WHITE CLOVER SEED PRODUCTION IN BRITISH COLUMBIA

by

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ABSTRACT

In the Creston valley in southeastern B.C., intermediate white clover is grown for seed and is a useful crop in farm field rotations. For several years seed yields have been declining, and despite good prices and markets, hectarage to white clover is declining.

In 1976, work was initiated to determine some of the factors responsible for the decline in seed yield and hectarage. At the same time, an exploratory study of the genetic variation in the seed stocks of the valley was instituted in the hope that a Creston strain might be characterized or selected.

In 1977, in the Creston valley, a series of replicated plots in six fields, representative of the edaphic, climatic and management regimes, were established to measure seed and forage yields and losses from multiple sources. At the University of B.C., four hundred individual plants representative of twenty sources, including some Creston sources, and encompassing substantial genetic diversity, were established from seed in replicated uniform nurseries. In an adjacent nursery one hundred and eighty Creston clones were established. Observation and measurement of a number of characters were taken on all plants several times during the growing season.

Average clean seed yields on the Creston experimental plots ranged from 468-972 kgs. per hectare (418-868 lbs per acre). Farm yields of clean seed, by contrast, ranged from 262-491 kgs per hectare (240 to 450 lbs per acre). It was estimated that of the loss in seed threshed (dockage), but not cleaned, 3-10% was insect damaged; loss attributable to farm harvesting procedures was estimated to reach 50%.

Losses in the developing crop are difficult to assess quantitatively but appeared to be very serious. To offset these losses, in recent years,

producers have been reducing the length of white clover ley and are now in most cases obtaining one seed crop only in the year after establishment; this practice, if carried on without counter selection, might result in a biennial habit.

Three species of weevil appeared to be the most serious pests, viz. the clover root curculio (Sitona hispidula Fab.), and the clover seed weevil (Miccotrogus picirostris (F)) and the lesser clover leaf weevil (Hypera nigrirostris Fab.). The population peaks of the adults apparently occur at different times in the season. Currently only one aerial application of malathion is applied in June to control the clover seed weevil. Almost all roots examined bore signs of larval feeding, doubtless due to the clover root curculio; root nodules, abundant in spring, diminished rapidly as the season progressed. Measurements of nitrogen fixation, using the acetylene reduction technique and the Kjeldahl N-determination, were incomplete.

Flower frequency and development, flower colour, leaf area, petiole length, leaf markings, plant height and weight, and prussic acid levels were some of the characters measured and observed on the individual plants, established in the U.B.C. nurseries from Creston and other sources. Not unexpectedly, the Creston stocks possessed a measure of distinction from most other stocks of intermediate white clover; nonetheless, there appeared to be ample variability in the Creston stocks within which to select strains to meet at least two needs of the region - viz.

a) plants useful in the revegetation of ranges and of unstable soils, and

b) plants well adapted to the arable long ley pastures of the humid and sub-humid areas.

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1. INTRODUCTION

The Creston valley in south eastern British Columbia is suitable for production of high quality clover seed. Furthermore the valley has become, during the last few decades, one of the more important areas for white clover seed production in Canada. The crop has been for several decades and still is an attractive and important constituent of the cropping systems in the Creston valley.

Within recent years, seed yields have declined in the first and subsequent years of production (see Table 1.1). One of the objectives of this research is a search for the major causes of the seed yield decline. Two important factors make the local industry worthy of close scrutiny and support. First, white clover, a leguminous crop with a high potential for the fixation of atmospheric nitrogen, fits in well with the agricultural cropping systems of the Creston valley. Second, the B.C. market for white clover seed is moderately lucrative and, in addition, markets are open in the United States and overseas.

A second objective of the research was to study the within strain variability with a view to determining the suitability of the Creston white clover stocks for designation as a land strain.

Studies of seed production in white clover have not been reported in Canada, therefore this study is exploratory and broad; it is hoped that additional studies will follow.

Table 1.1

Hectarage, average yield, total yield, price per kilogram and total value for white clover seed production in the Creston valley.

Year	Hectares	Average yield	Total yield	Price/kg	Total value
1966	771	607 kgs/ha	468,566 kg	-	-
1967	-	- -	301,869 kg	-	-
1968	918	492 kgs/ha	453,597 kg	77¢	$350,000
1969	891	358 kgs/ha	319,786 kg	90¢	290,000
1970	761	448 kgs/ha	341,105 kg	165¢	564,000
1971	709	560 kgs/ha	396,897 kg	71¢	280,000
1972	640	448 kgs/ha	286,673 kg	99¢	284,000
1973	607	280 kgs/ha	170,099 kg	143¢	243,750
1974	405	269 kgs/ha	108,863 kg	220¢	240,000
1975	324	403 kgs/ha	130,636 kg	198¢	259,000
1976	243	314 kgs/ha	76,204 kg	176¢	134,000
1977	243	426 kgs/ha	103,420 kg	176¢	182,400

2. REVIEW OF RELEVANT LITERATURE

2.1 General Biology of White Clover

White clover ranks as one of the world's more important forage plants. With its origin in the Mediterranean (Davies 1969) it now enjoys an almost worldwide distribution (Crowder 1960).

Variations in gross morphology are everywhere very noticeable; nevertheless within the genus Trifolium, a large genus, white clover (Trifolium repens) is a well defined species (Chromosome No. 2n = 32). Although the general biology of white clover is the subject of several early reports, notably a monograph by Erith (1924) and a bibliography (Imperial Bureaux of Pastures and Forage Crops 1939), it is useful to review some of the salient features.

2.1.1 Nomenclature

White clover is a Linnaean species and is universally recognized in technical nomenclature as Trifolium repens L.; other common names are white trefoil; white honeysuckle; shamrock; bubby-roses; quillet (Erith 1924); white man's foot.

2.1.2 Agronomic Races

Three forms of white clover are important agronomically; the race "sylvestre", known as wild white clover is a small but hardy plant. It enjoys a reputation for persistency in long-term pastures and hill pastures in Great Britain and other parts of the world. The race "hollandicum", known as "common white" or "white Dutch" (origin Holland), does not have the persistency of wild white clover but is a larger plant in form and is sometimes referred to as "intermediate" white clover. Place of origin for common white seed stocks has resulted in names such as New

Zealand white, Kentish white, Louisiana white (Hollowell 1948). Common white is suited to mixed leys of short to moderate duration (1-5 years). The race "giganteum", commonly known as "Lodi" or "Ladino" is similar in almost all respects to the above two forms mentioned but has a "gigas" or mammoth form. It yields a greater amount of dry matter than the hollandicum race and is commonly grown for hay and short-term pastures.

2.1.3 Strains

As mentioned previously white clover is greatly variable in its form and performance. However, it is possible to group plants that have certain salient features in common yet are not necessarily identical. For convenience it is customary to assign the name "strain" or less commonly "cultivar" (variety) (Hawkins 1953 and 1960). Hawkins (1959) lists several inherited measurable characteristics of white clover that are useful for strain classification and identification. When assigned a name or number, this tells us the plant's origin, type and suitability to certain uses (Ware 1925). A case in point is the Aberystwyth strain, S.184 wild white clover. English wild white clover, known for its persistency, is in form and behaviour quite variable. However by selection at the Welsh plant breeding station at Aberystwyth a pedigreed strain S.184 wild white clover was released. This strain has a greater degree of uniformity with improved performance (Jenkin 1943).

According to U.S.D.A. reports (1947) the use of the name White Dutch is misleading since it no longer represents any particular type. At one time it represented an intermediate type of white clover but is now merely a synonym for common white clover. This is important, since the use of nationality serves to describe the type of plant rather than just the country of origin (Ware 1925, Williams 1945). In Canada it is grown and marketed as White Dutch with no guarantee as to its origin, performance or uniformity.

