage production for wool and meat production is of chief interest.

This study was conducted to determine grazing practices which could be used to increase seed yields and to study the effects of sheep grazing on plant growth. A further objective was to compare the relative effects of sheep grazing and mechanical defoliation.

Four sheep grazing treatments were applied in 1979 and 1980 at a constant stocking rate of 5 and 7.5 ewes/ha, respectively. The duration of the grazing treatments was controlled with wire-mesh enclosure cages.

Extended grazing applied until early bur formation increased seed yields over the control by 51 and 27% in 1979 and 1980, respectively. The seed yield increase was due to an increase in the number of seed produced ( $r = 0.99$  in both years). Two grazing treatments of a shorter duration during this period were not as effective as the extended grazing treatment in increasing seed yields. Application of a short-term grazing treatment which was discontinued prior to flowering reduced seed yields 13% in 1980.

Extended sheep grazing reduced the leaf area index, canopy height, and the amount of light absorbed by the canopy. Once grazing pressure was removed, the canopy recovered to the levels of

the control in all parameters except canopy height.

Six mechanical defoliation treatments were applied at different times and with various frequencies. All treatments reduced seed yield below the level of an unclipped control. Seed yield was correlated with seed weight ( $r = 0.93$ ) and seed number ( $r = 0.69$ ). Final plant dry weight was reduced in all clip treatments. This differed from the sheep grazing treatments where none of the defoliation treatments reduced the final plant dry weight.

To successfully manage this crop with sheep grazing, it appears that the defoliation treatment must be discontinued soon enough to allow complete plant recovery. If this is not done, seed size may be reduced to a level that is not compensated for by increased seed numbers.

Water-soluble carbohydrate (WSC) and total nitrogen (N) levels in leaf, stem, and root tissues in the ungrazed and extended grazing treatments were affected differently depending on the phenological state of the plant and the availability of moisture from precipitation. Under limited moisture conditions in 1979, plants grazed for an extended period showed a decline in WSC and N in the stem tissue as the seed filled. The WSC and N contents of the leaf tissue were unaffected by the grazing treatment. Large differences in WSC and N occurred between the two years of study.

The more intense grazing and defoliation treatments induced higher levels of hardseededness, but lower levels of embryo dormancy and dead seed. Levels of hardseededness were similar in all

grazing and mechanical defoliation treatments.

Additional index words: Trifolium subterraneum L., water-soluble carbohydrates, total nitrogen, seed quality, hard seed, dormant seed, grazing management.

## SHEEP GRAZING EFFECTS ON SUBTERRANEAN CLOVER AND ITS SEED PRODUCTION

### INTRODUCTION

Subterranean clover (Trifolium subterranean L.), or subclover, has been grown in Oregon for seed production since 1941 (Rampton, 1945). The total acreage for seed reached a peak of 972 ha in 1951 (Oregon State College, 1954) and rapidly declined in the 1960's. The total acreage for seed production began to increase in the late 1970's and is now estimated to be more than 242 ha. Most of the seed fields in Oregon are being managed strictly for seed production with sheep grazing being of secondary concern.

In Australia, increased seed production of subclover following sheep grazing was first reported by Duggan (1940). It was stated that no specific grazing treatment could be applied, only that some grazing was required to prevent excessive top growth at the expense of seed production. Many experiments were conducted in Australia to investigate the effects of defoliation on the reproduction of subclover. Included were experiments with mechanical defoliation by Collins (1978), Collins and Aitken (1970), Hagon (1973), Rossiter (1972), and Walton (1975), and one experiment using sheep grazing by Rossiter (1961). The results of these experiments were not conclusive in respect to the effects of defoliation on seed production. Hagon (1973), using the variety 'Woogenellup,' found that seed yield and yield components, including number of flowers produced,

number of burs produced, seed size, and seed number, were not significantly affected by any of his defoliation treatments. Walton (1975) also showed that seed yield of several varieties was not affected by defoliation. Both of these studies were conducted in the field and given supplemental irrigation. Collins and Aitken (1970), also making their observations in the field, found that flowering in the variety 'Mt. Barker' was delayed and that the total number of inflorescences was reduced after defoliation. Rossiter (1972) showed that the number of inflorescences was increased in the variety 'Dwalganup' if defoliation was applied prior to or during early flowering. No mention was made of the effects of defoliation on final seed yield in these two latter experiments. Collins (1978), using the varieties 'Seaton Park', 'Yarloop', and 'Midland B', showed that the number of inflorescences was not greatly affected by defoliation. The rate of inflorescence production, as well as final seed yield, was increased in the defoliated treatments if the defoliation was discontinued prior to flowering in Yarloop and Midland B. Defoliation was continued into mid-flowering in Seaton Park without reducing seed yield. Defoliation later than mid-flowering reduced seed yields in all three varieties. Rossiter (1961) also studied the effects of mechanical defoliation on the seed yield of Dwalganup and Yarloop varieties and compared sheep-grazed to ungrazed treatments in Dwalganup, Yarloop, and 'Baccus March'. The results of the mechanically defoliated experiment were similar to those of Collins (1978) in that early

defoliation increased seed yields and late defoliation reduced yields. Unclipped controls were shown to have more total dry matter present at harvest than any of the defoliated treatments. All clip treatments reduced total dry matter, the amount of reduction depending on the lateness of the treatment. Sheep grazing increased seed yield and the total number of seeds in all three varieties. Rossiter's (1961) experiment was the only field study which did not use supplemental irrigation to insure adequate moisture throughout the growing season.

Explanations for the increased seed yields in defoliated subclover have usually been limited to the influence of the treatment on morphological characteristics of the plant (Collins and Aitken, 1970; Collins, 1978; Hagon, 1973) or the components of seed yield (Hagon, 1973; Rossiter, 1961, 1972).

This experiment was conducted to determine the effects of sheep grazing, as practiced in Oregon, on seed production of subclover. Grazing treatments were related to the phenological development of the crop. Various plant canopy parameters and environmental conditions were determined to measure plant responses to defoliation by sheep. The effects of mechanical defoliation were compared with sheep grazing in relation to seed yield, yield components, and seed quality.

#### MATERIALS AND METHODS

The experimental area was located in a 6-ha area within a

32-ha field on the Smith farm near Oakland, Oregon. This location was a hill-land site which was mapped as loamy mixed nonacid mesic shallow typic xerorthents, loamy mixed mesic shallow dystric xerochrept, fine mixed mesic ultic haploxeralfs, and fine-loamy over clayey mixed mesic ultic haploxeralf. Soil depth varied from 22 to more than 101 cm.

The field was seeded in the fall of 1978 with Mt. Barker variety subclover (Trifolium subterraneum L.) at a rate of 28 kg/ha.

Sheep grazing was controlled with enclosure cages 0.9 m high and 1.0 m in diameter. The stocking rates were 5 and 7.5 ewes/ha in 1979 and 1980, respectively. The grazing treatments were:

Grazing treatment	1979	1980
Ungrazed	None	None
Early grazing	3 May to 16 May	8 March to 3 April
Late grazing	16 May to 22 May	3 April to 19 April
Extended grazing	3 May to 22 May	8 March to 19 April

Climatic data were obtained from the Roseburg, Oregon weather station located 32 km south of the experimental site.

#### 1979

The treatments were arranged in a randomized block design with 20 replications. The four treatments of each block were arranged around a temporary marker stake which identified the block. Plot location was determined by random compass bearings and distances

from the marker. All treatments were no more than 12 m from the marker stake. Block placement was done in a systematic fashion. The locations of the blocks were recorded in respect to an adjacent fence line by compass bearing and distance. This information was used to relocate the plots in 1980.

Thirteen of the 20 replications were sampled on 25 April, 16 May, and 30 May for light destination within the canopy in the ungrazed and extended grazing treatments. Photosynthetically active radiation (PAR) was measured with a Li-Cor model LI-185 Quantum Radiometer Photometer. Readings were taken for the amount of incoming PAR (SKY), the amount reflected from the canopy at 0.5 m above the canopy (R), and the amount which penetrated the canopy to a height of 2 cm above the ground (P). The percent of PAR which was absorbed by the canopy (A) was calculated by the equation:

$$A = 100 - ((R/SKY) \times 100) - ((P/SKY) \times 100)$$

Total dry matter and canopy height were determined from a 2 x 2 dm area in each replication.

Four of the 13 replications were divided into leaf, stem, and inflorescence components and the inflorescences were further divided into flowers and burs. The leaves were measured for leaf area with a Li-Cor Portable Area Meter and then dried at 60 C for 2 days in a forced-air oven. The stems and inflorescences were dried similarly.

The samples taken on the three above mentioned dates, along with an additional sample taken on 14 June, were then ground in a

Wiley mill through a 40-mesh screen. The samples were analyzed for water-soluble carbohydrates (WSC) by the method of Yemm and Willis (1954) and total nitrogen (N) with low nitrate and nitrite content as described by Nelson and Sommers (1973). Two 2 x 2 dm areas were sampled in all of the experimental plots on 10 July for seed yield, seed size (g/100 seeds), number of seeds produced, and seed quality.

Final total plant dry weight was determined in the ungrazed and extended grazing treatments from the plant samples taken on 30 May.

#### 1980

One 2 x 2 dm area was sampled in four of the original 20 replications for leaf, stem, inflorescence, and root dry weight on 6, 20 March, 3, 17 April, 1, 15, and 29 May. The number of burs and flowers were also counted. WSC and N were analyzed for sample dates 6 March, 3 April, 1, 15, and 29 May.

Final plant dry weight, seed yield, number of seeds produced, seed size, and seed quality were determined from one 2 x 5 dm area sampled on 14 July. Seventeen replications were used for the ungrazed and extended grazing treatments with eight replications for the early and late grazing treatments.

The effects of mechanical defoliation on seed yield, seed yield components, and seed quality were studied in the same field as the sheep grazing experiment. Seven clipping treatments were applied and replicated four times in a randomized block design.

Each plot was 2 x 3 m in area. The dates of the defoliations were:

Defoliation treatment	3-5	3-19	4-3	4-17	5-1	5-15
Unclipped						
Single early		X				
Single mid				X		
Repeated early		X	X	X		
Repeated late				X	X	X
Single late						X
Continuous	X	X	X	X	X	X

All defoliations were done with a gasoline engine-powered, hand-held mower bar at a height of 5 cm.

Observations were made for time of flowering and start of bur filling. Two 2 x 5 dm areas were sampled on 14 July in all plots for plant dry weight, seed yield, seed yield components, and seed quality.

Subclover was grown in a greenhouse sward and allowed to flower and develop burs. Leaves located in proximal, medial, and distal positions which subtended inflorescences were removed by clipping. Each inflorescence was labeled to indicate its position on the stem. Inflorescences whose leaves were not removed were also marked. The plants were allowed to grow 28 days and then had the inflorescences harvested, dried at 60 C, and weighed.

#### SEED YIELD COMPONENTS AND SEED QUALITY FACTORS

The seed was threshed in a continuous belt thresher and further cleaned with a rub board, cleaning screens, and an aspirator. The

samples were weighed, all seeds counted, and seed size calculated by dividing seed weight by the number of seeds per plot.

Two replications of 100 seeds from each plot were germinated on standard blue germination blotters at 20 C for 7 days. Seeds which imbibed water during this time but which did not produce a radicle were considered to be embryo dormant. Seeds which were not imbibed were considered to be hard seed. Any seed which was attacked by fungi during this period was considered to be dead. Total soft seed was the sum of embryo dormant, germinated, and dead seed.

#### STATISTICAL ANALYSIS

The 1979 data for seed yield, number of seeds produced, and seed size were corrected for soil moisture differences caused by varying soil depths with the use of a concomitant variable as described by Cox (1958). The concomitant variable was based on a site moisture stress index taken on 30 March. The index values were 1 = no visible wilting; 2 = slight wilting; 3 = half of the plot wilted; 4 = most but not all of the plot wilted; and 5 = all plants in a plot completely wilted. The 1980 experimental data showed no site moisture stress differences and therefore were not corrected.

The 1979 sheep grazing experiment and the 1980 clipping experiment were analyzed as randomized block designs with two observations per experimental unit. The 1980 sheep grazing experiment was analyzed as an unbalanced block design. Least significant

differences (LSD) were determined in the 1979 sheep grazing and 1980 clipping experiments. A Student t-test was used to show mean differences in the 1980 sheep grazing experiment due to the unbalanced nature of the design. Pearson's correlation method was used to test for significant associations between the various data factor means. All differences reported are significant at the 5% level of probability unless otherwise indicated.

## RESULTS AND DISCUSSION

### GRAZING EFFECTS ON SEED YIELD AND YIELD COMPONENTS

Extended grazing until early bur formation increased seed yields over the ungrazed control by 51 and 27% in 1979 and 1980, respectively (Table 1). In 1979, early and late grazing increased seed yield 41% and 26%, respectively. In 1980, late grazing with a 12% increase, was intermediate to the ungrazed and extended grazing treatments. Early grazing discontinued prior to flowering reduced seed yields 13% below the level of the ungrazed control.

Differences in the number of seeds produced accounted for the seed yield differences between all treatments in both years. Over all treatments, seed yield was correlated with seed number ( $r = 0.99$  in both 1979 and 1980). Rossiter (1961) also showed that the number of seeds produced was the component of seed yield which accounted for the yield increase.

The seed yield response to the grazing treatments appears to be related to the phenology of the crop. The periods of sheep grazing in relation to the flowering and bur formation period are shown in Figure 1. The 1979 flowering period was short due to a harsh winter which delayed flowering until late April. A rapid decline in precipitation in mid-May brought the flowering period to an end. The flowering period in 1980 began in early April and continued until late June. Precipitation did not decline until early July.

All of the grazing treatments applied prior to or into the period of early bur filling increased seed yield over those of the ungrazed control. The longer extended grazing treatments in 1979 and 1980 were more effective in increasing seed yield than the shorter early and late grazing treatments in 1979 and the late grazing treatment in 1980. Grazing completed prior to flowering in 1980 resulted in decreased seed yields.

#### EFFECTS OF GRAZING ON PLANT DRY MATTER AND CANOPY STRUCTURE

Subclover is a prostrate growing plant which produces a series of basal runners along the soil surface. Reproductive development in the variety Mt. Barker does not occur on a runner until after the development of the ninth node during the early reproductive period (Aitken and Drake, 1941). Since the canopy is already established when flowering begins, defoliation at this time removes older leaves which subtend the inflorescences at each node. These leaves provide

for light between vegetative and reproductive foliage in the canopy. The shading imposed by the non-reproductive related vegetation may reduce the available light energy received by the reproductive related vegetation which is located in the lower regions of the crop canopy.

The production of leaf and stem material in the control was reduced in both 1979 and 1980 during periods of reduced precipitation (after day 129 [9 May] in 1979 and between day 114 [24 April] and day 128 [8 May] in 1980). This possibly reduced the development of the seeds by a reduction in active plant growth. The grazed treatment showed a rapid increase in stem and leaf material once grazing pressure had been removed, even during the periods of reduced rainfall (Figure 2). The continued development in the grazed treatment after the removal of grazing pressure was probably enhanced by the LAI being well below the optimum LAI of 4.5 for sub-clover. As the optimum LAI is approached, the rate of dry matter production is reduced (Davidson and Donald, 1958). The optimum LAI was approached in 1979 and exceeded in 1980 by the ungrazed control. This possibly reduced the availability of substrates for reproductive functions since more photosynthate would be required for respiratory maintenance processes.

Reduction of plant dry matter by grazing, especially leaf dry matter, may not be beneficial to seed production if the reduction is too severe. Since seed yield is also a function of seed size, enough photosynthetic tissue must be present in order to provide

a majority of the photosynthate used by that inflorescence for seed development. This was shown in soybean (Glycine max (L.) Merr.) by Belikov (1955a, 1955b), in pea (Pisum sativum L.) by Linck and Sudia (1962), and in P. arvense L. by Flinn and Pate (1970). Increasing the amount of light received by these leaves may stimulate their growth rate and make more photosynthate available for the newly differentiating fruits. Table 2 shows the effects of extended grazing on the destination of photosynthetically active radiation (PAR) in the canopy as well as on canopy height and leaf area index (LAI). After 14 days of grazing, the leaf area was reduced, the canopy height lowered, and the total amount of light absorbed reduced in the grazed treatment. Also, the amount of PAR which reached the lower parts of the canopy was increased. It is here that the leaves which subtend the newly formed inflorescences are located. Once grazing pressure was removed, the two treatments became similar in respect to the destination of light within the canopy, LAI, and total plant dry matter. The canopy height in the grazed treatment remained shorter than in the control. The taller canopy in the control resulted from newly formed leaves having to grow up through the established canopy. In the extended grazing treatment, the canopy was re-established at a lower height due to the removal of the pre-existing vegetative cover not associated with reproduction. Collins et al. (1978) showed that shading reduced the number of seeds and number of seeds per bur in greenhouse studies. This finding can be used to explain the competition effect

adequate substrate to fill the seed. In both 1979 and 1980, there was no relationship between seed size and plant dry weight in the control. This would be expected since the control received no defoliation. Final plant dry weight in the extended grazing treatment, however, is a function of the amount of regrowth following defoliation. Seed size was positively correlated with final plant dry weight in the extended grazing treatment both years (1979  $r = 0.73$ ,  $p = 0.01$ ; 1980  $r = 0.85$ ,  $p = 0.05$ ). The same was true for the early grazing treatment in 1980 ( $r = 0.85$ ,  $p = 0.01$ ). Hodgson and Blackman (1957) similarly showed that seed weight was reduced by defoliation treatments in Vicia faba. Rossiter (1961) found that late defoliation of subclover reduced seed size but did not affect the number of burs formed.