Understandably strains and varieties of white clover perform differently in the different geographic regions of Canada. The English wild white and the Danish strains lack persistence in places such as Beaverlodge and Ottawa, yet they enjoy moderate success at Agassiz, B.C. White Dutch and New Zealand strains perform well at Ottawa, yet poorly at Agassiz (Dominion Department of Agriculture 1941). Elliot and Howe (1977) give the relative hardiness and yields of white clover introductions as tested at Beaverlodge. The Louisiana strains showed poor hardiness and seed yield while those strains of an origin from a more temperate region displayed increased hardiness and seed yield.

2.1.4 Seed

The seed is small (1000 seeds weighing ca. 0.65 grams) and develops in a legume (the fruit) where the average number of seeds is two to three (Erith 1924). The testa is yellow in the majority of cases but in some cases is yellowish brown or brownish red. The seed coat in some seeds is so impermeable to moisture that germination is delayed for years. This condition known as "hard seed" exists in about 10% of mechanically harvested seeds in the common white (United States Department of Agriculture 1915). Robinson (1937) claims that this condition is brought about by over-ripeness of the flower heads at harvest. Germination is epigeal.

2.1.5 Aerial Development

The main stem is very short and the branches, known as stolons, are creeping and root at almost every node (i.e. shoot growth is indeterminate). It is from these nodes also that the leaf and flower buds originate. The leaf is palmate and trifoliate being born on a long petiole. Characteristic diurnal and nocturnal leaflet positions are probably attained by photonastic movement (Erith 1924). Brougham (1958) and Denne (1966)

suggest that petiole and leaf expansion continue for almost the entire life of those plant parts. The average life of a leaf and its petiole is approximately forty days after leaf expansion. Leaf size and petiole length do not differ greatly under nursery conditions but may change under biotic (grazing), edaphic and climatic influences (Caradus 1977, King 1961).

There are positive correlations between plant height, weight, rosette diameter and leaf area (Barcikowska 1976, Beinhart 1963). Leaves produced early in the growing season tend to be larger than leaves produced later in the season (Beinhart 1963). Defoliation is reported to reduce the size of leaves and the length of petioles (Carlson 1966, King et al. 1978). Observations by Davies (1958) suggest that good leafy cuts of white clover can be taken in June in Britain but subsequent crops are less leafy. He also claims that under his conditions, the intermediate White Dutch strains give the highest vegetative yields over a three-year period.

2.1.6 Reproductive Structures

The inflorescence is a dense raceme born on a long peduncle that arises from a leaf axil on a stolon or prostrate stem. It is made up of 10 to 80 florets; the petals are white but are occasionally pink. The species is almost always genetically self-incompatible and cross-fertilization is necessary (Atwood 1942); the most important pollinator is the domestic honey bee. Because of cross pollination and the self-incompatibility plant breeding in white clover is difficult. Most strains, that are in use, are the result of the process of natural selection with only incidental help by man (Hollowell 1948).

As well as sexual propagation, white clover is able to maintain itself asexually by means of stolons. This ensures dispersion and persistence of clones in local habitats. Work by Harberd (1963) showed that clones are

known to have survived longer than sixty years and to have spread over an area of twenty yards diameter.

2.1.7 Root Systems

White clover root systems are as varied as the forms occurring above ground. The wild races have a fibrous root system and no tap root on the main stem. On the other hand the Ladino race has a distinct tap root but few fibrous roots (Caradus 1977). The common or White Dutch is intermediate in that it has a goodly number of fibrous roots while adventitious roots occur at almost every node of the stolon.

2.2 Biological Nitrogen Fixation

The virtues of legumes in green manuring have been acknowledged by the ancients. However it was not until 1890 that Hellriegel and Wilfarth demonstrated conclusively that nitrogen fixation occurred symbiotically in the root nodules of legumes. Much of the early literature on nitrogen fixation has been reviewed by Wilson (1940).

Recently, especially with the institution of the International Biological Programme, several major texts have appeared on the broad subject of biological nitrogen fixation (Burns and Hardy 1975, Hardy and Silver 1977, Hardy and Gibson 1977, Lie 1971, Nutman 1976, Quispel 1974, Stewart 1966).

Nitrogen fixation by white clover occurs as a result of a symbiotic (Vincent 1974, 1976) relationship between the plant and bacteria (*Rhizobium trifolii*). The bacterial *Rhizobia* that infect white clover roots are specific to the clover (genus *Trifolium*) group. Cross innoculation by this group of bacteria on other leguminoid groups such as the alfalfa group or the lupin groups, will lead to degrees of ineffective nodules (Wilson 1940).

Annual amounts of nitrogen fixed by white clover are significant. Estimates by workers on the amount of nitrogen fixed by the white clover

system have been as high as 616 kgs of elemental nitrogen per hectare per annum (Dobson and Beaty 1977, Halliday and Pate 1976, Haystead and Low 1977, Jones et al. 1977, Kleter and Bakhuis 1972, Walker et al. 1954, Williams 1969). Non symbiotic biological systems, by comparison, have been documented to fix no more than 70 pounds per acre per year (Williams 1969). Under usual farm practices amounts of N fixed are commonly low - often below 170 kgs N per hectare.

Many conditions dictate the availability of fixed nitrogen associated species.

Excretion appears to be a ready source of transference (Mulder et al. 1977, Virtanen et al. 1937) whereas senescence of nodules, roots and top growth liberate nitrogen over a longer period of time (Walker et al. 1954). It is also suggested (Walker et al. 1954) that there is a direct relationship between the amount of nitrogen retained by clover and the contribution by clover to grasses. Clovers have been shown to make available to other associated species, notably grasses, as much nitrogen as they retain in their aerial parts. Fixed nitrogen from excretion and from decaying organic and other soil systems are confounded and it is difficult to assign quantitative expressions to the components.

It is considered that clover is inefficient in comparison to many other higher plants such as grasses in the sequestration of nitrogen from the soil (Jones et al. 1977). Long-term contributions by white clover, after ploughing, have been researched by Williams (1959). He claimed that over equal periods persistent varieties of white clover had a greater effect on increasing yields of crops following in a rotation than the less persistent varieties. He observed that wheat, under his conditions, grown on ground that was occupied by white clover for three years previously, yielded 30% higher than wheat grown on land occupied by white clover for only one year.

Although workers have claimed a 200% increase in forage production by including a legume (Dobson and Beaty 1977) in the forage mixture, the use of white clover in pasture and forage seed mixtures declined in many countries in the early 1950's (Williams 1969). On the other hand, for example, the use of inorganic nitrogen fertilizer doubled in Britain, between the years 1957 to 1967; there, the upper forage yield of a mixed clover-ryegrass ley is ca. 9000 kgs/ha while a pure ryegrass ley, using up to 400 units of inorganic nitrogen, will yield twice that figure, or ca. 18000 kg/ha of dry matter (Chestnutt and Lowe 1969, Cooper 1969, Williams 1969).

The reasons for the change, to the use of inorganic nitrogen fertilizer, are many and may continue as long as nitrogenous fertilizers are relatively inexpensive. It is to be noted however that artificial fixation of atmospheric nitrogen is a high energy consuming process.

Measurement of the level of nitrogen fixation on total nitrogen fixed has traditionally been by the Kjeldahl method (Burris 1974). Although this is an accurate means of total nitrogen measurement it is destructive of the plant material. In 1956 both Dilworth and Schöllhorn simultaneously, and independently observed the reduction of acetylene to ethylene in the presence of a biological nitrogen fixing agent (nitrogenase system)(Burris 1975). Koch and Evans (1966) published the first work in the use of the method followed by publications by Schöllhorn and Burris (1967) and Stewart et al. (1967). The method, known as the acetylene reduction method, has proved to be both sensitive and inexpensive with application both in the laboratory and the field. Sinclair et al. (1967) evaluated the technique and presented the limitations on its accuracy and application. Overviews of the technique have been given by Bergersen (1970), Burris (1974) and Hardy et al. (1968).