If a canopy of subclover were defoliated so extensively as to remove a great number of subtending leaves, then seed yields could be reduced substantially. Table 3 shows that removal of the subtending leaf of a bur in subclover reduces the size of the bur. This difference is especially great in young, newly formed burs. The effects of extensive defoliation on seed size can also be seen in the experiment using mechanical defoliation. These results are shown in Table 4. All of the mechanical defoliation treatments produced seed yields below the control. The more severe defoliation treatments, which included repeated late, continuous, and single late, all greatly reduced seed yield. The repeated early defoliation, which was applied before bur filling was active, did not reduce seed

yield as much as the later defoliations. Yield reductions were not as great in the treatments applied earlier. The intense nature of this type defoliation is more severe than that of the more gradual action of sheep grazing, so only general comparisons can be observed. Here, as in the sheep grazing experiment, seed size was correlated with final plant dry weight ( $r = 0.98$ ,  $p = 0.001$ ). The component of seed yield which accounted for the yield differences was seed size ( $r = 0.93$ ,  $p = 0.001$ ), whereas seed number accounted for the yield differences from sheep grazing. The number of seeds produced here only accounted for a small portion of the seed yield variation ( $r = 0.69$ ,  $p = 0.04$ ).

It therefore is shown that the timing and intensity of mechanical defoliation is important in minimizing the reduction of seed weight below a level which is not compensated by increased seed number. The findings of Hagon (1973) support this notion in that none of his defoliation treatments reduced seed yield. This may have resulted from none of the defoliation treatments reducing the final plant dry weight. Application of supplemental irrigation may have provided adequate moisture to accommodate regrowth following defoliation. It appears that any mechanical defoliation treatment must be timed to allow for sufficient regrowth of the plants to prevent seed size from being a limitation to seed yield.

## SHEEP GRAZING EFFECTS ON PLANT TISSUE ANALYSIS

No obvious relationship existed between seed yield and percent total nitrogen (N) or percent water-soluble carbohydrates (WSC). The gross analysis of plant parts appears to give general whole-plant relationships. Varying effects of the environment make it difficult to interpret these findings in the field. The results of plant tissue analysis for WSC and N are shown in Figures 3 and 4, respectively.

WSC and N levels in leaf, stem, and root tissues in the ungrazed and extended grazing treatments were affected differently, depending on the phenology of the plant and the availability of moisture from precipitation. Under limited moisture conditions in 1979, plants grazed for an extended period showed a decline in WSC and N in the stem tissue at seed fill. The WSC and N content of the leaf tissue were unaffected by the grazing. Large differences were seen between the 1979 and 1980 data for WSC and N. Moisture conditions were not limiting in 1980, so therefore no sheep grazing effect on WSC and N levels occurred as seen in 1979.

## DEFOLIATION EFFECTS ON SEED QUALITY

Even though significant differences in seed quality could be shown between the defoliation treatments, the differences have little practical significance. The levels of hardseededness in all treatments are adequate to ensure sufficient reseeding for

re-establishment of the following season's crop.

The effects of sheep grazing on the various seed quality factors are shown in Table 5. Mechanical defoliation effects on seed quality are given in Table 6.

The levels of hardseededness are comparable to those reported by Collins (1978) and Quinlivan (1976). However, it is interesting that virtually no burs were found to be buried in this experiment while a large portion of the burs were reported to be buried in Australia. Buried seed has been reported to have a higher hard seed content than unburied seed (Aitken, 1939; Collins et al., 1976).

The absence of buried burs in this study area may have been due to the more resistant nature of the soil surface to bur penetration. The seed size of Mt. Barker produced here is roughly 30% less than that of seed grown under optimum conditions in Australia (Collins et al., 1976; Collins, 1978).

Table 1. Effect of sheep grazing on the components of seed yield and final plant dry weight of subterranean clover.

Grazing treatment	Seed yield	Seed weight	Seeds	Burs	Plant dry weight
	kg/ha	g/100	— Number/ha —		kg/ha
<u>1979</u>					
Ungrazed	455 b*	.636 a	646 <sup>†</sup> c	643 <sup>†</sup> a	5842 a
Early grazed	642 a	.593 a	921 b	--	--
Late grazed	574 ab	.624 a	875 b	--	--
Extended grazing	689 a	.615 a	1046 a	703 a	5689 a
<u>1980</u>					
Ungrazed	804 bc	.716 a	1124 b	518 a	8768 a
Early grazed	700 c	.638 a	1036 b	--	7763 a
Late grazed	898 ab	.712 a	1273 ab	--	8216 a
Extended grazing	1027 a	.685 a	1487 a	610 a	8807 a

\*Values within each column for each year followed by a different letter are different at the 5% level of significance.

<sup>†</sup>Value x 100,000.

Table 2. Effect of sheep grazing on the destination of photosynthetically active radiation (PAR) within the canopy, canopy height, and leaf area index (LAI) of subterranean clover.

Grazing treatment	PAR			Canopy height	LAI
	Reflected	Penetrated	Absorbed		
	%			cm	
<u>25 April</u>					
Ungrazed	4.6 a*	7.8 a	87.6 a	9.8 a	2.0 a
Grazed	4.6 a	9.1 a	86.3 a	10.3 a	1.7 a
<u>16 May</u>					
Ungrazed	5.0 a	0.6 b	94.4 a	28.0 a	3.9 a
Grazed	4.0 a	5.6 a	90.4 b	19.6 b	2.5 b
<u>30 May</u>					
Ungrazed	4.2 a	0.4 a	95.4 a	27.2 a	3.7 a
Grazed	4.0 a	0.7 a	95.3 a	21.6 b	3.8 a

\*Values within each column for each sample date followed by a different letter are different at the 5% level of significance.

Table 3. Effect of removing the subtending leaf of a dependent inflorescence on the dry weight of a developing bur of subterranean clover.

Subtending leaf	Location of bur on runner		
	Proximal	Medial	Distal
	mg		
Present	34.3 a*	34.5 a	14.0 a
Removed	28.0 a	31.7 a	4.9 b

\*Values within each column followed by a different letter are different at the 5% level of significance.

Table 4. Effect of mechanical clipping on the components of seed yield and final plant dry weight of subterranean clover.

Clip treatment	Seed yield	Seed weight	Seeds	Burs	Plant dry weight
	kg/ha	g/100	No./ha x 100,000		kg/ha
Unclipped	1143 a	.736 a	1550 a	640 a	9360 a
Single early	1043 b	.713 a	1480 a	630 a	8420 ab
Single mid	1060 b	.702 a	1510 a	640 a	8060 b
Repeated early	1009 c	.618 b	1620 a	650 a	6790 c
Repeated late	885 d	.577 b	1530 a	660 a	5720 cd
Single late	814 e	.570 b	1430 a	620 a	6030 cd
Continuous	779 f	.573 b	1360 a	600 a	5420 d

\*Values within each column followed by a different letter are different at the 5% level of significance.

Table 5. Effect of sheep grazing on seed quality of subterranean clover.

Grazing treatment	Seed quality factors				Total soft
	Hard	Dormant	Dead	Germinated	
%					
<u>1979</u>					
Ungrazed	73 b*	4 a	11 a	12 a	27 a
Early grazing	78 a	3 ab	6 b	13 a	22 b
Late grazing	77 ab	3 ab	6 b	14 a	23 b
Extended grazing	77 ab	2 b	6 b	15 a	23 b
<u>1980</u>					
Ungrazed	81 b	6 b	4 a	9 b	19 b
Early grazing	68 c	11 a	5 a	16 a	32 a
Late grazing	78 b	7 b	4 a	11 b	22 b
Extended grazing	88 a	5 c	2 b	5 c	12 c

\*Values within each column followed by a different letter are different at the 5% level of significance.

Table 6. Effect of mechanical clipping on seed quality of subterranean clover.

Grazing treatment	Seed quality factors				Total soft
	Hard	Dormant	Dead	Germinated	
%					
Unclipped	88 b*	2 ab	1 a	9 a	12 a
Single early	88 b	3 ab	0 a	9 a	12 a
Single mid	88 b	4 a	1 a	7 ab	12 a
Repeated early	86 b	3 ab	2 a	9 a	14 a
Repeated late	96 a	0 b	1 a	3 b	4 b
Single late	95 a	1 b	0 a	4 ab	5 b
Continuous	96 a	1 b	0 a	3 b	4 b

\*Values within each column followed by a different letter are different at the 5% level of significance.

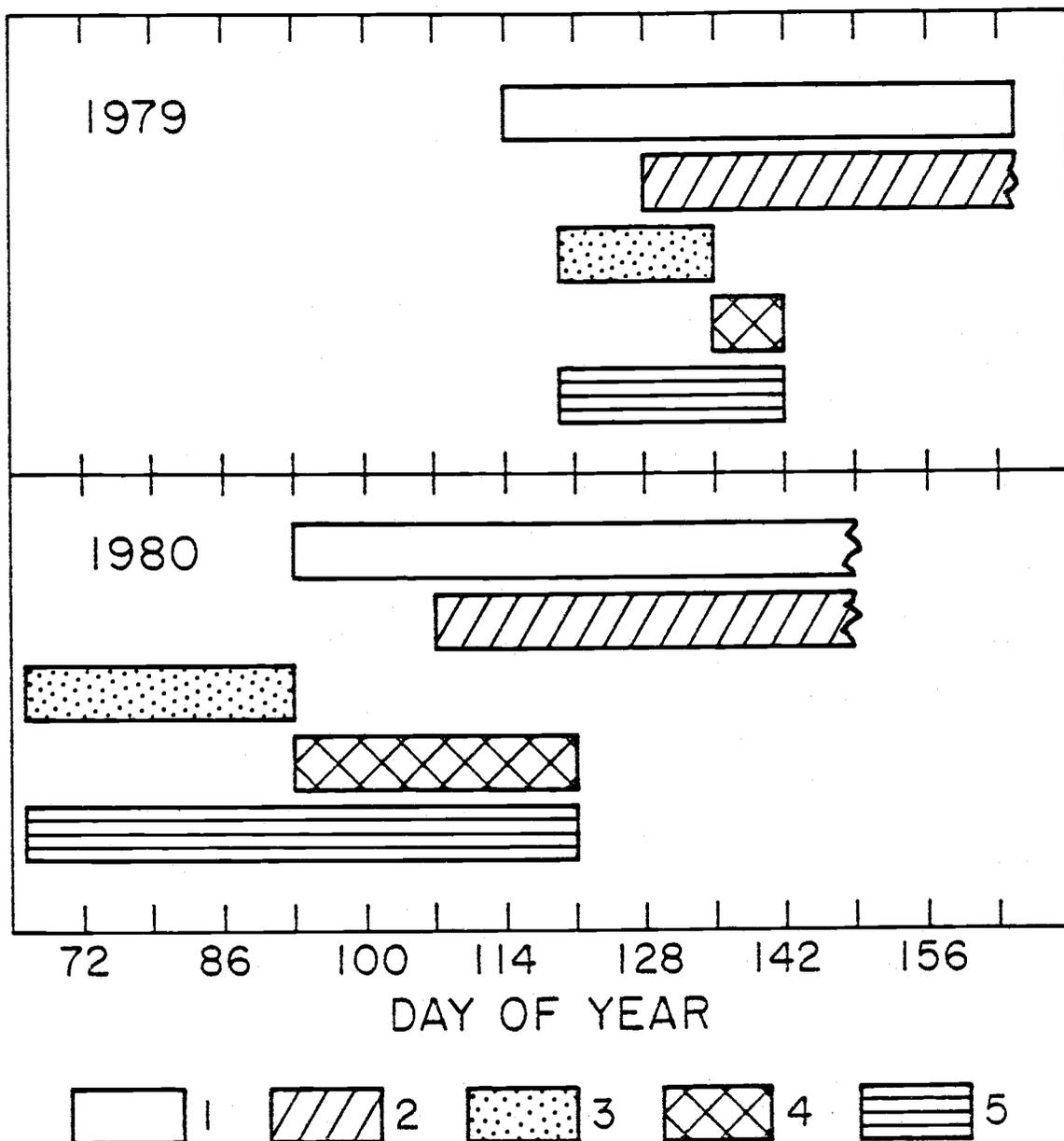


Figure 1. Period of grazing treatments in relation to flowering and bur filling of subterranean clover. 1 - flowering period; 2 - bur filling period; 3 - early grazing; 4 - late grazing; 5 - extended grazing.

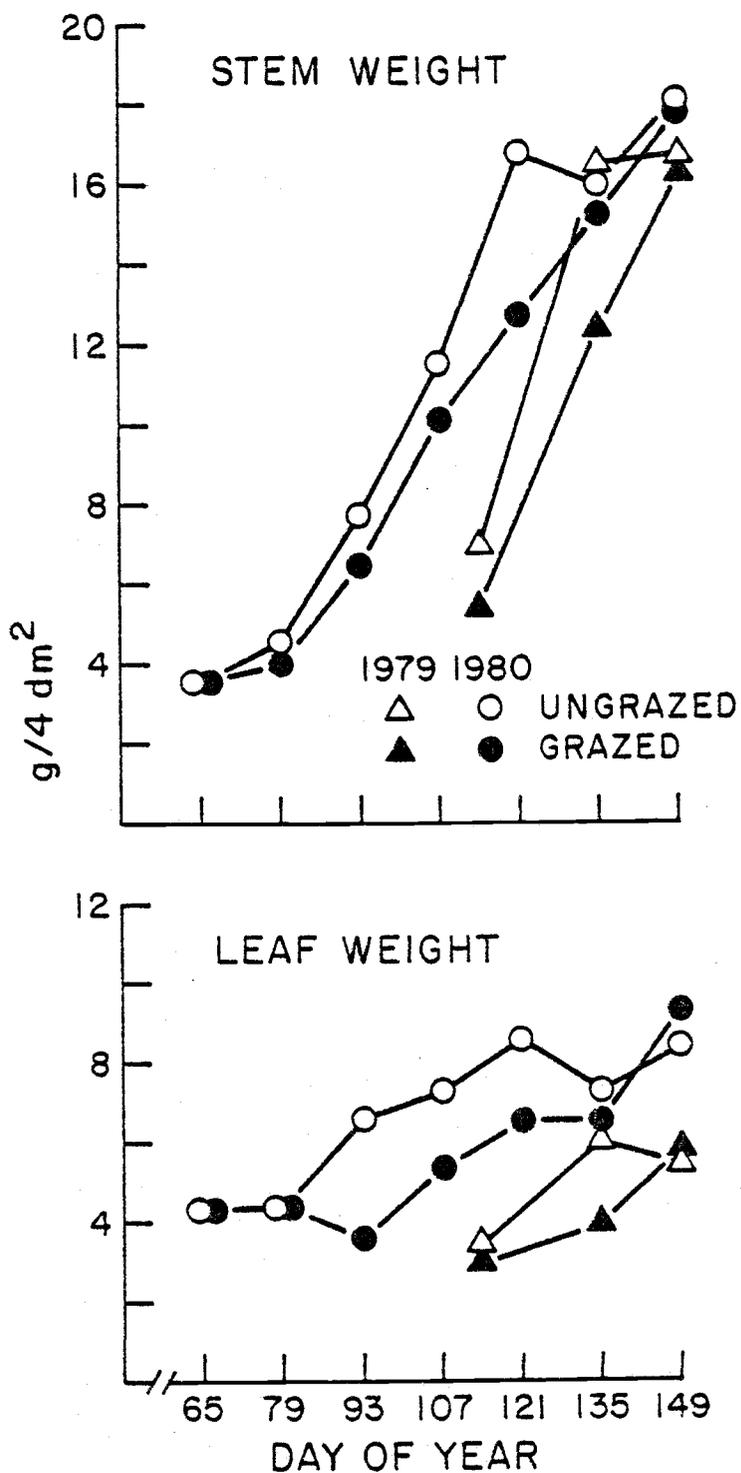


Figure 2. Effect of sheep grazing on stem and leaf dry weights of subterranean clover.