Several publications exist on the use of the acetylene reduction technique with various types of assay chambers ranging from 50 ml. syringes by

Hardy et al. (1968), 21 litre plastic waste paper baskets by Fishbeck et al. (1973) to plastic bags by Lee and Yoshida (1977). It has been used successfully under field conditions (Lee et al. 1977, Vaughn and Jones 1976) and in remote areas by Stutz and Bliss (1973).

White clover has been successfully studied by the technique (Halliday and Pate 1976, Haystead and Low 1977, Masterson and Murphy 1976, Moustafa et al. 1969, Sinclair 1973) and the same plants have been repeatedly sampled, i.e. the method may not involve destruction of the plant material tested.

The above authors have sampled nitrogenase activity over periods ranging from 30 minutes to twenty-four hours after acetylene introduction. The acetylene reduction technique has been used to measure qualitative responses by the nitrogenase system to such factors as defoliation or plant to plant variability. The authors caution that the interpretation of quantitative measurements can be misleading unless adequate controls are employed, these being ^{15}N isotopes or Kjeldahl nitrogen determinations.

Measurement of ethylene production has been by the use of gas chromatography either by direct introduction from the assay chambers or storage in serum vials. This method of storage has proved practical but limited in the length of time one can store samples before analysis. The publication by Mallard et al. (1977) outlines the construction and use of a gas chromatograph applicable to field use. It is capable of detecting ethylene concentrations as low as 10 parts per million.

2.3 Marker Genetics

2.3.1 Introduction

In the breeding and seed production of white clover it is very useful to have visible plant characters that can be used to identify plant stocks or materials. They can be used for a variety of purposes such as the ident-

ification of "mother stocks" and the field maintenance of certified stocks.

In white clover there are a number of very good distinctive markers governed by easily identifiable genetic systems. One of these is the ability of certain white clover lines to produce hydrocyanic acid (HCN) in varying amounts; detection of HCN can be usefully undertaken by employing the simple picric acid chemical test. There are also a series of leaf markings which are visible with the eye and which can be simply classified.

2.3.2 Cyanogenesis

A comprehensive review of cyanogenic glycosides, with special reference to white clover, has been published by Jones (1972). Corkill (1942 and 1971) and Daday (1954 a, b, c and 1955) have studied special features of cyanogenesis in white clover.

Cyanogenic glucosides can be found in over sixty of the flowering plant families. There is variation within families, genera and species with respect to presence or absence of cyanogenesis. White clover may be cyanogenic or acynogenic (Jones 1972). The presence of cyanoglucosides in white clover was apparently first reported by Mirande (1912).

Cyanogenesis in white clover occurs when the enzyme linamarase acts on (hydrolyses) the two glucosides linamarin and lotaustralin; gaseous hydrocyanic acid is released (Daday 1955, Melville and Doak 1940). The process is usually activated by stress on the plant or by mechanical damage; cold temperatures or mordants such as toluene or chloroform are also able to initiate this activity (Jones 1972).

Cyanogenesis is governed by two pairs of genes. One pair relates to the presence or absence of the cyanoglucosides (lotaustralin 80% and linamarin 20%) and the other pair to the presence and absence of the enzyme linamarase. In both pairs the gene for presence is dominant (Corkill 1942, Melville and Doak 1940, Pusey 1966) yet the amount of glucoside is governed

possibly by the presence of modifying genes (Corkill 1942).

Cyanogenesis is found mainly in the leaves and petioles but apparently is not associated with plant form (Atwood and Sullivan 1943, Daday 1955, Jones 1972); in New Zealand and the warmer areas of Europe cyanogenesis is associated with, but not necessarily the cause of, greater plant fitness (Bishop and Korn 1969). When cyanogenic plants are injured by frost the enzyme system is activated with a subsequent internal release of hydrocyanic acid (Daday 1965). In New Zealand the most productive and persistent types of white clover are the highest in cyanophoric properties (Atwood and Sullivan 1943, Corkill 1942, Daday 1955).

Because one cannot visually distinguish cyanogenic and acyanogenic plants or their seed, the New Zealand Department of Agriculture employs the picric acid test (Guignard test) as one of the methods used in its strain certification programme (Foy and Hyde 1937). The frequency of cyanogenic plants in a population is governed by geographic location and climate. Daday (1954 b) reported a decrease in the frequency of cyanogenic plants from the Mediterranean region northwards to northeastern Europe and which is strongly associated with the mean January temperatures (Daday 1954 c, 1965). This was also confirmed by DeAraujo (1976). Daday attributed this to a decrease in the glucoside gene frequency and to a lesser amount, the enzyme gene frequency. There is a slight diurnal and seasonal variation in the amounts of hydrocyanic acid released (Caradus and Evans 1977, Corkill 1942). The reasons for the clinal distribution of cyanogenesis in white clover may be found in repellent-defensive mechanisms (Wallace and Mansell 1975), but the interrelationship between the plant and its users are complex. Molluscs have been reported to consume up to 17% of the leaf area of acyanogenic plants in white clover (Angseesing and Angseesing 1973). However

different species of slugs differ in their preference for cyanogenic plants (Crawford-Sidebotham 1972); for example the species Agriolimax reticulatus is not deterred in its eating of cyanogenic plants (Bishop and Korn 1969). White clover has been reputed to cause death in cattle yet sheep require greater doses in order for it to be lethal (Todd 1969). High levels of hydrocyanic acid in white clover are of the order of 0.035 percent while the toxic level for sheep is ca. 0.09 percent (Bishop and Korn 1969) or ca. 2.4 mg/kg body weight (Jones 1972).

2.3.3 Leaf Markings

Leaf markings in the genus Trifolium are common (Brewbaker 1955). White clover displays two distinct types, white and red (Cahn and Harper 1976, Carnahan et al. 1955, Davies 1963). The inheritance systems for both are simple (Brewbaker 1955, Carnahan et al. 1955, Davies 1963) and are both used as valuable visual aids in the identification of individual clones and strains (Cahn and Harper 1976 a, b, Carnahan et al. 1955, Charles 1968, Corkill 1971, Harberd 1963). White clover clones are very rarely self-compatible (Corkill 1971); these leaf markings make it relatively simple in maintaining genetic purity and the identification of parental lines in the field.

There appears to be a seasonal effect in leaf mark expression. The white marks are at their sharpest expression between the months of April and July with the least expression from December to January. Cahn and Harper (1976 a, b) observed that sheep prefer plants with no leaf markings and show varying degrees of preference for different intensities of markings. Also plants with no leaf marks are suited to long periods of grazing (Charles 1968) and show more resistance to stolon rot by fungi (Cahn and Harper 1976 b).

The make-up of the white mark remains to be conclusively resolved, but a morphological explanation appears to be primary. It is recognized (Brewbaker 1955, Carnahan et al. 1955, Erith 1924, Hara 1957) that it is associated with the mesophyll tissue on the upper surface. The pallisade cells are less elongated and organized, yet appear to have a greater number of intercellular spaces.

The red markings on the leaf, due to the nature of their inheritance, are useful to plant breeders in the same way as the white marks. These red markings contain anthocyanin (Davies 1963) located in the epidermal tissue (Carnahan et al. 1955, Kirk et al. 1967); expression is at its greatest below 10°F.

The genetics of the white and red markings have been studied by Brewbaker (1955) and by Corkill (1971). The presence of marks is dominant to absence and the genes controlling colour are independent of the genes controlling cyanogenesis.

2.4.1 Seed Production - General Biology

An encompassing treatise on the management aspects of white clover, in the production of forage and seed as well as animal related factors for Britain, is available (Lowe 1969). It contains relevant articles authored by those working on various aspects of crop management. Bulletins with relative information about white clover for the farmer in Britain, the United States and Canada are available (Dominion Department of Agriculture 1941, United States Department of Agriculture 1915, Ware 1925).

The seeding of forages with a small grain companion crop is a common practice (Hughes et al. 1966), white clover being no exception. The choice of a grain crop and its seeding rate (spring wheat, oats or barley) rests largely on the edaphic, climatic, and economic situation of the area con-

cerned. Smith et al. (1954) found that under their conditions the seeding rates of spring oats, from 24 kgs/ha to 141 kgs/ha, had little effect on the establishment of alfalfa and red clover. The favourable effects enjoyed by the seedling clover and alfalfa plants under the low seeding rate of grain was cancelled out by the increased weed population. Under heavy seeding rates of grain, good weed control was obtained but moisture and shading were limiting factors for the legume seedlings.

Seed yields are closely related to the number of inflorescences per acre and the seeds per inflorescence (Hawkings 1956). Floral initiation in Britain, for Kent wild white clover, takes place when ca. 610 growing degree days have accumulated. The daylength threshold for floral bud initiation is ca. thirteen and a half hours (Hagger et al. 1963). This is also the stage when light penetration to the stolons is important (Zaleski 1969). Temperatures are also important in determining flowering and its intensity (Garrison and Bula 1961, Thomas 1962).

It is common practice in Britain to graze seed pastures or to defoliate in some other manner prior to the floral bud initiation stage. The belief is that defoliation allows light penetration to the stolons which, in turn, branch and provide a greater number of sites for floral buds (Haggar et al. 1963, Zaleski 1969). The addition of a nitrogen fertilizer encourages vegetative growth and consequently reduces the number of flower heads (Anslow 1962). If defoliation is delayed, peduncle length will be reduced and poor pollination may result (Thomas 1961).

The world average white clover seed yield is ca. 200 kgs/ha, with a range of 100 to 600 kgs/ha. Argentina, among countries, appears to have the highest yields per acre (FAO of the United Nations 1961). Seed production in Britain has a potential yield of ca. 760 kgs/ha (Davies 1969) yet only

a fraction of this potential is realized (Haggar et al. 1963, Hawkins 1960).

Harvesting losses in the form of shattering and seed head loss account for much of this loss while shrivelling and insect damage make up the balance (Davies 1969, Forster et al. 1962). Harvesting when the seed heads are very dry will result in heavy losses due to shattering on to the ground. The use of a vacuum-type harvester has given an 80% recovery rate of seed (Dexter and McKibben 1945).

The age of a seed crop is important in relation to its seed yield capacity. Haggar and Holmes (1963 a) reported that wild white clover, which in the first year of seed harvest yielded 132 kgs/ha, by the fifth year yielded only 60 kgs/ha of seed. Harvesting generally commences when 90% of the heads are "brown". A late harvest is reputed to give increased yield while an early harvest results in a lowering of persistence in future generations (Haggar and Holmes 1963 b). The colouring of seeds of white clover is not uniform; seedsmen prefer seed lots consisting largely of yellow seeds; a bright yellow colour is believed to be a result of harvesting conditions and to be associated with high viability of seed (Haggar and Holmes 1963 b, Ware 1925).

White clover is an open pollinated crop and honey bees are important pollinators. The presence of hives in a crop have given a 47% increase in seed yield (Haggar and Holmes 1963 a). The recommendations for numbers of hives per acre varies greatly from country to country. In Britain one hive for every one hectare is recommended (Davies 1969, Zaleski 1969) and in New Zealand one hive is recommended to every three hectares (Davies 1969). In the United States one hive per six hectares appears to be adequate (Anslow 1962) but Green (1957) found that one hive to every four hectares gave an acceptable seed set. Pankiw and Elliott (1959) working on alsike clover

in the Peace River Region of Canada recommended one hive to one half a hectare.

Demand for a persistent white clover is high (Haggar and Holmes 1963 b); and management is an important factor in persistence. It is for this reason that buyers of wild white clover, looking for seed with high persistence, insist that this seed be taken from old permanent pastures (Haggar and Holmes 1963 b). The tendency for seed to be taken from short term leys is reputed to be a guarantee of the loss of persistence. It is interesting to note that abundant flowering is usually associated with a lack of persistence (Davies 1969, Gibson 1957). The lack in replacement of photosynthetic surface when primordia become reproductive will result in poor carbohydrate storage (Beinhart 1963). The loss of other features of a strain are also possible when it is grown outside its region of adaptation (Garrison and Bula 1961).

Top growth yields of white clover grown in pure stands in northern latitudes range from 2 to 4.5 tons/ha (Allen et al. 1976, Chestnutt and Lowe 1969, Davies 1969, Ennik 1969). In Britain it is customary to grow white clover for seed with a companion grass that is also harvested for seed.

For white clover seed production, Chestnutt and Lowe (1969) recommend that available soil phosphorus be above 20 parts per million and potassium above 200 parts per million. In Britain, fertilizer applied to maintain these levels is between 224 to 448 kgs/ha of superphosphate (40 to 90 kgs of P_2O_5 per hectare) every two years and muriate of potash applied each fall at rates of 112 to 224 kgs/ha (67 to 134 kgs/ha K_2O) (Haggar and Holmes 1963 b). In the United States similar recommendations are made (Caradus and Evans 1977, United States Department of Agriculture 1947). Fall applications of fertilizer to white clover are justified since there is new nodal root

growth at that time which is capable of taking up the available nutrients (Caradus and Evans 1977).

Page and Thomson (1976) list several herbicides that can be safely applied to white clover; the current recommendations for conditions in British Columbia are made available to farmers through the field crop recommendations guide (British Columbia Department of Agriculture 1978).

2.4.2 Pests of White Clover Seed Crops

White clover, like all other forage legumes, is host to an array of parasites from the animal kingdom. Most are not-host specific and will attack species other than white clover.

The European clover seed weevil (Miccotrogus picirostris (Fab.) has been known to give a loss in white clover seed yields of up to 70% (Strickland 1956). Both larvae and adults are responsible for economic losses. The adults feed on the developing florets and forming seed pods, while the larvae feed on the immature and mature ovules (Swan and Papp 1972). This larval feeding is the more serious damage (Davidson and Peairs 1966, Detwiler 1923). Overwintering takes place in the adult and egg forms to give a rapid buildup of the parasite in one season (Morgan-Jones 1950).

The adult of the lesser clover leaf weevil (Hypera nigrirostris Fab.) is known to cause severe plant injury in years that are dryer than normal. The adults are mainly leaf feeders, while the larvae feed both on the leaves and flower buds (Swan and Papp 1972). Overwintering takes place in the adult form around the crowns of the clover plant. One generation a year is normal although two are possible (Davidson and Peairs 1966, Detwiler 1923).

The clover root curculio (Sitona hispidula Fab.) can be found in many parts of North America (Davidson and Peairs 1966, United States Department

of Agriculture 1947). The adults are leaf feeders but this damage is not considered to be of economic importance (Swann and Papp 1972). The larvae feed on all parts of the root system (root nodules, tap and lateral roots) in early summer and leave characteristic girdling scars especially on the tap root. Injury is cumulative and leads to the entry of pathogens into the plant (Dickason et al. 1958, Hill et al. 1969, 1971, Kilpatrick and Dunn 1961) resulting in the loss of plant vigour and persistence (Dickason et al. 1968, Hill et al. 1971, Pescho 1975, Underhill et al. 1955). Two generations a year are possible (Stein 1970).

Other insects of economic importance to white clover come from the families Aphididae, Curculionidae, Eurytomidae, Cicadellidae, Miridae and Pentatomidae (Davidson and Peairs 1966). Biological control of the injurious insect pests of white clover is exercised by the beetles, true bugs (Homoptera), flies and wasps, fungi, virus and bacteria (Davidson and Peairs 1966).

Chemical control is recommended and is practical for all of the above insect pests (Dickason et al. 1958, 1968, Neal and Ratcliffe 1975, Turner 1957, Underhill et al. 1955) with varying degrees of success. Plant morphological characteristics offer limited control or immunity in some plant species, notably a measure of resistance in Medicago species to the alfalfa weevil (Hypera postica Gyll) (Liang and Sorenson 1977). This resistance is apparently associated with pubescence and vascular differences.