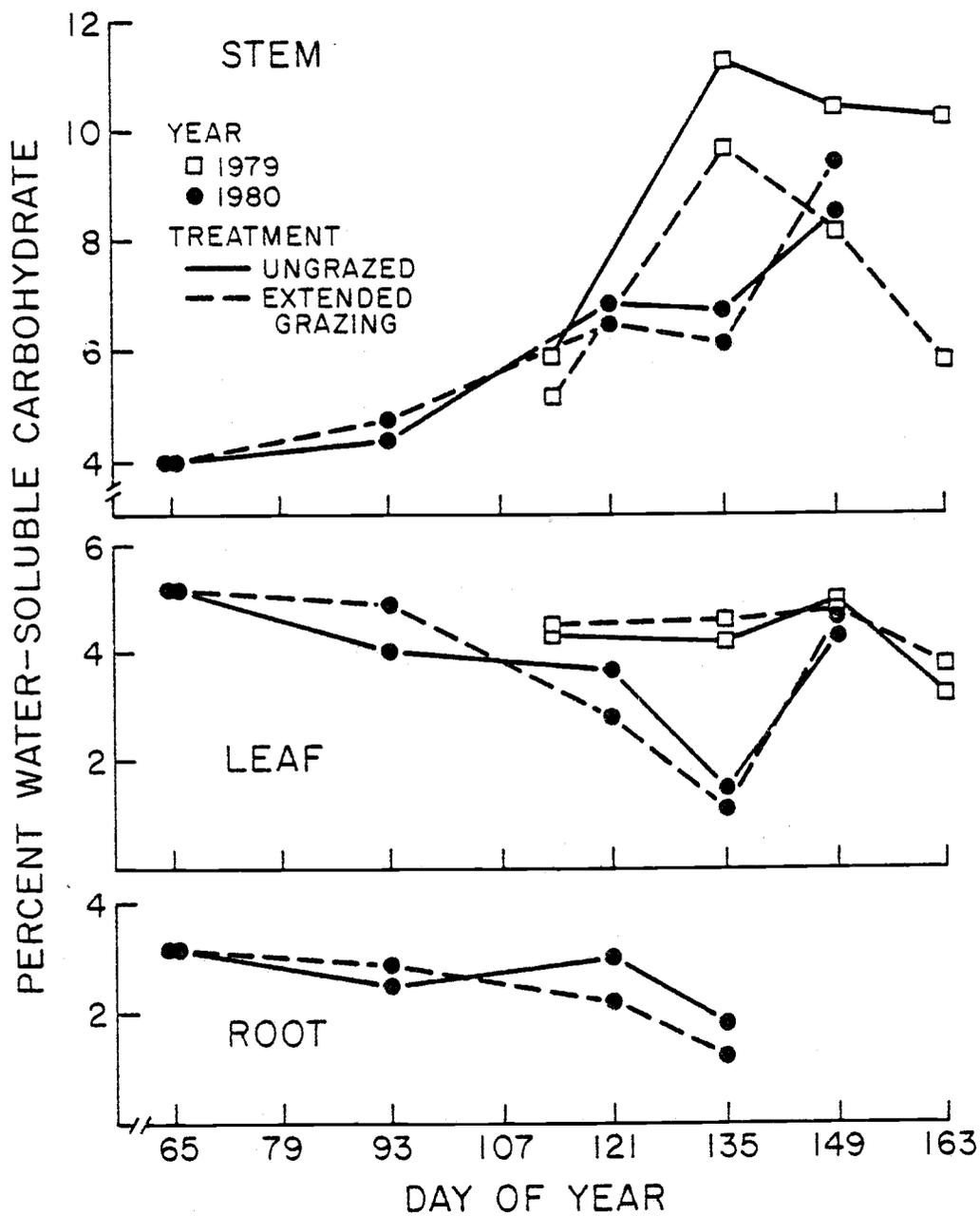


Figure 3. Effect of sheep grazing on percent water-soluble carbohydrate in stem, leaf, and root tissues of subterranean clover.

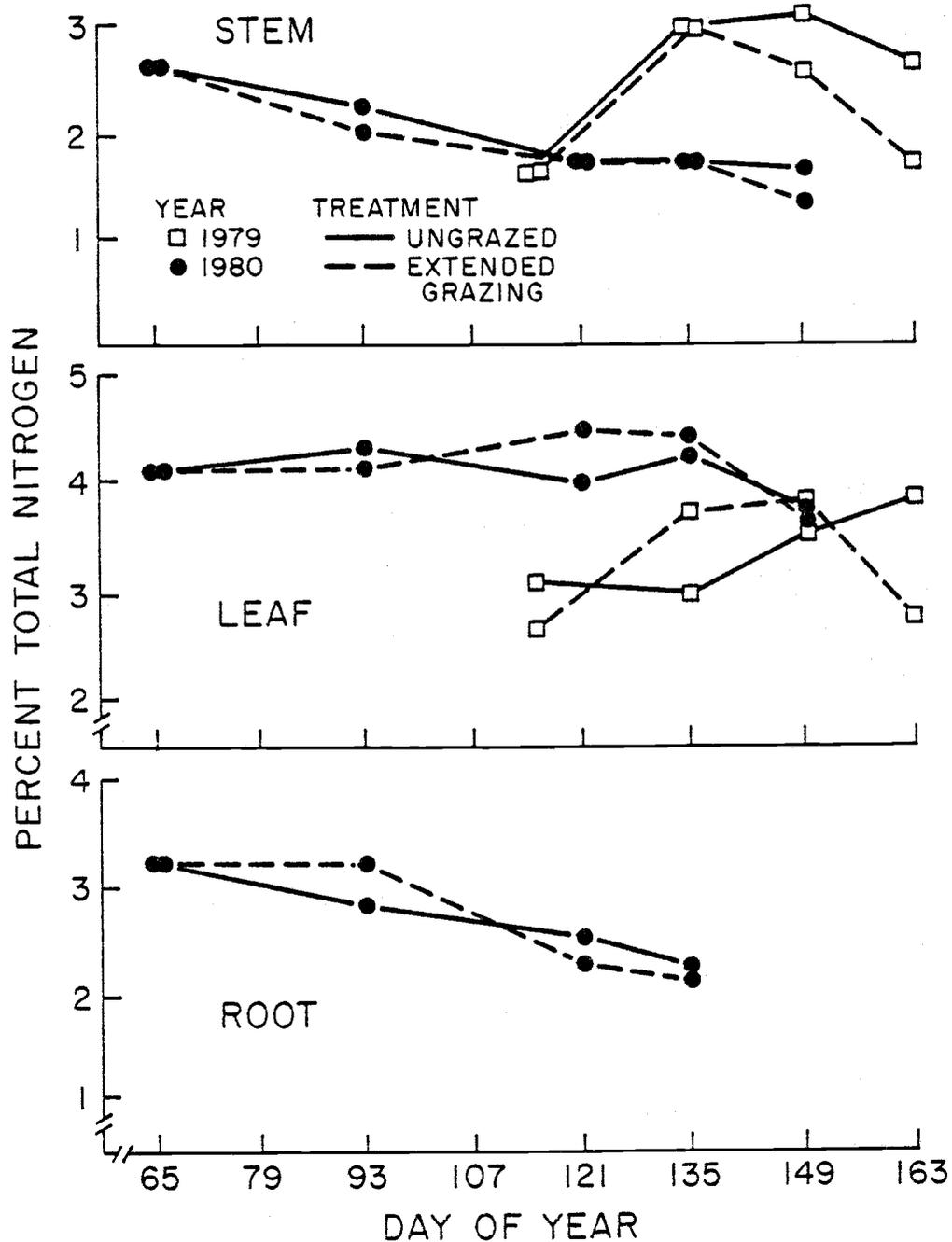


Figure 4. Effect of sheep grazing on percent total nitrogen in stem, leaf, and root tissues of subterranean clover.

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## MANUSCRIPT II

Effect of Moisture Stress and Sheep Grazing  
on Subterranean Clover Seed Production

## ABSTRACT

Subterranean clover (Trifolium subterraneum L.), or subclover, is a winter annual pasture legume of Mediterranean origin. The potential seed yield of this crop is often not reached because of a limitation in the amount of effective rainfall in late spring. This study was conducted to investigate the effects of natural moisture stress in the field on plant growth during reproductive development. The effects of sheep grazing at different moisture stress levels were also studied.

The field was indexed for moisture stress to characterize the amount of variable moisture stress present in the experimental area which was subjected to four levels of defoliation by sheep grazing. The variable moisture stress was due to varying soil depths which lay over a mudstone layer.

Seed yield, seed number, and seed size (g/100) showed negative correlations of  $-.84$ ,  $-.80$ ,  $-.84$ , respectively, with increasing moisture stress. Flower production was unaffected by extended grazing, but was reduced when precipitation declined during the previous 14-day period. The number of new burs formed was not influenced by this reduction in precipitation.

Seed yields were increased following grazing, regardless of the level of moisture stress. The extended grazing treatment gave the highest seed yield. Comparisons between this treatment and the control showed leaf area index, leaf dry weight, and total stem dry

weight to be reduced equally over the five moisture stress levels. Following recovery from grazing, the canopy height of the grazed treatment was at a lower level than the control, but the amount of reduction was similar at the different moisture stress levels. Total plant dry weight of the control was not reduced with increasing moisture stress; however, the grazed treatment caused a reduction with increasing moisture stress.

Contrary to general theories regarding the effect of moisture stress on hard seed formation, the amount of hard seed produced was unaffected by increasing moisture stress.

Additional index words: Trifolium subterraneum L., hardseededness, seed yield, flowering period, canopy height, leaf area index.

EFFECT OF MOISTURE STRESS AND SHEEP GRAZING  
ON SUBTERRANEAN CLOVER SEED PRODUCTION

INTRODUCTION

Subterranean clover (Trifolium subterraneum L.), or subclover, is a winter annual pasture legume of Mediterranean origin. It is adapted to regions which exhibit mild, moist winters and dry summers such as found in many areas of the western Pacific region of the United States.

The effects of water stress on plant development and structure of subclover grown in the greenhouse were documented by Andrews et al. (1977). They also showed that prolonged moisture stress greatly reduced seed yields in two early maturing varieties, 'Northam A' and 'Geraldton.' Short-term stress during late flowering resulted in reduced seed numbers in the short-flowering period Geraldton. No effect of short-term stress could be shown in the longer flowering Northam A. The effects of stress on reproductive development of other legumes has been shown in soybeans by Doss et al. (1974) and Sionit and Kramer (1977) who showed early seed formation and pod-filling were the critical periods of development. Field peas were most sensitive during flowering and pod-fill (Hiler et al. 1972).

Aparicio-Tejo et al. (1980) showed the influence of moisture stress on nitrogen fixation, stomatal response, and transpiration of subclover.

Sheep grazing is known to increase seed yields in subclover (Rossiter, 1961). However, there is no published information regarding the effects of sheep grazing on seed yields under varying moisture stress conditions.

The study was carried out in a field with variations in soil depth which limited the amount of moisture available to the crop during the reproductive stage of development.

#### MATERIALS AND METHODS

The experimental site was a first-year seeded field of 'Mt. Barker' subterranean clover (Trifolium subterraneum L.) planted in the fall of 1978 at a rate of 28 kg/ha on the Smith farm near Oakland, Oregon. The site location was mapped as i) loamy mixed nonacid mesic shallow typic xerorthents, ii) loamy mixed mesic shallow dystric xerochrept, iii) fine mixed mesic ultic haploxeralfs, and iv) fine-loamy over clayey mixed mesic ultic haploxeralf. These four soils ranged in depth from 0-25.4, 25.4-50.8, 50.8-101.6, and 101.6 + cm, respectively.

Moisture stress levels in the field were designated with the use of a site moisture stress index. This index was derived from a visual rating of every plot in the experimental area on 30 May 1979. Plots which showed no visible signs of wilting were assigned an index rating of 1; plots which showed approximately 25% wilting, a rating of 2; plots moderately wilted, a rating of 3; those

showing a large degree of wilting, but not completely wilted, a rating of 4; and the plots which were completely wilted, a rating of 5. The depth of the soil in each plot was determined with a soil probe which reached a maximum depth of 106 cm. Precipitation data were acquired from the Roseburg weather station located 32 kilometers south of the experimental area.

Flower and bur number were sampled on 25 April, 16, 30 May, and 14 June 1979. One 2 x 2 dm area was sampled in 4 plots of both the control and extended grazing treatments.

Twenty replications of four grazing treatments were established in late winter 1979. There were a variable number of moisture stress index levels within each treatment.

The grazing treatments, in order of increasing grazing intensity, consisted of an ungrazed control; a late grazed treatment applied after the peak of flowering (16 May to 22 May); an early grazed treatment applied between early and peak flowering (3 May to 16 May); and an extended grazing treatment applied between early flowering and the decline in flowering (3 May to 22 May). The stocking rate was five ewes/ha. Grazing was controlled with wire mesh enclosure cages 0.9 m high and 1.0 m in diameter. Plot sizes were 1.0 m in diameter.

All measurements of canopy components were made on 30 May 1979, after an adequate period of regrowth following the removal of grazing pressure. Four plots of the control and extended grazing treatment were sampled for leaf area, leaf dry weight, and

total stem dry weight. Leaf area was measured with a Li-Cor Portable Area Meter. These plots, plus an additional 19 plots from both grazing treatments, were measured for canopy height and plant dry weight. The sample was taken from a 2 x 2 dm area per plot.

Seed harvest was on 10 July from two 2 x 2 dm samples per plot. Data were collected for seed yield, seed size, and number of seeds produced from all 80 of the experimental plots. Seed size was determined by dividing seed yield per plot by the number of seeds produced per plot. Percent hard seed was determined by germinating two replications of 100 seeds on standard germination blotters for 7 days at 20 C. Any seed which did not imbibe water was considered to be hard.

All seed yield data were analyzed as an unbalanced block design with the site moisture stress index used as a treatment effect. Mean separation was done by comparing each moisture stress level and moisture stress x grazing treatment combination with an unpaired t-test.

Due to the unbalanced nature of the design, the number of samples was too small to perform mean separation of the canopy components over the different moisture stress levels. Therefore, only linear regression analysis was used to determine trends.

All comparisons made are significant at the 5% level of probability unless otherwise indicated.

## RESULTS

### SOIL DEPTH / SITE MOISTURE STRESS INDEX

The range of moisture stress levels in the field was due to variability in soil depth to an underlying mudstone layer. The mudstone layer ranged from a minimum of 22 cm to more than 106 cm beneath the soil surface. The means of the soil depth for each grazing treatment were negatively correlated with the site moisture stress index ( $r = -.91$ ,  $p = .001$ ).

### FLOWERING

Flowering was unaffected by the extended grazing treatment, but was related to the precipitation patterns in 1979. This was established by comparing the flowering patterns to the weekly accumulated precipitation (Figure 1). Flower formation was reduced by the decline in spring precipitation after 9 May 1979. Peak flowering in that year was on 16 May and was effectively stopped by 13 June. The production of new burs was not affected greatly by the short-term declines in precipitation (Figure 1).

## SEED YIELD AND YIELD COMPONENTS

Seed yield, seed size, and number of seeds were all reduced as moisture stress increased (Table 1). The four grazing treatments affected seed yield and number of seeds differently over the different moisture stress levels. With extended grazing, the yield advantage over the ungrazed control was greater at the lower moisture stress levels than at the higher stress levels (Figure 2). The same response was seen for number of seeds (Figure 3). Early and late grazing produced results which were intermediate to extended grazing and the control. Over all treatments, seed number was correlated with seed yield ( $r = .99$ ,  $p = .001$ ).

Sheep grazing reduced seed size equally over the different moisture stress levels and was negatively correlated with moisture stress ( $r = -.85$ ,  $p = .001$ ).

Hard seed content was unaffected by moisture stress (Table 1).

## PLANT CANOPY

Plant dry weights in the ungrazed control were unaffected by increasing moisture stress but were reduced under extended grazing with each successive increase in moisture stress (Figure 4).

Leaf dry weight, leaf area index, and total stem dry weight were reduced with increasing moisture stress under both the ungrazed and extended grazing treatments (Figure 5). The height of

the canopy in both of these grazing treatments was reduced as moisture stress increased. The canopy under extended grazing was approximately 7 cm shorter than that of the ungrazed control (Figure 6).

## DISCUSSION

Increasing moisture stress during the reproductive phase of development had the expected result of decreasing seed yield, seed size, and number of seeds produced. These field results are in agreement with those of Andrews et al. (1977) under greenhouse conditions.

Moisture stress under these field conditions did not occur until flowering because the soil moisture remains near water-holding capacity until precipitation declines in late spring or early summer. It is at this time, as is often the case in western Oregon, that soil moisture tensions increase rapidly unless precipitation resumes (S.H. Sharrow, personal communication). It is soon after the decline in precipitation that the rate of flower production decreases (Figure 1). The decrease in flower production was observed within 14 days of the decline. Clarkson and Russell (1976) showed results similar to these with several annual species of Medicago.

Stoddart et al. (1975) suggest that plants which grow in arid regions benefit from grazing by having the total transpirational surface area of their canopies reduced, which thus conserves

moisture. Since the leaf surface area of the grazed treatment was similar to that of the control over all moisture stress levels, this notion does not seem to be wholly supported. In addition to this, King and Evans (1967) showed subclover had little increase in transpiration with leaf areas in excess of LAI 3.0. Since even the most stressed plot had a LAI of nearly 3.0, it appears that little moisture would be conserved at this level as compared to a higher LAI.

The benefits of sheep grazing in conserving moisture may be due to the modification of the entire vegetative canopy and not just the transpirational surface area. The canopy height in the grazed treatment was reduced below that of the control at all moisture stress levels (Figure 6). This was most likely due to a reduction in petiole length following the removal of grazing pressure. When all of the canopy components are examined together, it is shown that there is a reduction in total plant dry weight with increasing moisture stress in the extended grazing treatment and no reduction in the control (Figure 4). This suggests that less vegetative material is being generated in the grazed plots as moisture stress increases. Since the control has not been reduced in total dry matter by grazing, it enters the reduced precipitation period with a large amount of canopy material which must be maintained. The grazed treatment enters this period with less total vegetative material to support and is therefore able to partition more of the available moisture to the development of seed. This may explain why there was an increase in the number of seeds produced in all of the grazing treatments (Figure 2).

The fact that moisture stress had no effect on the amount of hard seed produced (Table 1) was surprising in light of Aitken's (1939) conclusions that formation of hard seed was dependent on the length of the growing season. Quinlivan (1965) also determined that increasing the length of the growing season increases hard seed formation. The present findings are not in agreement with these conclusions and support those from Taylor and Palmer's (1979) greenhouse experiment.