Biological control has been effectively used in North America, notably in the state of New York where 1.9 million acres of alfalfa are protected from the alfalfa weevil (Gyrisco 1977).

Molluscs can become economic pests in clover in certain years. The

common slug (Agriolimax reticulatus) can cause economic damage by leaf feeding on clover. Chemical control is possible by the use of two kgs/ha of actual metaldehyde injected into the irrigation water. A natural control in white clover is through the repelling factor of cyanogenesis (Howitt 1961).

Nematodes (eelworms) can become a pest of white clover. Their feeding on the roots give rise to poor plant growth and poor nitrogen fixation. The principal species are the root lesion nematode (Pratylenchus sp.) (Willis and Thompson 1977), clover root nematodes (Heterodera trifolii and Meloidogyne hapla) and the stem eelworm (Tylenchus sp.) (Goodey 1950, Willis and Thompson 1977, Yeates 1976, Yeates et al. 1977). Although chemical control is increasing in its practical application on a field scale, crop rotation is still the recommended method (Yeates et al. 1976).

2.4.3 Diseases of White Clover Seed Crops

More than thirty diseases in white clover limit the production of forage and seed for this crop (United States Department of Agriculture 1947). "Clover sickness", a catch all term, is of a practical concern to many growers and farmers (Board of Agriculture and Fisheries 1913, United States Department of Agriculture 1924). It involves all the cultivated clovers yet the causes remain obscure (Mann 1950). Usually the symptoms are either a decline in plant yield and stand density or the poor establishment of a clover crop when reseeded within four years of a preceeding clover crop. Several suggestions for the decline have been put forward. These usually involve a combination of clover rot (Sclerotinia trifoliorum) and stem eelworm (Dillon Weston 1950). The variety from Denmark, "Pajberg Milka", is resistant to clover rot (Aldrich 1969). O'Rourke (1969) considers that root rot complex of white clover to be the most limiting factor in the establishment, production and persistence of this crop in Ireland. He considers that the

unaccountable failure in clover persistence can be attributed to two Fusarium species that are resident in the soil. Damage by these fungi is considered to occur only when the plant is subjected to stress in some form; at no other time does damage occur.

3. DESCRIPTION OF STUDY AREAS

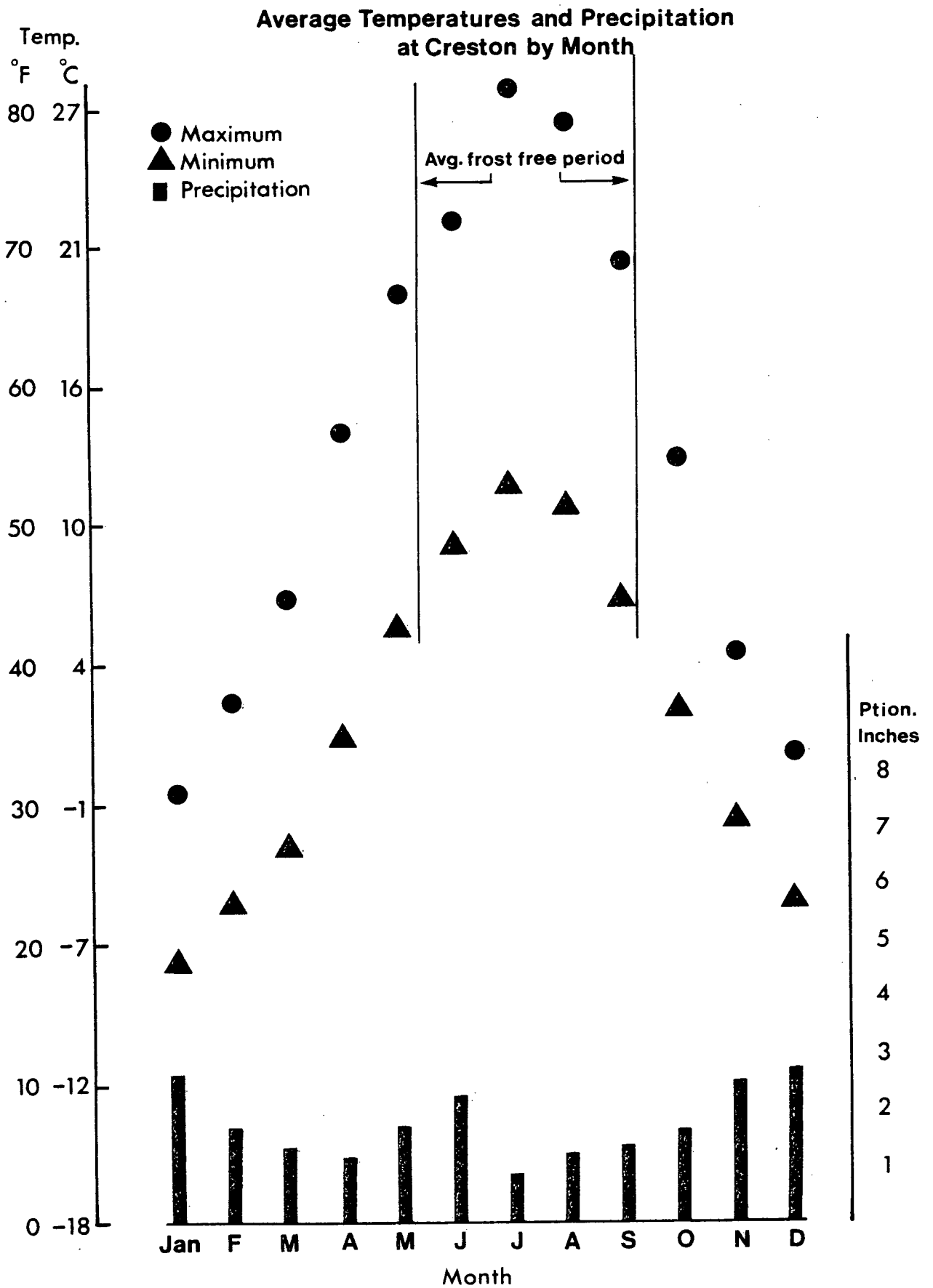
3.1.1 Creston

The Creston valley is situated in south eastern British Columbia and is approximately 806 kilometers due east of Vancouver. The area occurs in the Purcell Trench south of Kootenay lake at elevations between 530 to 760 meters above sea level. The agricultural part of the valley runs in a south-east to north-west direction, is about 24 kilometers long and is 6 to 10 kilometers wide. It comprises a total arable hectarage of 18,802 hectares. The Creston flats on which white clover seed production takes place are alluvium and comprise a total arable hectarage of 9,120 hectares (Wittneben and Sprout 1971).

The climate of the Creston area falls into Köppen's world classification as Humid Continental with warm summers (Department of Mines and Technical Surveys 1957). The Creston flats receive about 2800 growing degree days a year and about 120 frost free days with 175 to 200 millimeters of precipitation during that period. Annual snowfall is about 1400 millimeters. The climatic data is presented in Figure 3.1.1 (British Columbia Department of Agriculture 1974; Connor 1949). Six fields for study were chosen for their geographic position; all fields chosen had a companion crop seeded with the white clover; general management in all fields chosen was considered to be average or better than average. The fields were located on the alluvial flats with a distance of 10 kilometers between the southernmost and northernmost field. The remaining four fields occurred in between. Four fixed but roughly randomly chosen sampling areas were located within each field. Photographs of each field and sampling area are found in Figures 3.1.2, 3.1.3, 3.1.4 and 3.1.5.

Figure 3.1.1

Average temperature, precipitation, and
frost free period at Creston, B.C.


Average Temperatures and Precipitation at Creston by Month
Temp.
°F °C
80 27
70 21
60 16
50 10
40 4
30 -1
20 -7
10 -12
0 -18
Maximum
Minimum
Precipitation
Avg. frost free period
Ption. Inches
8
7
6
5
4
3
2
1
Jan F M A M J J A S O N D
Month

3.1.2 Comment on Field Management

The individual farm field is the result of a complexity of factors, biological, economic and human. Only a few of the current management factors, over which the producer may have some control and which may be considered to have relevance to seed production, are recorded for the individual fields selected for this study. In the interests of brevity the management record is by phrase and includes a few occasional observations on the development of the seed crop.

Field 1: "Wyndell south".

This field is located at the south end of the valley adjacent to the Agriculture Canada Research Sub Station and belongs to Wyndell Farms Ltd. Field acreage - 65 acres (26 hectares).

1976 - Barley seeded at 90 lbs per acre (100 kgs/ha) with white clover underseeded at 3 lbs per acre (3.4 kgs/ha).

- Fertilizer applied - 22 lbs (25 kgs) N; 66 lbs (74 kgs) P_2O_5; 66 lbs (74 kgs) K_2O as 8-24-24 at seeding time.

1977 - May 15th to 20th - 2 pints per acre (2.3 li/ha) MCPB (Tropotox) for Canada thistle (Cirsium Arvense (L.) Scop.) and perennial sowthistle (Sonchus arvensis (L.)) control (Frankton 1955).

- June 7th to June 30th - Bloom 30% to 100% respectively.
- 48 hives of bees introduced (1 hive to 1.4 acres, 0.6 hectares).
- June 22nd - 2 pints per acre (2.3 li/ha) Malathion for clover seed weevil control.
- July 2nd - Bloom "browning".
- August - swathed, combined and mouldboard ploughed.
- Clover seed yield 400 lbs/ac after cleaning (448 kgs/ha).

Figure 3.1.2 Aerial photograph of field 1.

Figure 3.1.3 Aerial photograph of field 2.

Field 2: "Eastman".

This field is located in the middle of the valley and belongs to Eastman Farms Ltd. Field acreage - 55 acres (22 hectares).

1976 - Spring wheat seeded at 90 lbs/ac (100 kgs/ha) and clover underseeded at 3 lbs/ac (3.4 kgs/ha).

- Fertilizer applied - 6 lbs (6.7 kgs) N; 20 lbs (22.4 kgs) P_2O_5; 20 lbs (22.4 kgs) K_2O as 8-24-24 at seeding time per acre.
- June 5th - 10 oz/ac (685 g/ha) of 2,4-D for general weed control.
- October 10th - 6 oz/ac (411 g/ha) MCPA for general weed control.

1977 - May 27th - 3 pints/ac (3.4 li/ha) MCPB (Tropotox) for dandelions, Canada thistle and perennial sowthistle control.

- June 1st to July 2nd - Bloom 30% to 100%.
- 45 hives of bees introduced (1 hive to 1.2 acres, 0.5 ha).
- June 22nd - 1 lb/ac (1.1 kgs/ha) Malathion for clover seed weevil control.
- August 20th - swathed, combined and chisel ploughed.
- clover seed yield 400 lbs/ac after cleaning (448 kgs/ha).

Fields 3 and 4: "Huscroft"

These fields are located on the eastern side of the valley south of the village of Wyndell and belong to Huscroft Bros. Farm. Field acreage - 80 acres (32 hectares).

1976 - May 14th - Field 3 (eastern section) - Barley seeded; Field "4" (west section) Spring wheat seeded. On both the cereal was seeded at 90 lbs/acre (100 kgs/ha) and clover underseeded at 4 lbs/acre (4.5 kgs/ha).

- Fertilizer applied - 11 lbs (12.3 kgs) N; 22 lbs (24.6 kgs) P_2O_5; 39 lbs (43.7 kgs) K_2O; 2 lbs (2.2 kgs) of Boron; as 17-34-0 and 0-0-60 at seeding time.

Figure 3.1.4 Aerial photograph of fields 3, 4 and 5.

1977 - May 13th - Field "4" 2.25 pints per acre (2.6 li/ha) MCPB (Tropotox) for perennial sowthistle and dandelion control.

- June 7th to June 22nd - 40% to 100% Bloom.
- 64 hives of bees introduced (1 hive to 1.3 acres, 0.5 hectares).
- June 22nd - 1.5 pints (1.7 li/ha) Malathion for clover seed weevil control.
- July 2nd to July 15th - perennial sowthistle density high in places.
- August - swathed, combined and chisel ploughed; seed yield 400 lbs/ac after cleaning (448 kgs/ha).

Field 5: Ogilvie.

This field is located diagonally across the road north of Huscroft field and belongs to Ogilvie farms. Field acreage - 50 acres (20 hectares).

1976 - Spring wheat, seeded at 90 lbs/acre (100 kgs/ha) and clover underseeded.

- Fertilizer 56 lbs (62.7 kgs) N; 96 lbs (107.5 kgs) P_2O_5; 20 lbs (22.4 kgs) k_2O as 11-48-0; 34-0-0 and 0-0-60 at seeding time per acre.
- Avadex BW (triallate) and MCPB (Tropotox) for wild oats and general weed control.

1977 - May - heavy infestation of dandelions, perennial sowthistle and Canada thistle; no weed control undertaken.

- June 7 to June 22nd - 30% to 100% bloom.
- 40 hives of bees introduced (1 hive to 1.3 acres, 0.5 hectares).
- June 22nd - 2 pints (2.3 li/ha) Malathion for clover seed weevil control.
- July 2nd - Blooms "browning".
- August - swathed and combined; clover seed yield 240 lbs/acre after cleaning (269 kgs/ha).

- October - chisel ploughed.

Field 6: "Wyndell North".

This field is located at the north end of the valley about one mile north-west of the village of Wyndell and belongs to Wyndell Farms. Field acreage - 60 acres (24 hectares).

1976 - Barley seeded at 90 lbs/acre (100 kgs/ha) and clover under-seeded at 3 lbs/ac (3.4 kgs/ha).

-Fertilizer applied - 12 lbs (13.4 kgs) N; 36 lbs (40.3 kgs) P_2O_5; 36 lbs (40.3 kgs K_2O as 8-24-24 at seeding time per acre.

1977 - May 15th to 20th - 2 pints per acre (2.3 li/ha) MCPB (Tropotox) for Canada thistle and perennial sowthistle control.

- June 7th to July 2nd - 10% to 100% Bloom.
- 44 hives of bees introduced (1 hive to 1.4 acres, 0.6 hectares).
- June 22nd - 2 pints/acre (2.3 li/ha) Malathion for clover seed weevil control.
- July 2nd - heavy infestation of perennial sowthistle controlled by clipping.
- August to September - swathed, combined and mouldboard ploughed.
- Clover seed yield 450 lbs/acre (500 kgs/ha).

3.1.3 Soil Series by Fields

The soils of the Creston Valley area where virtually all of the white clover is grown are developed on reclaimed (dyked) alluvium deposited in recent millennia by the Kootenay River as it debouched into Kootenay Lake; old channelling and variable depositional patterning is still visible in the generally flat terrain; some modification by ploughing, "floating" and drainage is to be seen in the soil profiles. To some degree soil variability is reflected in the soils record by field and soil series description given below.