Moisture stress during seed development did not have an effect on hardseededness. The length of the seed development period was shortened in the stressed plots. This should have reduced the amount of hard seed produced in the stressed plots if Aitken's (1939) and Quinlivan's (1965) conclusions were true. Since this was not the case, the general theories regarding factors which determine hard seed development in subclover are not true under all conditions.

Table 1. Effect of moisture stress on subterranean clover seed yield and its components.

Component	Moisture stress index <sup>†</sup>				
	1	2	3	4	5
Seed yield (kg/ha)	984 a <sup>‡</sup>	872 a	461 b	397 b	217 c
Seed size (g/100)	.74 a	.72 a	.62 b	.52 c	.50 c
Number of seeds/4 dm <sup>2</sup>	567 a	490 a	300 b	270 b	162 c
Hard seed (%)	76 a	79 a	78 a	78 a	73 a

<sup>†</sup>Moisture stress index values range from 1 - lowest to 5 - highest stress.

<sup>‡</sup>Means average over four grazing treatments within each line followed by a different letter are significantly different at the 5% level of probability according to an unpaired t-test for mean to mean comparisons.

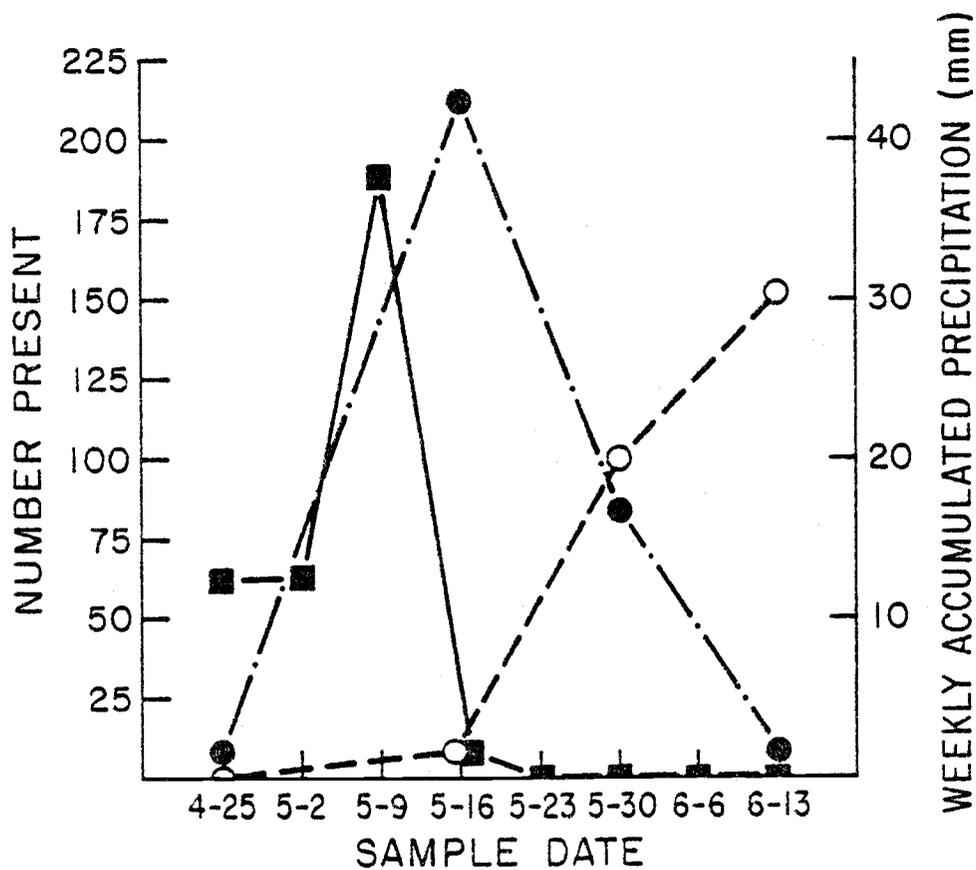


Figure 1. Relationship of flower and bur formation of subterranean clover to weekly accumulated precipitation in 1979. ● - flowers; ○ - newly formed burs; ■ - preceding seven-day accumulated precipitation.

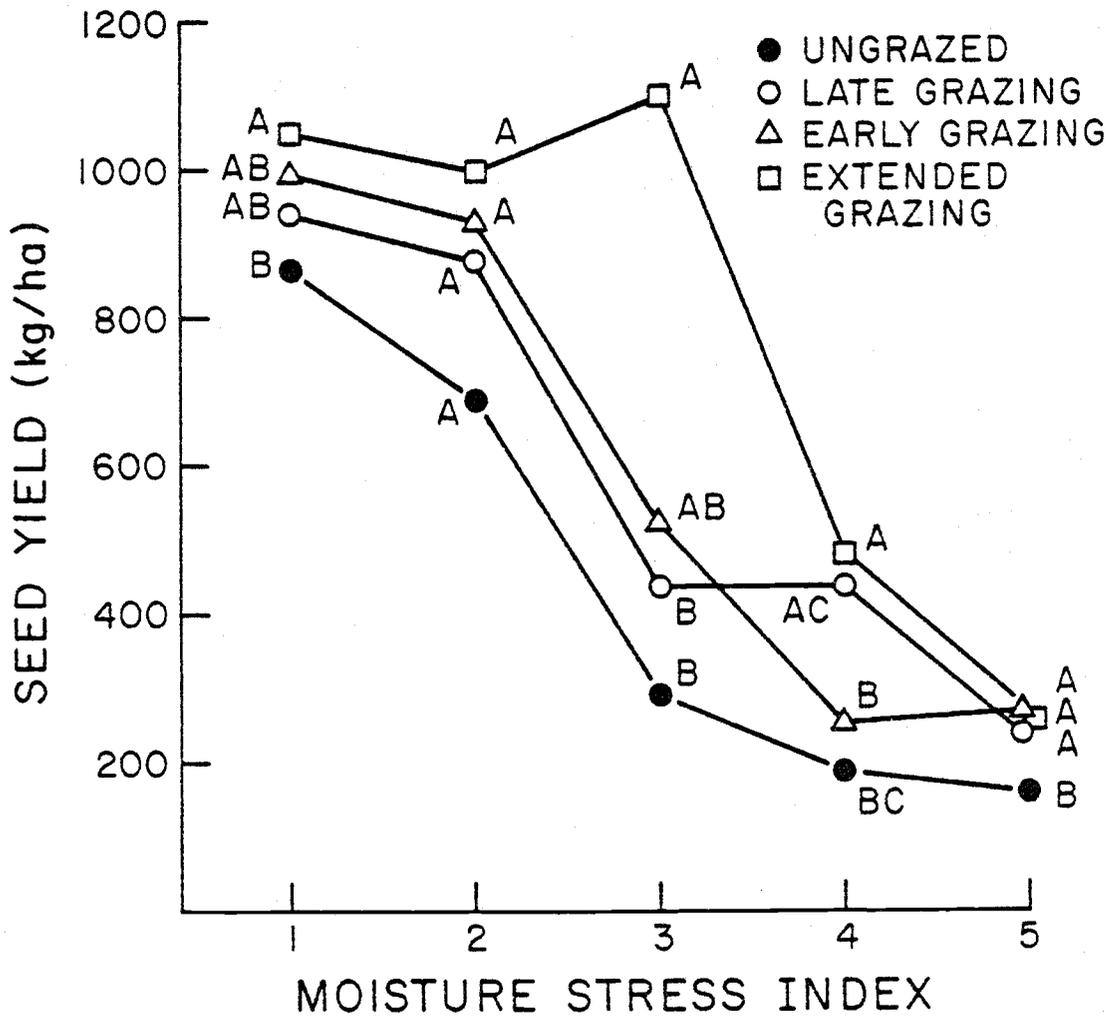


Figure 2. Moisture stress and sheep grazing effects on seed yield of subterranean clover. Significant differences ( $P = .05$ ) between grazing treatments within each moisture stress level are present when data points are marked with different letters.

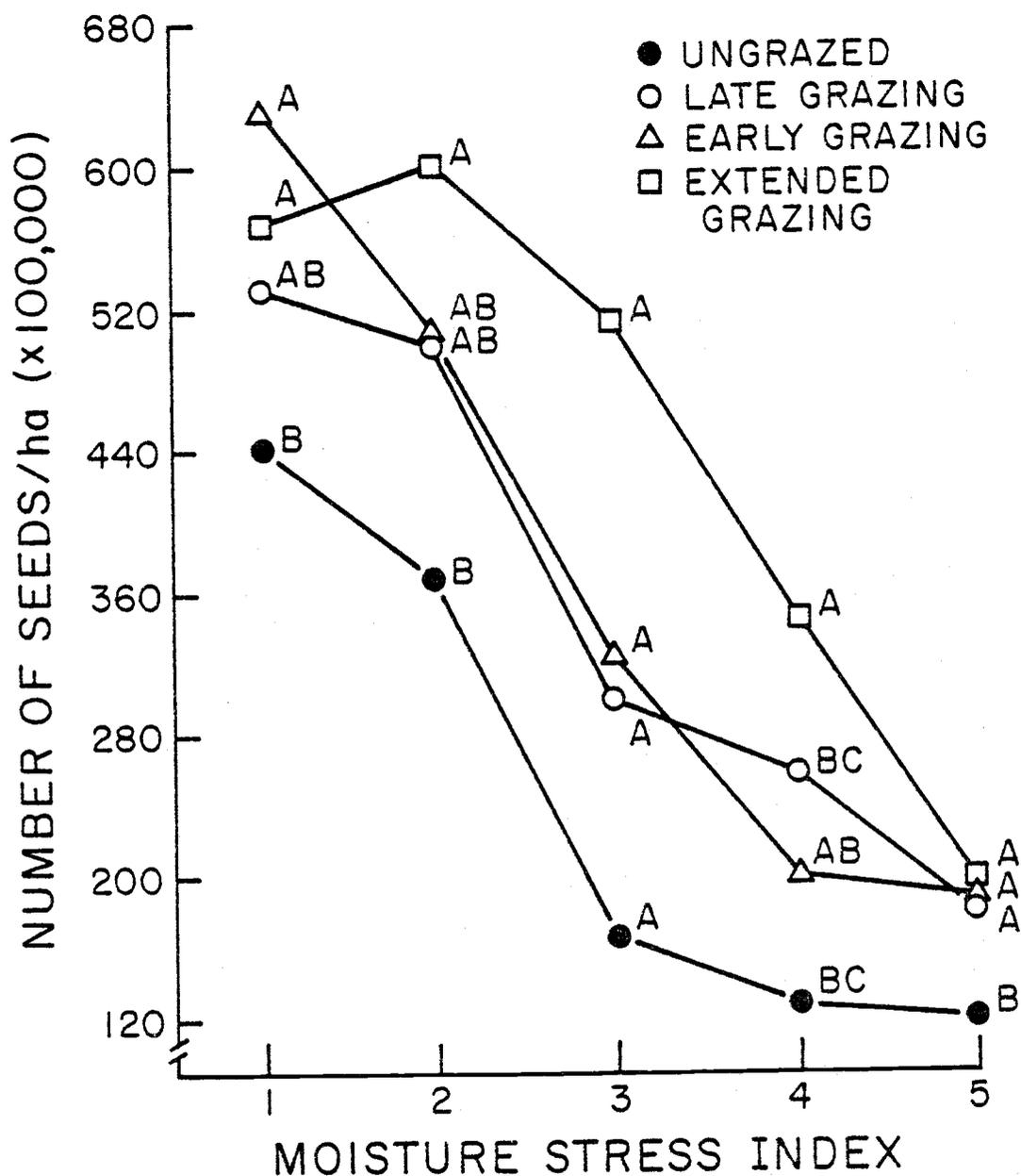


Figure 3. Moisture stress and sheep grazing effects on number of seeds produced in subterranean clover. Significant differences ( $P = .05$ ) between grazing treatments within each moisture stress level are present when data points are marked with different letters.

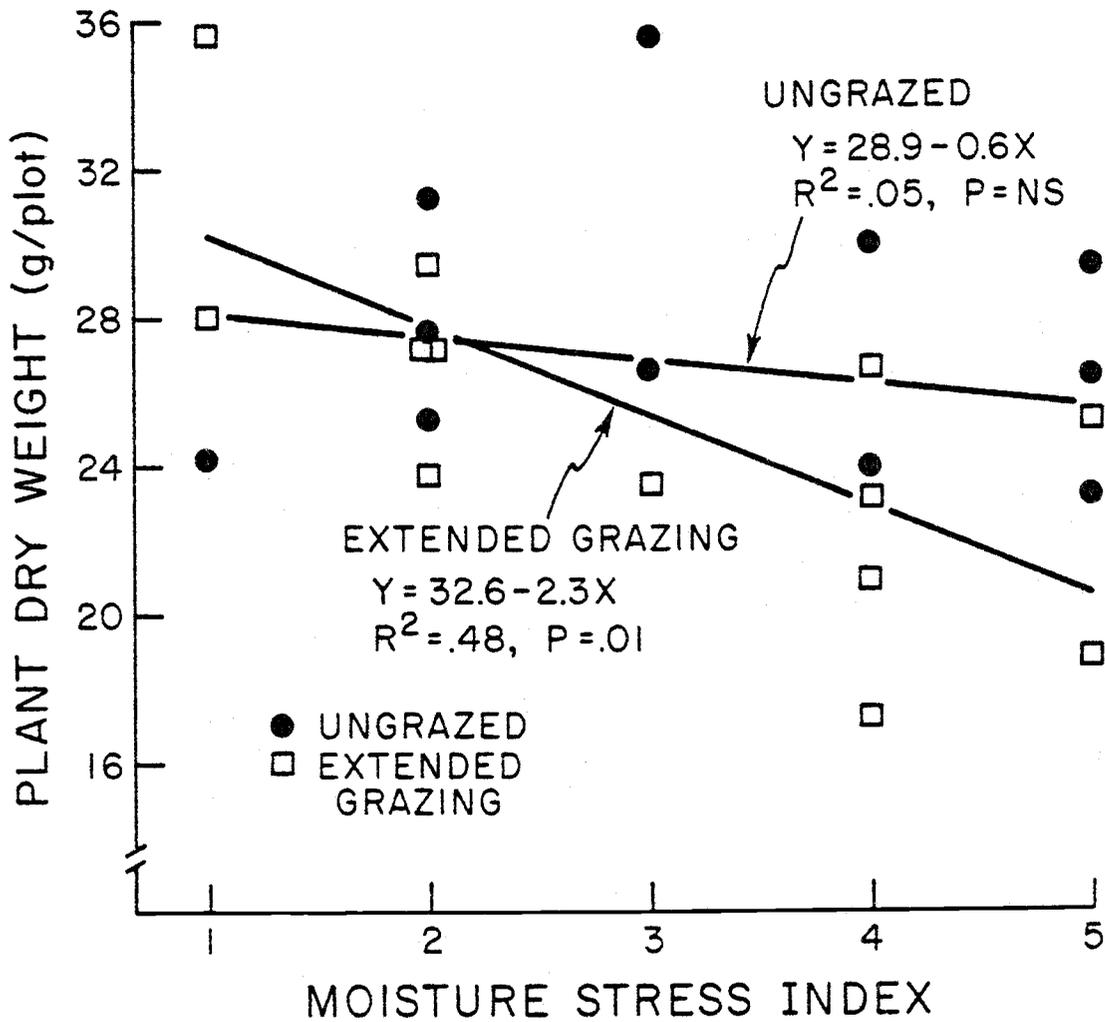


Figure 4. Moisture stress and sheep grazing effects on plant dry weight of subterranean clover.

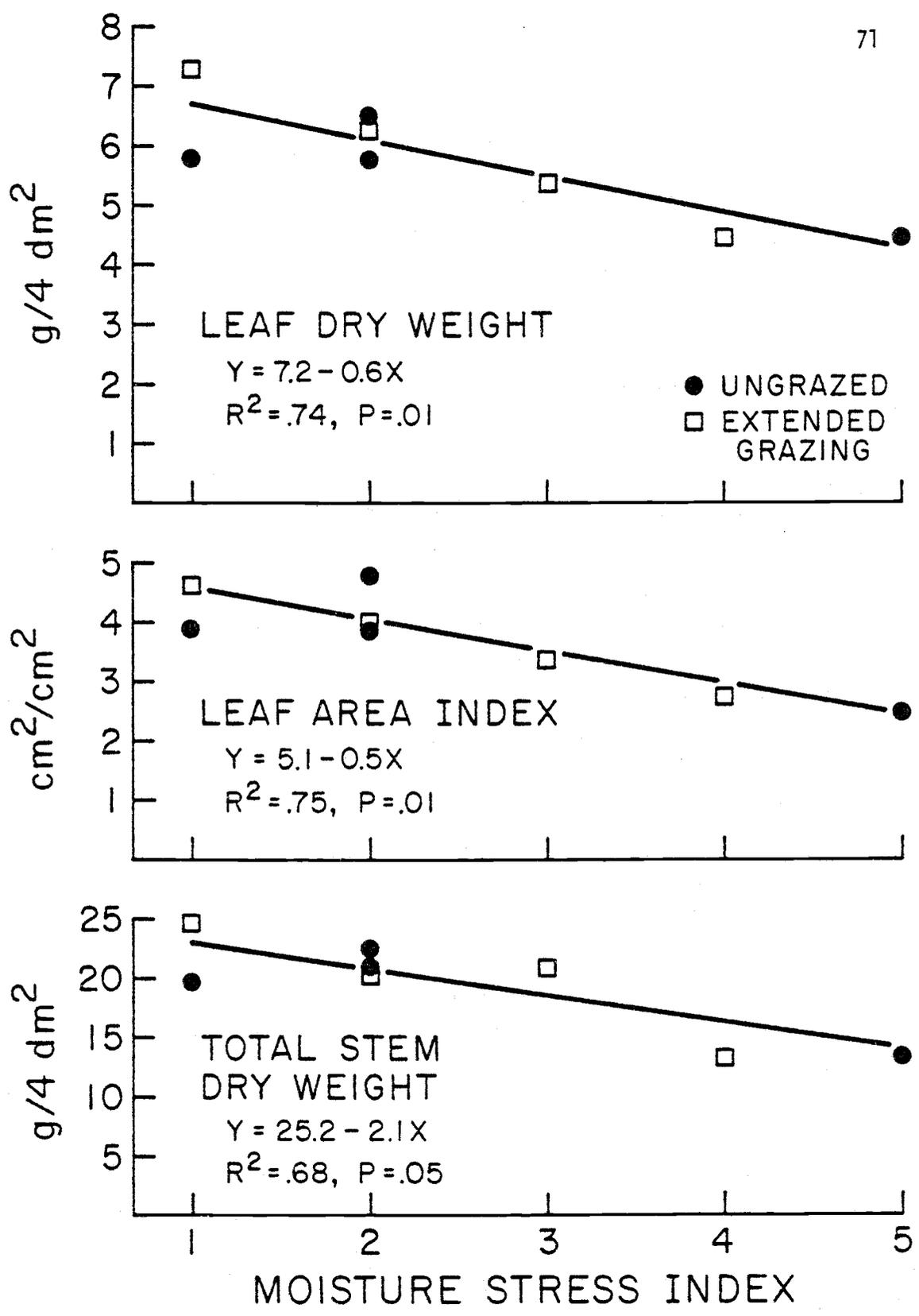


Figure 5. Moisture stress effects on leaf dry weight, leaf area index, and total stem dry weight of subterranean clover.