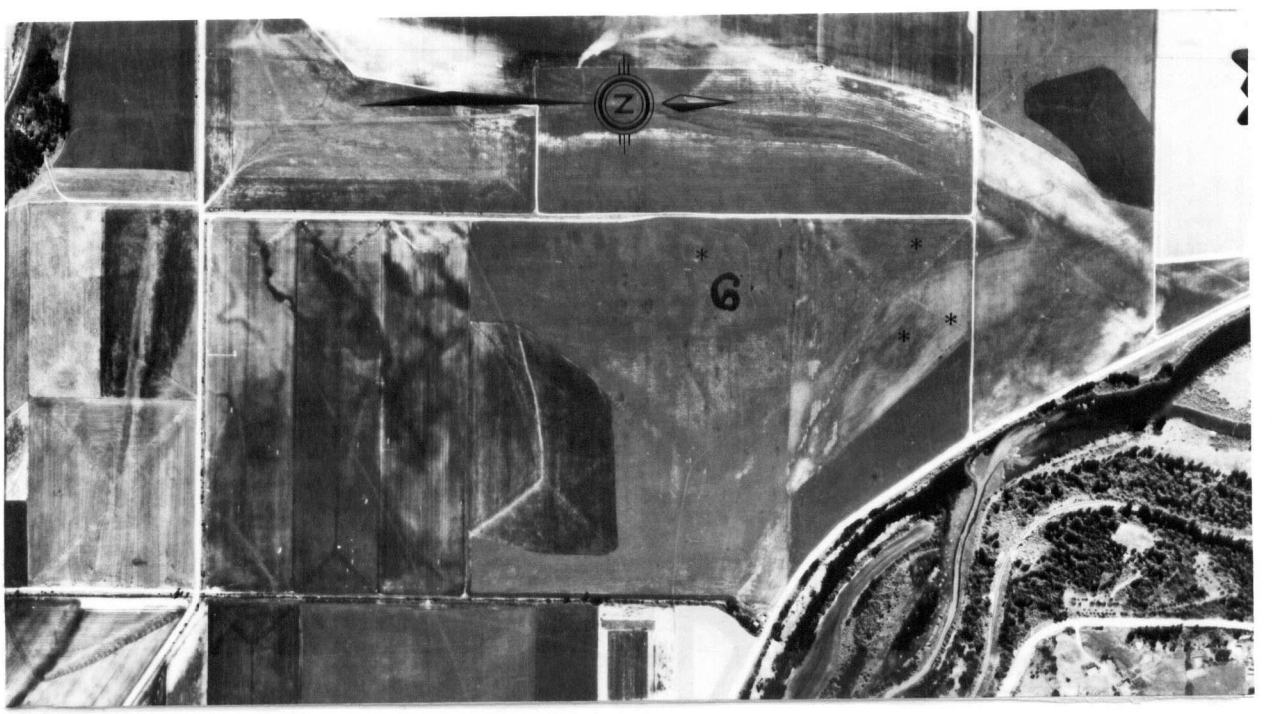


Figure 3.1.5 Aerial photograph of field 6.

			Soil Series (Wittneben and Sprout 1971)
Field 1	(65 acres) (30 hectares)	All sampling areas	Benny
Field 2	(55 acres) (22 hectares)	All sampling areas	Kuskanook 7/10
			Buckworth 2/10
Field 3 & 4	(80 acres) (32 hectares)	All sampling areas	Kuskanook 7/10
			Buckworth 3/10
	(areas with		Buckworth 8/10
	early maturity)		Kuskanook 2/10
Field 5	(50 acres) (20 hectares)	Sampling area 1	Rykerts
		Sampling areas 2 & 3	Buckworth
		Sampling area 4	Nicks
Field 6	(60 acres) (24 hectares)	Sampling areas 1 & 2	Benny 7/10
			Rykerst 3/10
		Sampling areas 3 & 4	Nicks 8/10
			Kuskanook 2/10

Soil Series Description

Benny	Great soil group	Regosol
	Sub group	Gleyed orthic regosol
	Parent material	Alluvium
	Texture	Fine sandy loam
	Drainage	Imperfect
	pH	7.9 moderately alkaline
	Calcium	25 m.e. per 100 gms high
	Magnesium	1.35 m.e. per 100 gms. Med.

	Potassium	0.13 m.e. per 100 gms.
	White clover suitability	High
Buckworth	Great soil group	Gleysol
	Sub group	Carbonated rego gleysol
	Parent material	Alluvium
	Texture	Very fine sandy loam
	Drainage	Poor
	pH	7.9 moderately alkaline
	White clover suitability	Medium
Kuskanook	Great soil group	Gleysol
	Sub group	Carbonated orthic gleysol
	Parent material	Alluvium
	Texture	Silty clay loam
	Drainage	Moderately to poorly drained
	pH	8.0 moderately alkaline
	Nitrogen	0.195% (low)
	Phosphorous	10.5 ppm (low-med)
	Potassium	0.1 me/100 gms (L)
	Calcium	27.55 me/100 gms
	Magnesium	1.76 me/100 gms (med)
	White clover suitability	Medium
Nicks	Great soil group	Gleysol
(Field 5, 6)	Sub group	Low humic eluviated gleysol
	Parent material	Alluvium

	Texture	Clay loam
	Drainage	Poor to moderately poor
	pH	7.8 (mildly alkaline)
	Nitrogen	0.162% (low)
	Phosphorous	4,2 ppm (low)
	Potassium	0.11 me/100 gms
	Calcium	14.88 me/100 gms
	Magnesium	3.01 me/100 gms (med)
	White clover suitability	Low
Rykerts	Great soil group	Regosol
(Field 5, 6)	Sub group	Gleyed orthic Regosol (Cal)
	Parent material	Alluvium
	Texture	Fine loamy sand
	Drainage	Imperfect
	pH	7.5 mildly alkaline
	Nitrogen	0.133% (low)
	Phosphorous	5 ppm (low)
	Potassium	0.07 me/100 gms
	Calcium	18.73 me/100 gms
	Magnesium	0.92 me/100 gms
	White clover suitability	Medium to high