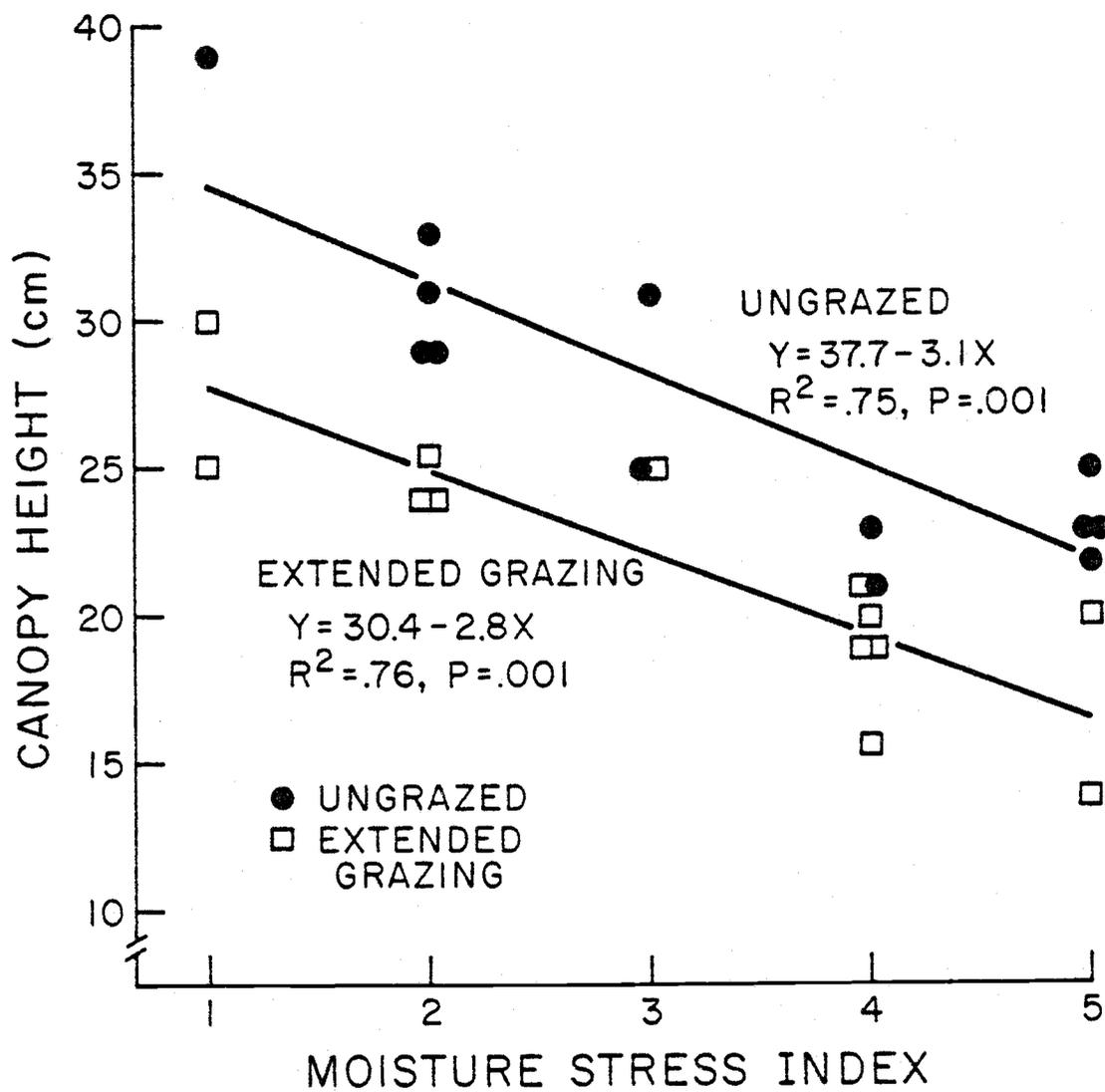


Figure 6. Moisture stress and sheep grazing effects on canopy height of subterranean clover.

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## MANUSCRIPT III

Effect of Specific Leaf Weight on the Prediction of  
Leaf Area from Leaf Dry Weight in Subterranean Clover

## ABSTRACT

Equations were developed which described the relationship between leaf area and leaf dry weight in samples of subterranean clover (Trifolium subterraneum L.). The samples were obtained from field and greenhouse plots grown in 1979 and 1980.

Discrepancies were shown between the equations which predicted leaf area from leaf dry weight. These differences were accounted for by variations in the specific leaf weight of the various samples. The origin (field and greenhouse) and treatment (defoliation and time of sampling) of the samples were shown to cause differences in specific leaf weight.

Careful consideration should be given to selecting the leaf samples which are to be used to estimate leaf area from leaf dry weight. Not only should the location and treatment of the sample be considered, but also the position of the leaves within the canopy.

Additional index words: Trifolium subterraneum L., prediction equations, defoliation, canopy structure.

EFFECT OF SPECIFIC LEAF WEIGHT ON THE PREDICTION OF  
LEAF AREA FROM LEAF DRY WEIGHT IN SUBTERRANEAN CLOVER

INTRODUCTION

Methods for estimating leaf area in several crops have been well documented and described in reviews by Aase (1978) and Ogbuehi and Brandle (1981). These include accurate measurements with air-flow planimeters (Jenkins, 1959) and electronic leaf area meters (Hatfield et al., 1976) and less accurate estimates based on leaf length (Kemp, 1960) or mid-rib length (Davidson and Donald, 1958; Wiersma and Bailey, 1975).

An even simpler method is to use the linear relationship between leaf dry weight and leaf area. Watson (1937) described this method and used it for estimating the leaf area of several crops. Even though the results of this method are generally satisfactory, there are some conceptual problems involved.

Marshall (1968) was concerned that such estimates may be in error if the specific leaf weight (SLW -- grams dry weight of leaf/cm<sup>2</sup> leaf area) of all samples was not constant under all conditions during the sampling period. Aase (1978), using his results and citing those of Frey and Moss (1976), concluded that SLW remained constant throughout the growing season. Aase therefore dispelled the concerns of Marshall, at least for wheat.

This study was conducted to investigate whether prediction of leaf area from leaf dry weight in a broadleaf crop, subterranean clover (Trifolium subterraneum L.), would produce results as

satisfactory as those shown by Aase (1978) for a cereal. A simple linear regression model for all samples was developed which represented the relationship between leaf area and leaf dry weight. Sets of data collected in the field and greenhouse were broken out of this full model. These sets were further divided into subsets which described the effects of defoliation, time of sampling, phenological stage of development, and position of leaves within the canopy on SLW. SLW differences were used to explain the differences in the linear regression results.

#### MATERIALS AND METHODS

Forty-six samples of 'Mt Barker' subterranean clover (Trifolium subterraneum L.) were collected from field and greenhouse plots.

Four samples of grazed and ungrazed plots in the field were collected on three sampling dates in 1979 (25 April, 16 May, and 30 May).

Swards of Mt. Barker were planted in the greenhouse to a density of 100 plants/m<sup>2</sup> in 1979 and 1980. One sample was taken from the 1979 sward during vegetative, early flowering, and early bur fill (14 March, 10 April, and 25 May, 1980). Two samples were taken from the 1980 sward during late bur fill (15 January 1981); one plot had been defoliated to a height of 10 cm during flowering and the other was not.

Leaves were samples in 5 cm increments from the soil surface to the top of the canopy in all greenhouse plots.

The field samples were taken from a 2 x 2 dm area and the greenhouse samples taken from a 3 x 3 dm area.

Leaf area was measured with a Li-Cor Portable Area Meter. Unfolded leaves were included with the greenhouse sample sets. The leaves were dried in a forced-air oven at 60 C for approximately 48 hours and then weighed.

Leaf area was regressed on leaf dry weight for all samples by the least-squares method. Sample sets taken from the field and greenhouse were likewise analyzed and tested with the model for all samples combined. The subset samples were also handled in this manner and tested with their respective sample set models for significant differences.

Specific leaf weight (SLW) was determined by dividing the sample leaf weight by its leaf area. Analysis of variance was used to test for differences between sample subset SLW results.

## RESULTS AND DISCUSSION

The relationship between leaf area and dry weight for all samples is shown in Figure 1. The regression equation given accounts for 93% of the variation. The regression equations for the sample sets and those of the subsets are given in Table 1.

The variations in the prediction of leaf area from leaf dry weight were accounted for by differences in the specific leaf weight (SLW) of the samples. The mean SLW of the field and greenhouse sets was 4.3 and 3.7 mg/cm<sup>2</sup>, respectively (P = .05). The relative position of the greenhouse derived data to that of the field data (Figure 2) resulted from this difference in SLW. The

difference in SLW of the two sets is probably due to the greenhouse set growing under lower light intensities than the field set.

Regression of leaf area on leaf dry weight of the subset samples likewise revealed that their regression equations differed from that of all combined samples (Figure 3). Differences could also be shown between the subset equations and their respective set equations in most cases (Table 2).

The time of sampling in relationship to phenology affected the SLW of the samples taken in the greenhouse (Figure 4). The increase in SLW with time is in agreement with the hypothesis of Watson (1937) that the leaf area to leaf weight ratio should decrease with time of development. This is different from the conclusions of Frey and Moss (1976) with barley and Aase (1978) with wheat where SLW remained constant throughout the growing season.

SLW of the samples not only changed with phenological development, but also changed with position of the leaves within the canopy (Figure 5). As the plant developed, more leaf material was added to the canopy, resulting in greater degree of shading in the lower regions of the canopy. It would be expected that shaded leaves would have a lower SLW much the same as the common practice of shading tobacco leaves to produce thinner leaves (low SLW). Figure 5 shows that this effect is greatest in the latest sample date in the 5-10 cm level. The higher SLW with

time in the 0-5 cm level is due to a higher portion of the leaves found in that level being unfolded. Few fully expanded leaves are found in this level since peduncle elongation soon moves leaves to a higher position within the canopy.

This same relationship was shown when clipped and unclipped plots in the greenhouse were compared (Figure 6). Leaves located in the lower levels of the canopy have a smaller SLW as compared to the leaves near the top of the canopy. The lowest level shows a higher SLW than the levels above them due to the greater portion of unfolded leaves. The generally lower SLW values of the unclipped plots were probably due to the greater amount of shading which occurred from the greater leaf area in that treatment (2537 and 2227 cm<sup>2</sup>, respectively).

In the field, defoliation by sheep grazing did not affect the SLW of the grazed treatment (Figure 7). However, the SLW of the samples decreased with time, which is opposite of the effect observed in the greenhouse samples (Figure 4).

The results shown here indicate that a great deal of variability results in the SLW of samples obtained from the field and greenhouse. The location of leaves within the canopy, as well as the time of sampling, phenological stage of development, and defoliation also influence the SLW of the samples.

These findings show that there is a great deal of variation in the ability to predict leaf area from leaf dry weight and are in agreement with those of Ogbuehi and Brandle (1981) using soybean. Much of this is due to variability in SLW.

Based on the limited amount of literature which describes the prediction of leaf area by this means, it appears that leaf area of cereals may be accurately predicted in this manner. The results of this study and that of Ogbuehi and Brandle (1981) indicate that at least in the case of subterranean clover and soybean, careful consideration must be given to the selection of leaf samples which are to be used to derive equations to predict leaf area from leaf dry weight.

Table 1. Regression equations for predicting leaf area from leaf dry weight in subterranean clover.

Source of samples	Regression equation	Number of samples	R <sup>2</sup>
All samples combined	$Y = 20.3 + 244.9 X$	46	.93
<u>Field samples</u>			
Combined	$Y = -229.1 + 288.1 X$	24	.91
Ungrazed	$Y = -249.8 + 288.6 X$	12	.88
Grazed	$Y = -246.8 + 296.1 X$	12	.93
<u>Greenhouse samples</u>			
Combined	$Y = 22.3 + 265.1 X$	22	.91
Vegetative	$Y = -139.7 + 293.5 X$	5	.97
Flowering	$Y = 48.5 + 248.7 X$	5	.90
Early bur fill	$Y = -12.1 + 372.1 X$	4	.99
Late bur fill	$Y = 10.4 + 283.2 X$	8	.99

Table 2. F values and levels of significance for the comparison of regression equations for predicting leaf area from leaf dry weight in subterranean clover.

Source of samples	All samples combined	Field samples combined	Greenhouse samples combined
<u>Field samples</u>			
Combined	8.7 <sup>†</sup> (.005) <sup>‡</sup>	--	--
Grazed	21.1 (.001)	9.6 (.005)	--
Ungrazed	9.1 (.01)	2.9 (NS)	--
<u>Greenhouse samples</u>			
Combined	38.8 (.001)	--	--
Vegetative	91.4 (.005)	--	17.5 (.025)
Flowering	46.0 (.01)	--	8.2 (NS)
Early bur fill	2183.5 (.001)	--	262.2 (.001)
Late bur fill	200.1 (.001)	--	38.6 (.001)

<sup>†</sup>F value

<sup>‡</sup>Level of significance

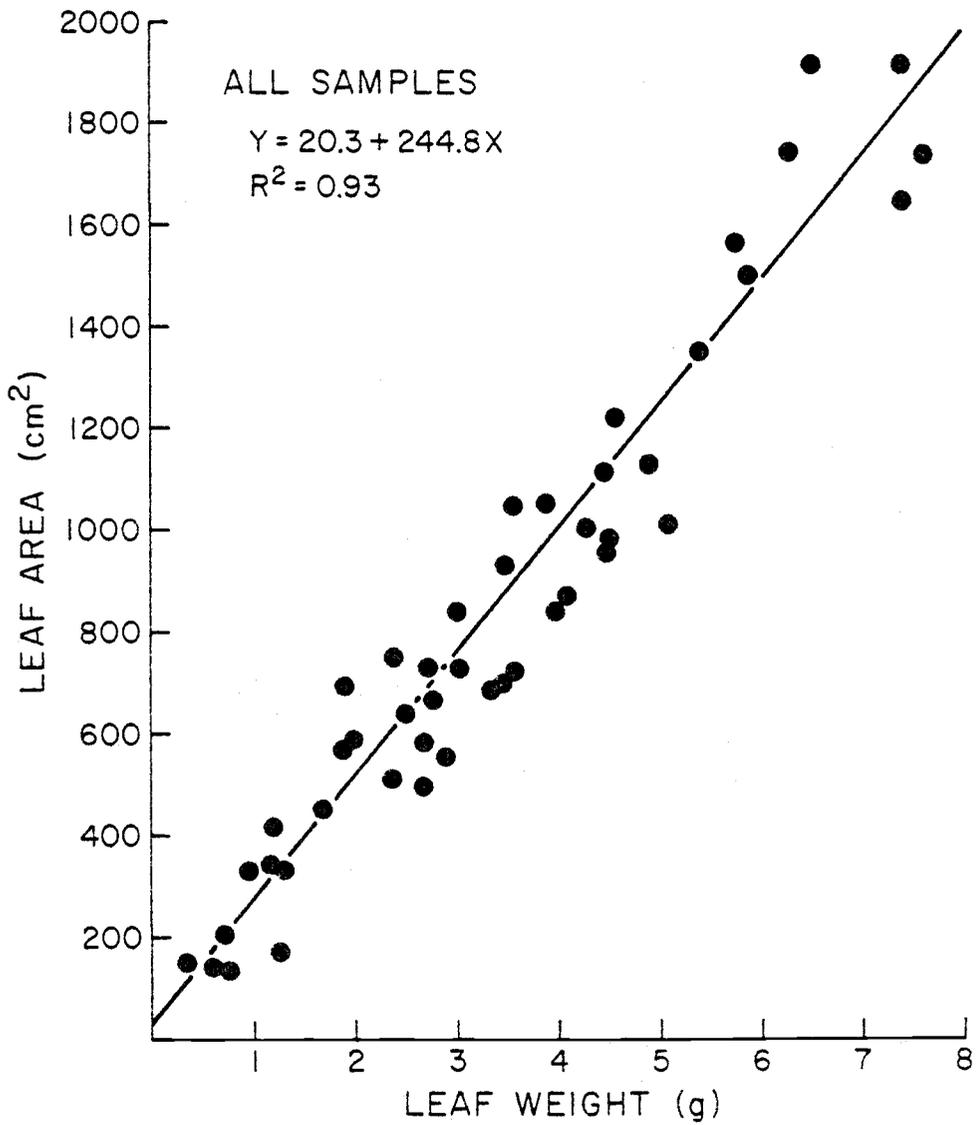


Figure 1. Leaf area vs. leaf dry weight for 46 samples of subterranean clover.

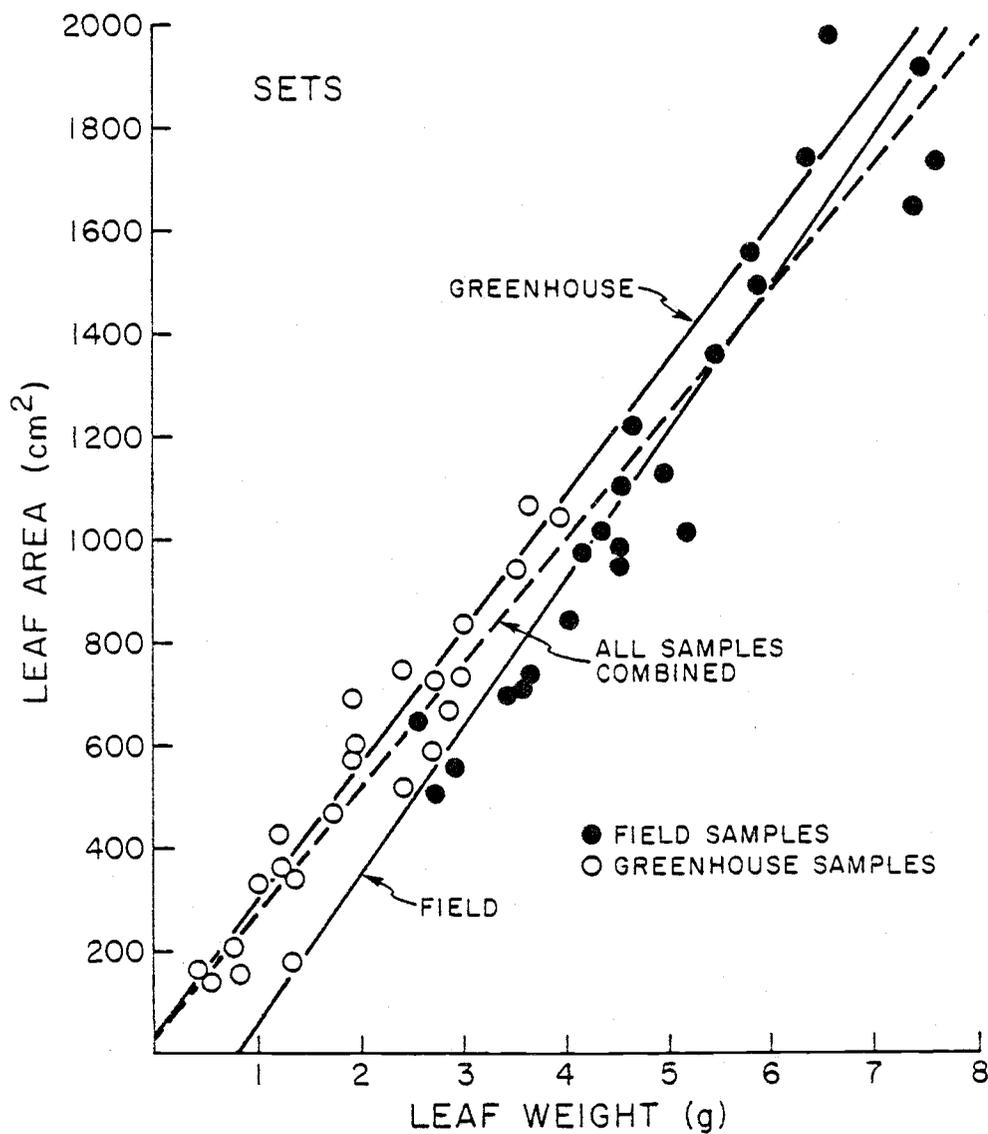


Figure 2. Leaf area vs. leaf dry weight obtained from field and greenhouse samples of subterranean clover.

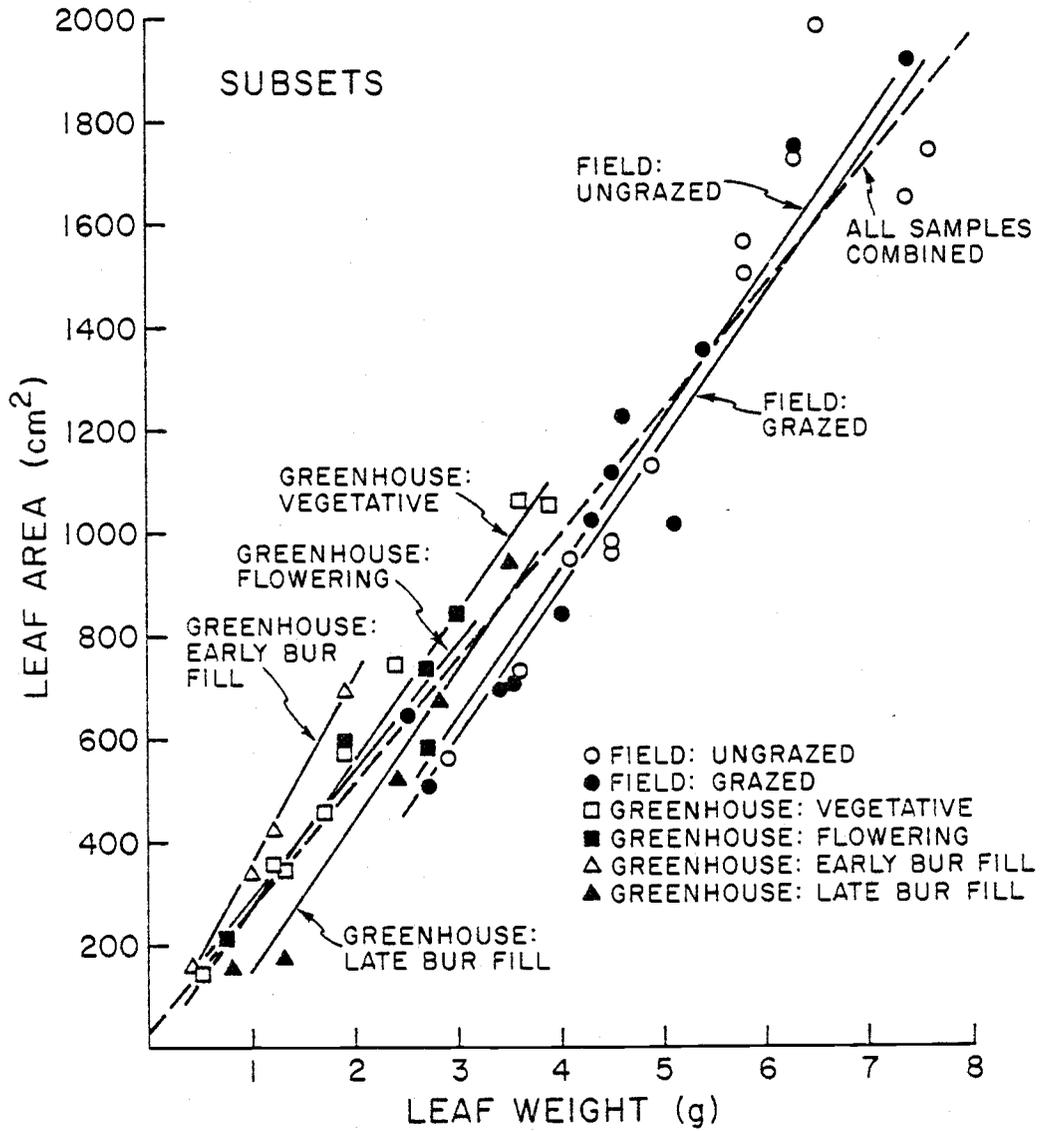


Figure 3. Leaf area vs. leaf dry weight for samples of subterranean clover obtained from various sources.

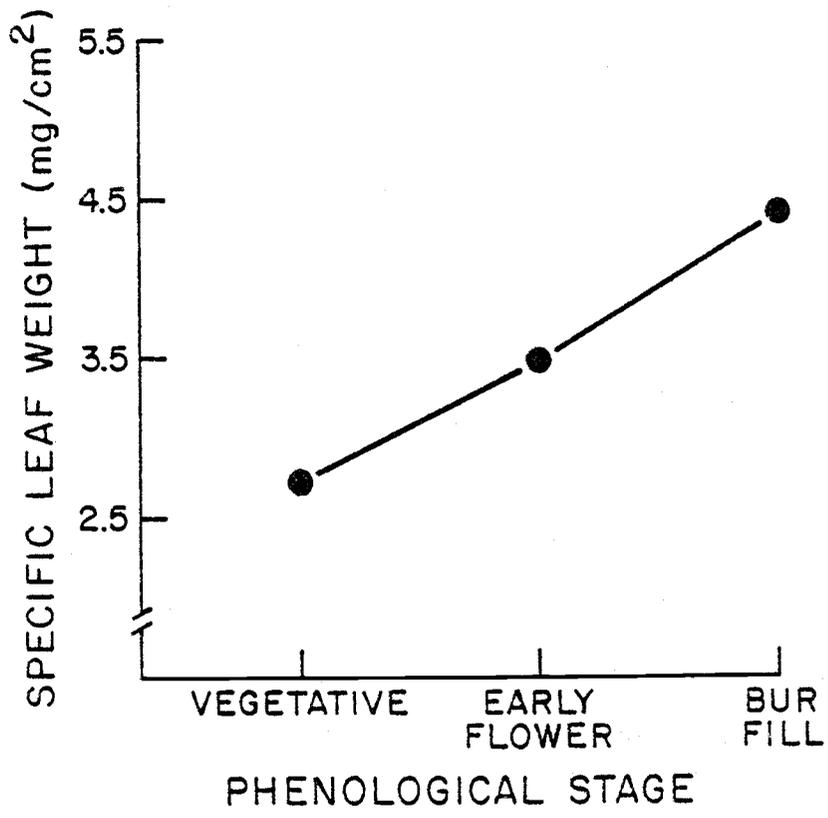


Figure 4. Effect of phenological stage on the specific leaf weight of subterranean clover grown in the greenhouse.

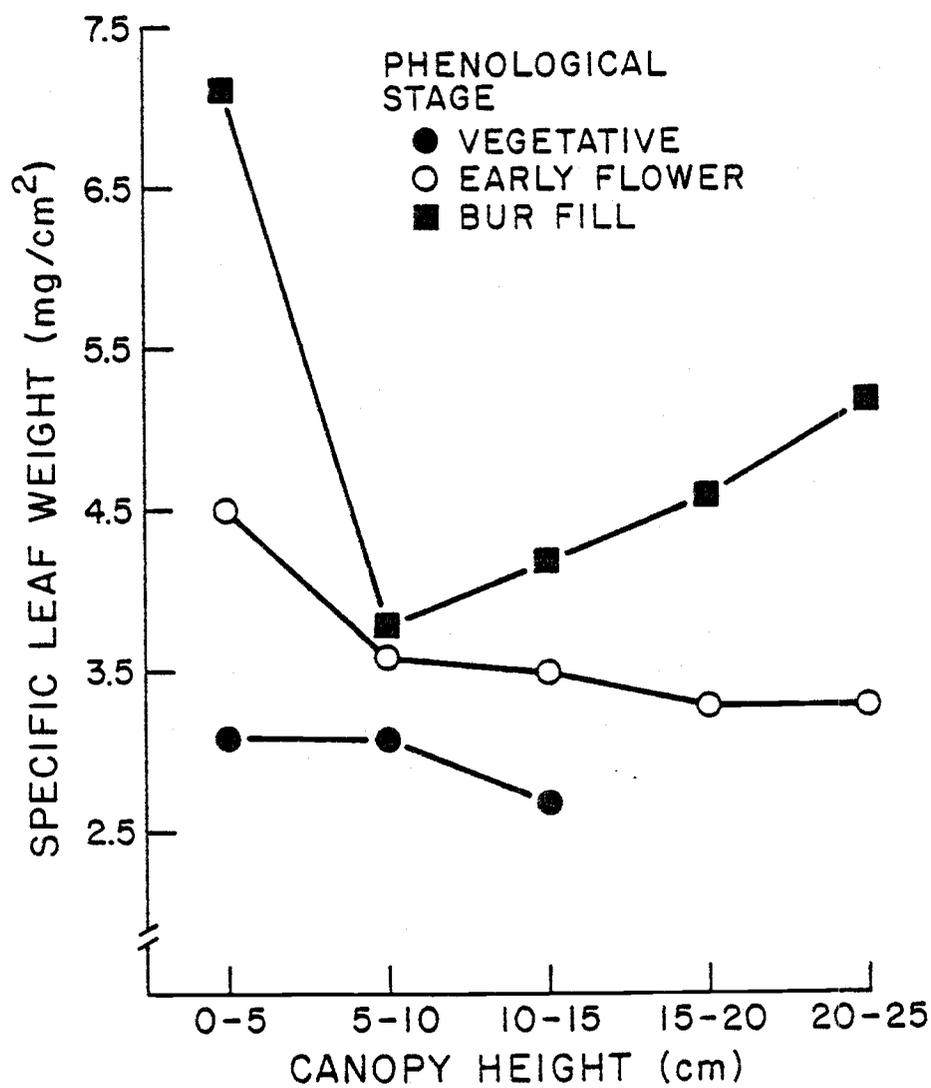


Figure 5. Effect of phenological stage and leaf location within the canopy on the specific leaf weight of subterranean clover grown in the greenhouse.

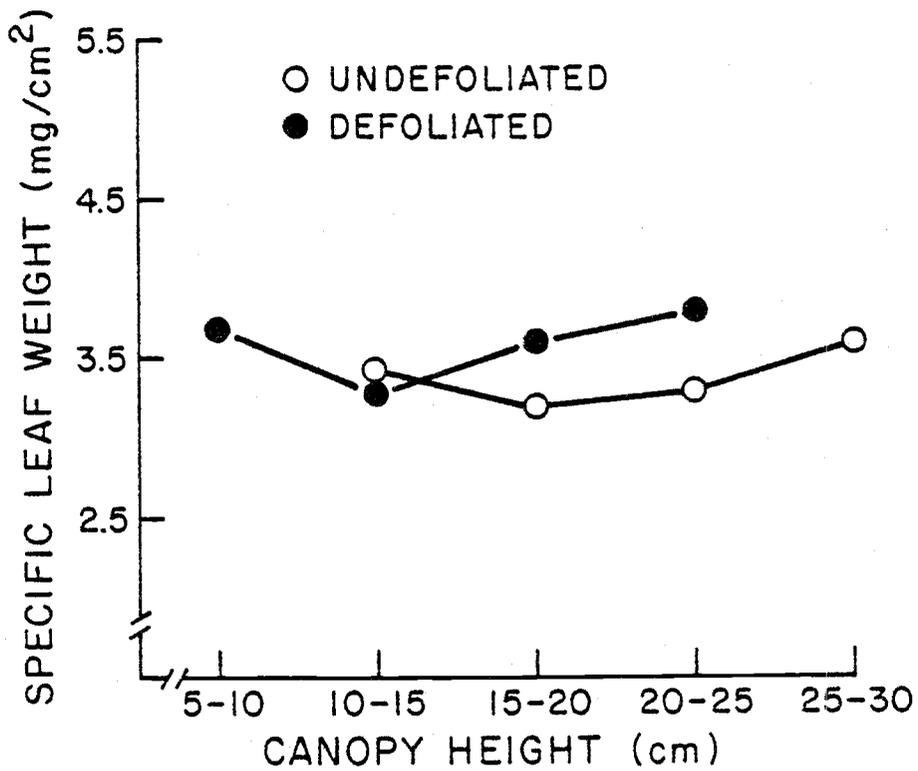


Figure 6. Effect of defoliation and leaf location within the canopy on the specific leaf weight of subterranean clover grown in the greenhouse.

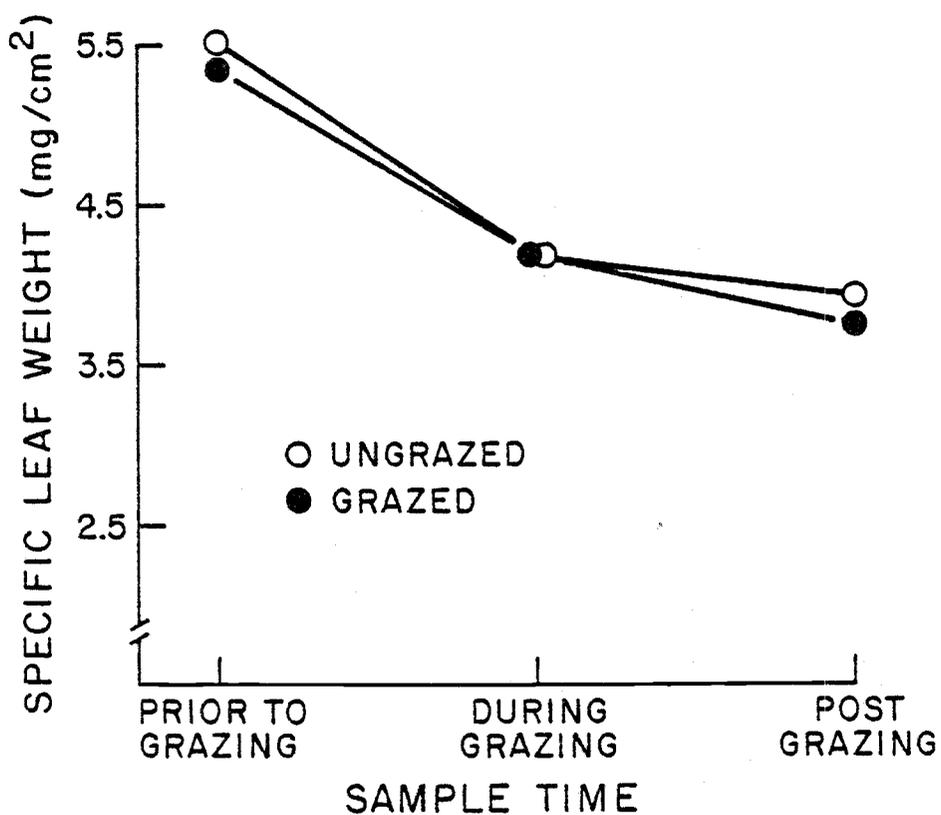


Figure 7. Effect of time of grazing on the specific leaf weight of subterranean clover grown in the field.

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MANUSCRIPT IV

Production of Subterranean Clover Seed  
in Western Oregon

PRODUCTION OF SUBTERRANEAN CLOVER SEED  
IN WESTERN OREGON

INTRODUCTION

Subterranean clover (Trifolium subterraneum L.). or subclover, is a winter annual pasture legume which is adapted to much of the hill-land pasture region west of the Cascades. Subclover is native to the Mediterranean and western European regions. It has been successfully cultivated in Australia since the late 1800's as a pasture crop with seed being produced there commercially since 1906 (1).

Subclover was introduced into the United States from Australia in the 1920's. The first plantings were made in Oregon in 1922 on the Agricultural Experiment Station at Corvallis (5). Some of the earliest commercial plantings for pasture were made in the late 1920's in Douglas county. Some stands of those early plantings can still be found -- a credit to the persistent nature of this plant.

The first commercial seed fields of subclover in Oregon were planted in 1941 (5), with production reaching a peak in 1951 with 2400 acres. The average yield at that time was 82 lbs/acre. The main areas of production were in the Willamette Valley with a small amount in the lower river valleys of south central Oregon (2). Acreage for seed production fell to less than 50 acres between the late 1950's and mid-1970's, but began to increase again in the late 1970's. The number of acres increased to approximately 305 in 1980 and more than 500 in 1981. Most of this acreage has shifted from

the traditional seed-producing areas in the Willamette Valley to the valleys around the Umpqua River in Douglas County. There is some production in Lane County.

### Description

Subclover is established in the fall following the first effective rainfalls. It characteristically has large percentages of hard seed and seed with dormant embryos. This insures that stands will not be greatly reduced if early-season rainfall is followed by dry periods.

Depending on the severity of the winter temperatures, subclover grows slowly during the winter months. A rapid increase in growth is observed between March and late May due to the presence of adequate moisture and increased temperatures. Growth is terminated as late spring rainfalls decline. The time of flowering is highly variable and dependent on warmer temperatures. Adequate moisture levels are necessary for active flower development.

Subclover is self-pollinated and, therefore, requires no bee pollinators.

Subclover grows in a rosette form during the winter months. As temperatures increase, main basal runners are formed and elongate along the ground. These runners may in turn form lateral runners. Flowers develop at the stem nodes toward the ends of the basal runners and along most of the lateral runners. The flowers are not easily observed unless one looks through the

leaves and near the ground. Flowers are cream-colored with some varieties showing red markings. The flowers develop into a bur which contains 2 to 4 pods. Each pod contains one purple-colored seed. On very sandy soils, the burs may bury themselves somewhat like peanuts. On clayey soils, however, the burs usually are on the soil surface. The earlier the maturity of the variety, the more likely the variety will bury its burs.

The varieties which are best adapted to Oregon conditions, in order of maturity from latest to earliest, are: Tallarook, Nangeela, Mt. Barker, and Woogenellup. These varieties have the potential to produce more than 1000 pounds of seed per acre.

## SEED PRODUCTION

### Precautions

Readily available markets have not been developed outside of Oregon. Presently, most of the subclover seed being used in the United States is imported from Australia. Nearly all of the seed produced in Oregon is being sold in local markets. Before producing a crop of subclover seed, some inquiries are needed to determine the proper variety to grow and to secure a buyer.

Some consideration should also be given to the method and equipment that will be used to harvest the seed. Most conventional methods used to harvest clover seed are not adequate for subclover.

### Field Preparation

Care should be taken during field preparation prior to seeding.

It is very important that the field be as smooth as possible. This care not only benefits stand establishment, but also may greatly improve the ease of harvest. The soil surface should be free of unnecessary ridges which may hinder the harvest of burs.

### Time of Planting

Subclover will germinate at relatively low temperatures. It is probably best to establish first-year fields in late September or early October just prior to the start of heavy rainfall. Planting into a moist seedbed which has been well worked and smooth is most desirable. Early planting can be done if sprinkle irrigation is used to establish the crop. However, there are cases reported of spotted cucumber beetle attacking early established fields in the Willamette Valley.

### Inoculation

The seed should be inoculated just prior to the time of planting. Inoculant which is specific for subclover should be used. General clover and alfalfa inoculants give results which are inferior to the specific inoculants. Care should be taken to purchase fresh inoculant which is not outdated. The inoculant package should be stored in a cool, dark place and should not be opened until ready for use.

No matter how careful the precautions taken, planting seed into a dry seedbed may result in the death of the inoculum which will result in ineffective nodulation. Also, mixing the inoculant

with the seed a day or more before planting may result in unsatisfactory inoculation results.

### Seeding Rate and Planting

Subclover has a larger seed than other clovers. Therefore, seeding rates of 10 to 20 lbs/acre are desirable. It is best to plant the seed with a grain drill set at the shallowest possible planting depth. The seed should be planted no deeper than 1/2 inch.

### Fertilization (3)

Nitrogen (N) is not required as a fertilizer since this element is supplied by nitrogen-fixing bacteria in the root nodules. Soils which have a low pH test value will not allow effective root nodulation. If the pH in the top 2 to 3 inches of soil is below 5.5, apply 1 to 2 tons per acre of lime.

Phosphorus (P) should be banded 1/2 to 1 inch to the side or below the drilled seed row of new seedings. Otherwise, broadcast applications can be used for new stands. P can be applied to established stands by broadcasting in the fall. P should be applied in the following amounts if the OSU soil test values are:

Phosphorus	Apply this amount of P <sub>2</sub> O <sub>5</sub>
ppm	lbs/acre
0 to 10	60 to 90
10 to 20	40 to 60
20 to 40	30 to 40

Potassium (K) should be broadcast in the fall prior to seeding new stands or if required in established fields. On new or established fields, K should be applied in the amount shown for the following OSU soil test values:

Potassium	Apply this amount of K <sub>2</sub> O
ppm	lbs/acre
0 to 75	60 to 100
75 to 150	40 to 60
over 150	none

Sulfur (S) should be applied annually to furnish 20 to 30 lbs/acre. On the 'Red Hill' soils, 40 to 50 lbs/acre of S every 3 years gives good results.

Boron (B) responses are not always apparent in subclover. B should be broadcast if needed and never banded. B should be applied in the amount shown for the following OSU soil test values:

Boron	Apply this amount of B
ppm	lbs/acre
0 to 0.5	1 to 2
0.5 to 1.0	1
over 1.0	none

All of these figures are based on forage responses. No data are available for specific responses for seed production.

#### Forage Management

Sheep grazing may increase seed yields as much as 50%, depending

on the year and the time of the grazing treatment. Experiments in Oregon and Australia have shown that without some grazing, seed yields are not optimized. Light grazing with 2 to 3 ewes per acre from just prior to flowering until early bur formation gives the best results. Sheep grazing should be monitored to leave enough time following the grazing period to allow the crop to fully recover from the defoliation. Periods of limited rainfall following grazing may reduce regrowth and in turn reduce seed yields.

Excessive grazing, grazing during bur formation, or grazing very early in the season may reduce seed yields. Consideration should be given to the trade-off between added income from grazing fees and the possible reduction in seed yields due to heavy grazing. Intense grazing may also stimulate bur burial which makes seed harvest more difficult.

Mechanical defoliation has not increased seed yields in experimental plots.

#### Weed Control (4)

Next to harvest loss, weed control is the major concern in the production of subclover seed. Not only do the weeds compete with the crop for light, mineral nutrients, and water, but they also reduce the efficiency of the seed harvest.

Winter annual grasses and broadleaf weeds pose the main problem in subclover seed fields. Annual grasses such as ripgut brome, wild oats, rattail fescue, dogtail grass, small quacking grass, and annual ryegrass are a few of the annual grassy weeds. Common annual

broadleaf weeds are filaree, common chickweed, galium (bedstraw), black medic, vetch, Italian thistle, mustard, and dog fennel.

Some perennial weeds which are commonly found in subclover seed fields include Kentucky bluegrass, bulbous bluegrass, perennial ryegrass, bentgrass, St. Johnswort, and sheep sorrel.

Annual grasses and volunteer cereals can be controlled with EPTC (Eptam) prior to planting or with propham (Chem-Hoe) or pronamide (Kerb) after crop emergence (at least three trifoliolate leaves). EPTC should be applied at the rate of 3 lbs/acre active ingredient and incorporated into the soil immediately after spraying. Propham should be applied at 4 lbs/acre active ingredient, while pronamide should be applied at 0.75 to 1.0 lb/acre active ingredient.

Propham is degraded rapidly by soil micro-organisms, so application should be delayed until soil temperatures have cooled. December applications are often more effective than earlier applications, but propham should be applied while the grasses are still small.

Pronamide is more effective on seedling grasses, but requires rainfall or irrigation to move it into the root zone of the weeds. Conditions are usually favorable for pronamide by late November. Later applications may require the higher rate of herbicide.

Perennial grasses such as bentgrass are controlled by EPTC at a rate of 3 lbs/acre active ingredient or by pronamide at 1.5 lbs/acre active ingredient. Time of application is the same as for annual grasses.

Product labels should be followed closely for best performance of the herbicide.

These herbicides primarily control grasses, but also control some broadleaf weeds such as chickweed. However, general broadleaf weed control will usually not be adequate.

There are no registered herbicides for the control of broadleaf weeds in subclover seed fields. Field observations have shown that weeds such as filaree and black medic increase in frequency in successive years. Starting with a clean field and use of crop rotations with such crops as small grains, may help reduce the incidence of these weeds in subclover seed fields.

#### Irrigation

No experimental information is available on the effects of irrigation on subclover seed production in Oregon. Information from Australia indicates that late irrigation during bur filling may be harmful to seed yields. Even though moisture stress is avoided, seed yields may be reduced from rotting of the seed. Excess watering also produces large amounts of vegetative growth which hinders seed harvest. Irrigation may be of some benefit during periods of drought.

#### Seed Harvest

Presently, harvest loss is the greatest limitation to seed yield. As much as 50% of the seed crop can be lost during harvest. There is no fully effective harvest procedure which will easily recover all of the seed.

All varieties are ready for harvest at about the same time because the active growth of the crop ceases with the end of spring precipitation. Large amounts of vegetative growth may delay drying by shading the soil surface and preventing the burs from drying.

There are several methods of harvesting this crop. Careful consideration should be given before producing a seed crop to determine which method is best suited to your conditions.

The Horwood-Bagshaw vacuum harvester is the most commonly used machine at the present time. When used correctly, much of the potential seed yield can be realized. This machine represents a sizeable investment and is primarily limited in use to subclover.

Before using this machine, the field must be cleared of the dried stems and leaves which cover the seed burs lying near the ground. This is done with conventional hay-making equipment. Once the hay crop has been removed, the field may be allowed to dry further or may be prepared for the vacuum harvester. Preparation for harvest involves dragging equipment such as a spike-toothed harrow which has been turned over, or a length of cyclone fencing, through the field several times. This process breaks the stems and burs from the crown of the plant, but care must be taken not to shatter too many of the seeds from the burs. The number of passes over the field and the time of day regulate the effectiveness of this process. Since a vacuum is used to pick up the burs, it is very important that the field be flat and free of mounds and ridges. Rough ground, weed residue, and stems still attached to the crown

will prevent the burs from being picked up.

The vacuum harvester is then pulled through the field. The vacuum should be set to pick up whole burs and not individual seeds. Because the Horwood-Bagshaw harvester has an operating speed of approximately 1/2 acre per hour, it is usually not economical to make more than two passes over a field unless a portion of the field is especially full of burs. This machine is not well suited to steep slopes.

Another method of harvesting seed is to use a standard windrower with conventional guards on the mower bar to cut the dried plants. The mower bar can be tilted 12 to 15° below horizontal to aid in cutting close to the ground. The windrow is then picked up with a combine as in other seed crops. This method is much faster than the vacuum harvester and uses standard seed harvesting equipment that can be used for many crops. However, seed losses can be high and the field must be fairly flat to insure even cutting of the standing crop.

The Murphy Pickup has been used to pick up additional seed following the windrow method of harvesting. This attachment is mounted on a conventional harvester in place of the header. Rotating rubber flails create a vacuum that is capable of picking up any burs left on the soil surface.

#### Field Preparation for Following Year's Crop

Most harvest methods leave more than 20% of the seed crop in the field. This means that in a field which had the potential of

producing 1000 pounds of seed per acre, 200 pounds will remain in the field. Not all of this seed will germinate immediately since much of it is dormant, but more than enough is present to re-establish the crop.

The harvest operations tend to leave the seed unevenly distributed throughout the field. For this reason, it is probably best to harrow or lightly disc the field sometime prior to germination in the fall. This operation will not only better distribute the seed in the field, but will also allow for better contact between the seed and the soil, facilitating better stand establishment.

Since subclover is an annual crop, soil amendments and fertilizers can be applied at this time if needed.

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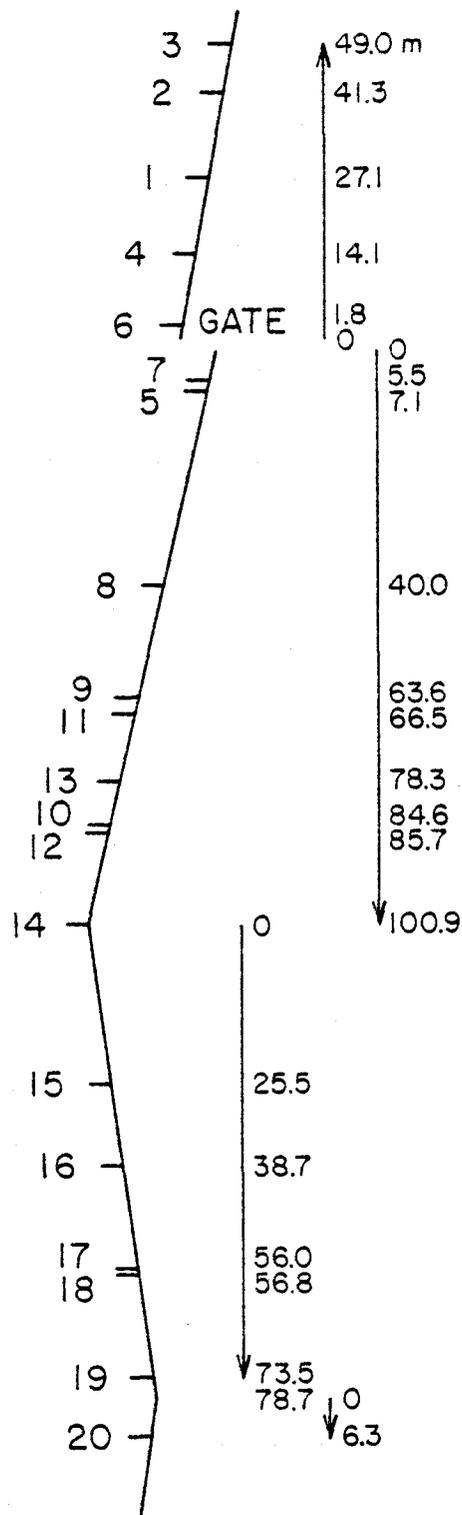
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A P P E N D I X



Appendix Figure 1. Location of block markers on the fence bordering the experimental area on the Winston Smith farm, Oakland, Oregon.

Appendix Table 1. Replication and treatment locations on the Winston Smith farm, Oakland, Oregon.

Position along fence	Repli- cation	Bearing (degree)	Distance (meters)	Treat- ment	Bearing (degree)	Distance (meters)
3	1	126	24.7	1	267	5.5
				2	208	7.3
				3	113	8.2
				4	142	6.4
2	2	132	41.2	1	110	4.6
				2	310	5.5
				3	185	5.5
				4	38	3.7
1	3	138	68.6	1	334	5.5
				2	274	5.5
				3	222	7.3
				4	116	4.6
4	4	129	65.9	1	30	7.3
				2	154	7.3
				3	168	11.9
				4	294	6.4
5	6	128	70.4	1	335	3.7
				2	182	4.6
				3	26	8.2
				4	20	4.6
6	7	125	33.8	1	204	4.6
				2	328	5.5
				3	102	5.5
				4	48	3.7
7	5	144	47.6	1	104	6.4
				2	300	6.4
				3	42	7.3
				4	236	5.5
8	8	123	74.1	1	315	9.1
				2	270	8.2
				3	225	5.5
				4	5	7.3
9	9	114	75.9	1	122	6.4
				2	238	8.2
				3	34	10.1
				4	308	6.4
10	11	121	67.7	1	345	4.6
				2	190	9.1
				3	136	3.7
				4	262	5.5

Appendix Table 1 (continued)

Position along fence	Repli- cation	Bearing (degree)	Distance (meters)	Treat- ment	Bearing (degree)	Distance (meters)
11	13	124	44.8	1	240	5.5
				2	239	10.1
				3	215	6.4
				4	124	2.7
12	10	125	44.8	1	205	4.6
				2	351	5.5
				3	98	3.7
				4	45	4.6
13	12	128	28.4	1	244	7.3
				2	112	5.5
				3	116	8.2
				4	194	7.3
14	14	127	33.8	1	48	2.7
				2	150	4.6
				3	109	7.3
				4	129	0.9
15	15	124	65.9	1	140	7.3
				2	324	6.4
				3	335	6.4
				4	198	4.6
16	16	118	56.7	1	84	3.7
				2	274	3.7
				3	220	7.3
				4	358	4.6
17	17	116	54.0	1	258	7.3
				2	312	3.7
				3	54	8.2
				4	4	6.4
18	18	114	35.7	1	8	4.6
				2	317	3.7
				3	46	4.6
				4	90	4.6
19	19	124	40.2	1	318	4.6
				2	348	5.5
				3	114	7.3
				4	13	9.1
20	20	134	29.3	1	206	5.5
				2	138	3.7
				3	162	9.1
				4	197	0.9

Appendix Table 2. Frequency of site moisture stress index values within sheep grazing treatments.

Grazing treatment	Moisture stress index				
	1	2	3	4	5
Ungrazed	3	4	2	2	9
Early grazing	7	5	1	4	3
Late grazing	3	5	3	4	5
Extended grazing	4	3	1	7	5

Appendix Table 3. Mean soil depth (cm) for treatment and locations from sheep grazing

Grazing Treatment	Site moisture stress index				
	1	2	3	4	5
Ungrazed	97.8	81.3	71.1	49.5	40.9
Early grazing	100.3	77.7	53.3	50.8	45.2
Late grazing	79.5	70.1	55.9	42.4	53.3
Extended grazing	83.8	72.4	55.9	50.3	(46.2) <sup>1</sup>

<sup>1</sup>Missing plot

Appendix Table 4. Anova for regression of mean soil depth with site moisture stress index.

Source	MS	DF	R <sup>2</sup>
Regression	33.38	1	.83
Residual	0.37	17 <sup>1</sup>	

<sup>1</sup>Degrees of freedom for residual reflects one degree of freedom less for missing plot.

Appendix Table 5. Soil fertility levels in October 1979 on the Winston Smith farm, Oakland, Oregon.

Component	Swale	Hill top
pH	5.9	5.8
P (ppm)	20	17
K (ppm)	312	293
B (ppm)	0.37	0.37
Ca (meg/100 g)	9.2	6.5

Appendix Table 6. A partial list of plants found in the experimental study area during the 1978-80 crop seasons on the Winston Smith farm, Oakland, Oregon.

*Agrostis stolonifera*  
*Avena fatua*  
*Briza minor*  
*Bromus diandrus*  
*Bromus mollis*  
*Cynosurus echinatus*  
*Hordeum murinum*  
*Lolium multiflorum*  
*Poa bulbosa*  
*Triticum vulgare*  
*Vulpia muros*

*Alchemilla arvensis*  
*Allium* sp.  
*Barbarea vulgaris*  
*Boisduvalia* sp.  
*Brassica* sp.  
*Cerastium vulgatum*  
*Chrysanthemum* sp.  
*Galium aparine*  
*Geranium* sp.  
*Hypericum perforatum*  
*Lotus* sp.  
*Medicago lupulina*  
*Plantago lanceolata*  
*Rumex acetosella*  
*Senecio vulgaris*  
*Sherardia arvensis*  
*Sonchus oleraceus*  
*Taraxacum vulgare*  
*Trifolium* sp.  
*Trifolium subterraneum*  
*Veronica* sp.  
*Vicia* spp.

Appendix Table 7. Anova for sheep grazing effects on seed quality. 1979.

Component	Source	MS	DF
Seed weight	Treatment	.0081	3
	Error	.0018	80
Seed yield	Treatment	1418.03	3
	Error	27.28	80
Seed number	Treatment	337559.00	3
	Error	5490.21	80
Hard seed	Treatment	224.62	3
	Error	60.40	80
Dormant seed	Treatment	26.31	3
	Error	6.93	80
Germinated	Treatment	21.92	3
	Error	21.75	80
Dead seed	Treatment	216.79	3
	Error	22.50	80

Appendix Table 8. Anova for sheep grazing, moisture stress, and sheep grazing x moisture stress effects in 1979.

Source	DF	MS
Seed yield		
Grazing	3	1418.03
Moisture stress	4	6219.02
Grazing x moisture	12	2333.74
Error	80	27.28
Number of seeds		
Grazing	3	337,559.00
Moisture stress	4	1,016,059.19
Grazing x moisture	12	339,129.04
Error	80	5,490.21
Seed weight		
Grazing	3	.0081
Moisture stress	4	.2112
Grazing x moisture	12	.1512
Error	80	.0018

Appendix Table 9. Sheep grazing experiment. Correlation table for all factors, 1979.

	Seed weight	Seed number	Hard seed	Dead seed	Dormant seed	Germinated seed
Yield	.55 <sup>NS</sup>	.99 <sup>**</sup>	.94 <sup>*</sup>	-.47 <sup>NS</sup>	-.33 <sup>NS</sup>	-.90 <sup>*</sup>
Seed weight		.48 <sup>NS</sup>	.57 <sup>NS</sup>	.14 <sup>NS</sup>	.05 <sup>NS</sup>	-.83 <sup>NS</sup>
Seed number			.95 <sup>*</sup>	-.56 <sup>NS</sup>	-.29 <sup>NS</sup>	-.93 <sup>*</sup>
Hard seed				-.66 <sup>NS</sup>	.01 <sup>NS</sup>	-.97 <sup>*</sup>
Dead seed					-.39 <sup>NS</sup>	-.80 <sup>NS</sup>
Dormant seed						-.04 <sup>NS</sup>

NS, \*, \*\* represent not significant and significant at the 5 and 1% levels of probability, respectively.

Appendix Table 10. Moisture stress effects on seed yield of subterranean clover over four grazing treatments in 1979.

Site moisture stress Index	Seed yield (kg/ha)
1	983.5
2	871.8
3	460.8
4	397.0
5	216.8

Appendix Table 11. Unadjusted and adjusted seed yields for moisture stress differences in the field.

Treatment	Seed yield (kg/ha)	
	Unadjusted	Adjusted
Ungrazed	389.5	459.8
Early grazing	707.8	620.3
Late grazing	574.8	578.4
Extended grazing	645.3	694.8

Appendix Table 12. Anova for regression of seed yield in 1979 on moisture stress.

Component	Source	MS	DF	R <sup>2</sup>
All samples	Regression	26711.70	1	.70
	Residual	73.11	158	
Ungrazed	Regression	4793.81	1	.78
	Residual	36.38	38	
Early grazing	Regression	8384.08	1	.69
	Residual	96.87	38	
Late grazing	Regression	4892.86	1	.74
	Residual	46.34	38	
Extended grazing	Regression	6315.87	1	.69
	Residual	73.61	38	

Appendix Table 13. Moisture stress effects on number of seeds of subterranean clover over four grazing treatments in 1979.

Site moisture stress index	Number of seeds/ha
	x 100,000
1	536.7
2	496.5
3	298.3
4	262.8
5	183.3

Appendix Table 14. Unadjusted and adjusted number of seeds for moisture stress differences in the field.

Treatment	Unadjusted	Adjusted
	x 100,000	
Ungrazed	570.3	646.5
Early grazing	1085.0	920.5
Late grazing	867.5	875.3
Extended grazing	1002.5	1045.8

Appendix Table 15. Anova for regression of number of seeds in 1979 on moisture stress.

Component	Source	MS	DF	R <sup>2</sup>
All samples	Regression	4031750.0	1	.64
	Residual	14373.1	158	
Ungrazed	Regression	647278.0	1	.70
	Residual	7180.2	38	
Early grazing	Regression	1359650.0	1	.75
	Residual	12203.4	38	
Late grazing	Regression	735542.0	1	.68
	Residual	8979.9	38	
Extended grazing	Regression	865775.0	1	.59
	Residual	15893.7	38	

Appendix Table 16. Moisture stress effects on seed weight of subterranean clover over four grazing treatments in 1979.

Site moisture stress index	Seed weight (g/100)
1	.7396
2	.7220
3	.6246
4	.5171
5	.4974

Appendix Table 17. Unadjusted and adjusted seed weights for moisture stress differences in the field.

Treatment	Unadjusted	Adjusted
	(g/100)	
Ungrazed	.6078	.6363
Early grazing	.6342	.5930
Late grazing	.6230	.6244
Extended grazing	.6031	.6145

Appendix Table 18. Anova for regression of seed weight (g/100) in 1979 on moisture stress.

Component	Source	MS	DF	R <sup>2</sup>
All samples	Regression	1.657	1	.72
	Residual	0.004	158	
Ungrazed	Regression	0.565	1	.74
	Residual	0.005	38	
Early grazed	Regression	0.461	1	.81
	Residual	0.003	38	
Late grazed	Regression	0.283	1	.68
	Residual	0.003	38	
Extended grazing	Regression	0.370	1	.71
	Residual	0.004	38	

Index Table 19. Anova for regression of plant components in 1979 on moisture stress.

Component	Source	MSE	DF	R <sup>2</sup>
Leaf area index	Regression	3.911	1	.75
	Residual	0.218	6	
Specific leaf weight	Regression	0.011	1	.75
	Residual	0.002	6	
All samples	Regression	400.511	1	.51
	Residual	16.077	24	
Ungrazed	Regression	251.475	1	.75
	Residual	7.712	11	
Grazed	Regression	185.145	1	.76
	Residual	5.448	11	
Leaf weight (all samples)	Regression	5.040	1	.74
	Residual	0.299	6	
Stem weight (all samples)	Regression	66.38	1	.68
	Residual	5.10	6	

Appendix Table 20. Anova for sheep grazing effects on seed quality in 1980.

Component	Source	MS	DF
Seed weight	Treatment	0.0038	3
	Error	0.0034	22
Seed yield	Treatment	23.74	3
	Error	5.19	22
Seed number	Treatment	534,259.67	3
	Error	36,053.40	22
Plant weight	Treatment	27.87	3
	Error	95.28	22
Hard seed	Treatment	491.43	3
	Error	11.69	22
Dormant seed	Treatment	33.25	3
	Error	4.52	22
Germinated seed	Treatment	148.03	3
	Error	9.60	22
Dead seed	Treatment	26.35	3
	Error	2.94	22

Appendix Table 21. Sheep grazing experiment. Correlation table for all factors, 1980.

	Seed weight	Seed number	Plant weight	Hard seed	Dead seed	Dormant seed	Germinated seed
Yield	-.62 <sup>NS</sup>	.99**	.69 <sup>NS</sup>	.91*	-.94*	-.83 <sup>NS</sup>	-.91*
Seed weight		-.71 <sup>NS</sup>	-.30 <sup>NS</sup>	-.51 <sup>NS</sup>	.77 <sup>NS</sup>	.31 <sup>NS</sup>	.52 <sup>NS</sup>
Seed number			.62 <sup>NS</sup>	.86 <sup>NS</sup>	-.94*	-.76 <sup>NS</sup>	-.87 <sup>NS</sup>
Plant weight				.83*	-.79 <sup>NS</sup>	-.96*	-.93*
Hard seed					-.94*	-.98*	-.99***
Dead seed						.85 <sup>NS</sup>	.95*
Dormant seed							.97*

NS, \*, \*\*, \*\*\* represent not significant and significant at the 5, 1, and .1% levels of probability.

Appendix Table 22. Anova for mechanical defoliation effects on seed quality.

Component	Source	MS	DF
Seed yield	Treatment	14.98	6
	Error	5.03	28
Plant dry weight	Treatment	1826.36	6
	Error	74.76	28
Number of burs	Treatment	3937.65	6
	Error	10349.40	28
Number of seeds	Treatment	60929.2	6
	Error	10346.2	28
Seeds/bur	Treatment	0.056	6
	Error	0.087	28
Seed weight	Treatment	0.043	6
	Error	0.001	28

Appendix Table 23. Mechanical defoliation experiment. Correlation table for seed yield factors.

	Plant weight	No. of burs	No. of seeds	Seeds/bur	Seed weight
Yield	.94***	.49 <sup>NS</sup>	.69 <sup>NS</sup>	.58 <sup>NS</sup>	.93***
Plant weight		.22 <sup>NS</sup>	.42 <sup>NS</sup>	.36 <sup>NS</sup>	.98***
No. of burs			.89**	.46 <sup>NS</sup>	.19 <sup>NS</sup>
Seeds/bur				.78*	.37 <sup>NS</sup>
Seeds/bur					.31 <sup>NS</sup>

NS, \*, \*\*, \*\*\* represent not significant and significant at the 5, 1, and .1% levels of probability.

Appendix Table 24. Mechanical defoliation experiment. Correlation table for seed quality factors.

	Plant weight	Hard seed	Germinated	Dormant seed	Dead seed
Yield	.94***	-.88**	.89**	.72*	.69*
Plant weight		-.80*	.83**	.60 <sup>NS</sup>	.79*
Hard seed			-.98***	-.88**	-.43 <sup>NS</sup>
Germinated				.79*	.50 <sup>NS</sup>
Dormant seed					.10 <sup>NS</sup>

NS, \*, \*\*, \*\*\* represent not significant and significant at the 5, 1, and .1% levels of probability.

Appendix Table 25. Percent water-soluble carbohydrate in the leaves of subclover at different levels within the canopy of greenhouse samples.

Phenological stage	Canopy level (cm)					
	0-5	5-10	10-15	15-20	20-25	25-30
	% WSC					
Vegetative	5.3	5.7	4.0	-	-	-
Early flowering	5.5	4.6	5.2	3.7	4.9	-
Early bur fill	3.0	2.9	4.4	4.7	2.7	-
Late bur fill (undefoliated)	-	-	5.2	4.3	8.6	9.3
Late bur fill (defoliated)	-	5.8	7.6	8.5	7.6	-

Appendix Table 26. Association of inflorescences with subtending leaves within the canopy of subclover during late bur fill of undefoliated and defoliated plots.

Canopy level	Flowers	Burs	Leaf area	% of total leaf area
cm	———— % ————	————	cm <sup>2</sup>	%
Undefoliated				
25-30	0 <sup>1</sup>	8 <sup>2</sup>	145	6
20-25	19	8	580	23
15-20	10	34	747	29
10-15	71	50	1065	42
5-10	0	0	0	0
0-5	0	0	0	0
Defoliated				
25-30	0 <sup>3</sup>	0 <sup>4</sup>		
20-25	29	8	347	16
15-20	4	6	462	21
10-15	21	5	362	16
5-10	45	81	1057	47
0-5	0	0	0	0

<sup>1</sup>Based on 21 flowers.

<sup>2</sup>Based on 24 burs.

<sup>3</sup>Based on 24 flowers.

<sup>4</sup>Based on 116 burs.

Appendix Table 27. Association of inflorescences with subtending leaves within the canopy of subclover sampled in early flowering and bur fill.

Canopy level	Flowers	Burs	Leaf area	No. of leaves	% of total leaf area
cm	———— % ————		cm <sup>2</sup>		%
Early flowering					
20-25	0 <sup>1</sup>	0	218	22	7.3
15-20	3	0	590	72	19.7
10-15	5	0	850	120	28.4
5-10	3	0	739	139	24.7
0-5	89	0	593	204	19.8
Early bur fill					
20-25	0 <sup>2</sup>	0 <sup>3</sup>	155	23	6.2
15-20	3	12	526	102	21.1
10-15	0	8	680	148	27.3
5-10	22	50	945	224	38.9
0-5	75	30	181	206	7.3

<sup>1</sup>Based on 37 flowers.

<sup>2</sup>Based on 32 flowers.

<sup>3</sup>Based on 40 burs.

Appendix Table 28. Effect of inflorescence location and defoliation on bur weight of subclover.

Treatment	Location		
	Proximal	Medial	Distal
	mg		
Undefoliated	39	41	7
	39	28	15
	25		13
			8
			23
	$\Sigma$ 103	69	84
	$\bar{x}$ 34	35	14
	n 3	2	6
Defoliated	37	31	1
	19	31	1
		33	2
			2
			13
			5
			10
	$\Sigma$ 56	95	34
	$\bar{x}$ 28	32	5
	n 2	3	7