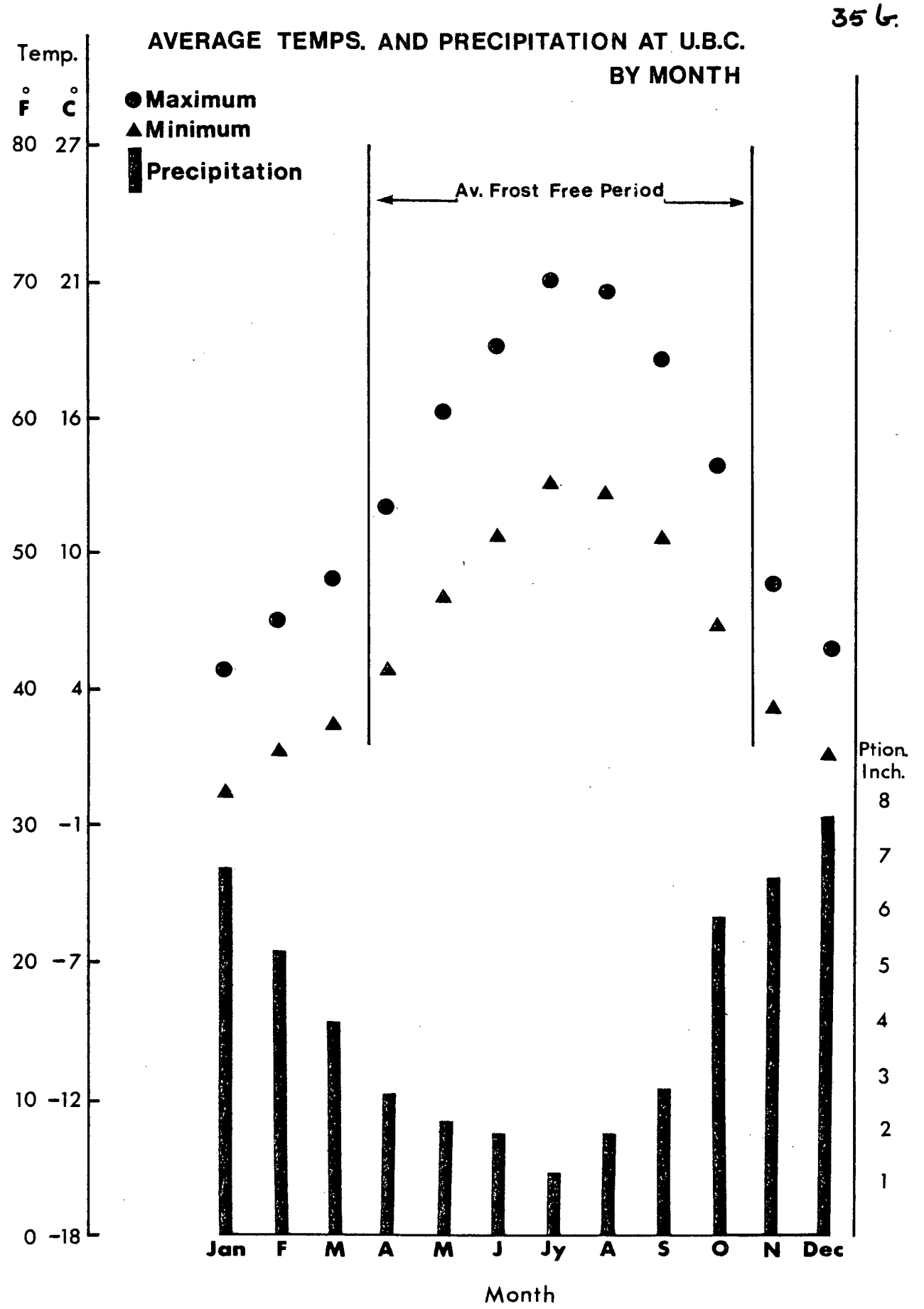
3.2.1 UBC Study Area

The area used for the study of white clover was located at the University of B.C. at Point Grey. The area is situated on the west coast of the Lower Mainland and experiences a humid, mesothermal climate, marine west coast as found in Köppen's World Classification (Department of Mines and Technical Surveys, 1957). The average climatic data are presented graphically in Figure 3.2.1. The average frost-free period is approximately 260 days, with an approximate precipitation in the growing season of 275 mm (B.C. Department of Agriculture 1974; Connor 1949). The soil is an upland soil developed on glacial material. The sub-drainage is restricted, with an acid soil reaction of about pH 5.0 (Kelly and Spilsbury 1939). The area is approximately 107 meters above sea level and the topography is undulating. The area experiences the same number of daylight hours as does Creston.

The land used for the trials was previously uncropped except for fall rye and Austrian winter peas grown as a green manure crop over the winter. Barnyard manure was applied in the spring and this together with the green manure was ploughed under in the spring of 1977. The area was harrowed, "floated", and rocks were removed prior to planting of experimental material.

Figure 3.2.1

Average temperature, precipitation, and frost free period at the University of British Columbia.


AVERAGE TEMPS. AND PRECIPITATION AT U.B.C.
BY MONTH
Temp.
°F °C
Maximum
Minimum
Precipitation
Av. Frost Free Period
80 27
70 21
60 16
50 10
40 4
30 -1
20 -7
10 -12
0 -18
Ption.
Inch.
8
7
6
5
4
3
2
1
Jan F M A M J Jy A S O N Dec
Month

4. OBSERVATIONS AND EXPERIMENTS

A. For Creston-based Studies

Selection of the fields and locations took place on the 11th of May, 1977. The fields constitute roughly a north-south transect of the valley; sites within fields were located at random but clustering was avoided.

4.1 Sampling for Insects

No one method of assessing insect kind and abundance is without difficulty and criticism. Accordingly, several approaches to assessment and monitoring were undertaken with the assistance of a student resident in Creston employed by the B.C. Ministry of Agriculture.

4.1.1 Sweeps

4.1.1.1 Materials and Methods

Three sweeps in each location in each field on each separate date were made with a 15 inch net. Sweeping took place prior to 08:00 hours to avoid bee activity. Samples were placed in plastic bags and frozen. The sweeps took place on the following dates in the summer of 1977: May 19th, June 7th, June 15th, June 20th, June 24th, June 29th, July 8th and July 15th. The average numbers of weevils collected for each field were converted to 20 sweeps per field.

4.1.1.2 Observations and Results

The results of the sweeps are represented by Fig. 4.1.1. The most striking feature is that there are three species of weevils present in all six fields (clover root curculio, clover seed weevil, lesser clover leaf weevil). There appears little overlap between the adults among species, however, larvae of the clover root curculio are in evidence in June.

Table 4.1.1

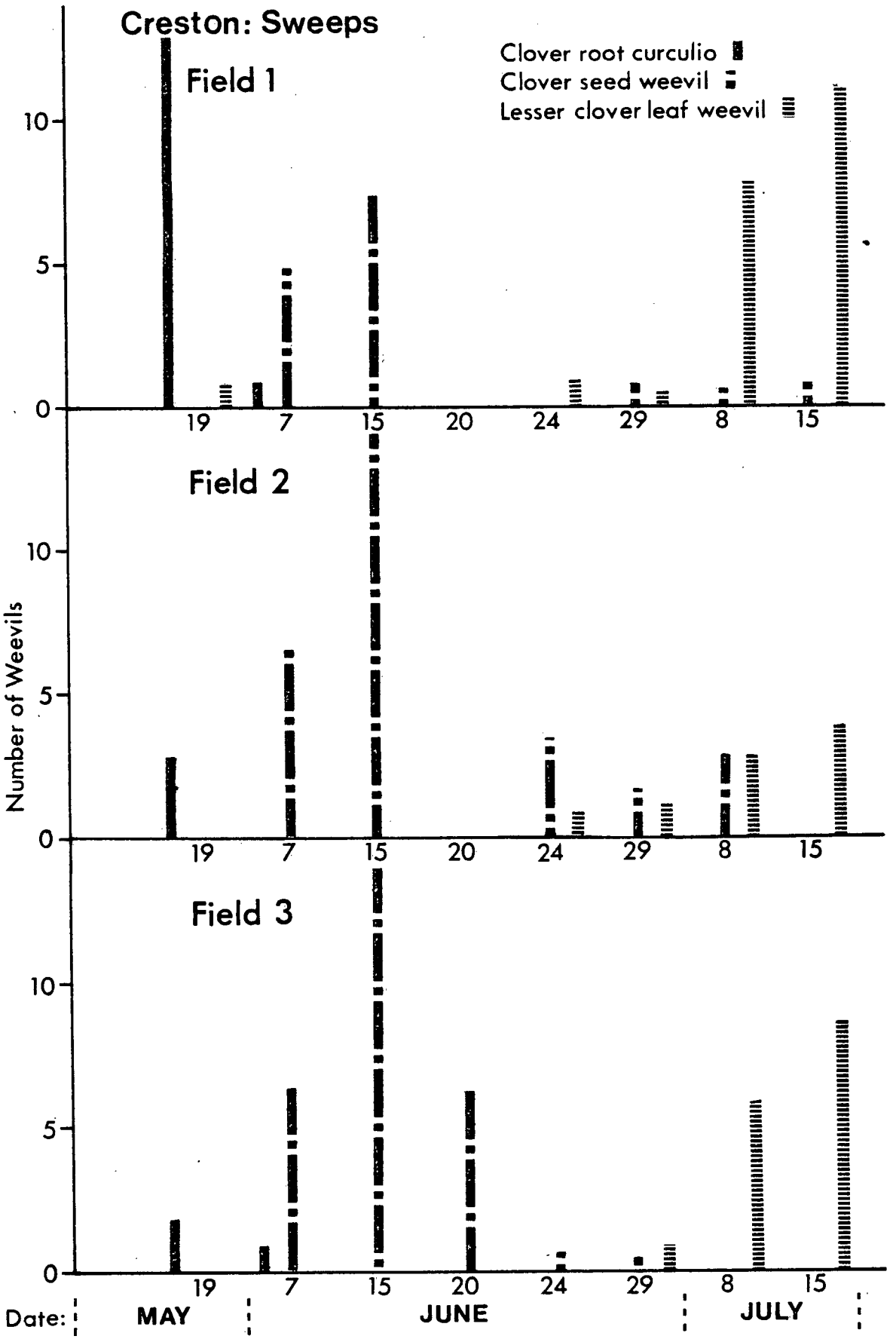
Average number of weevils collected by sweeping the foliage at Creston; numbers presented for each species by field and date

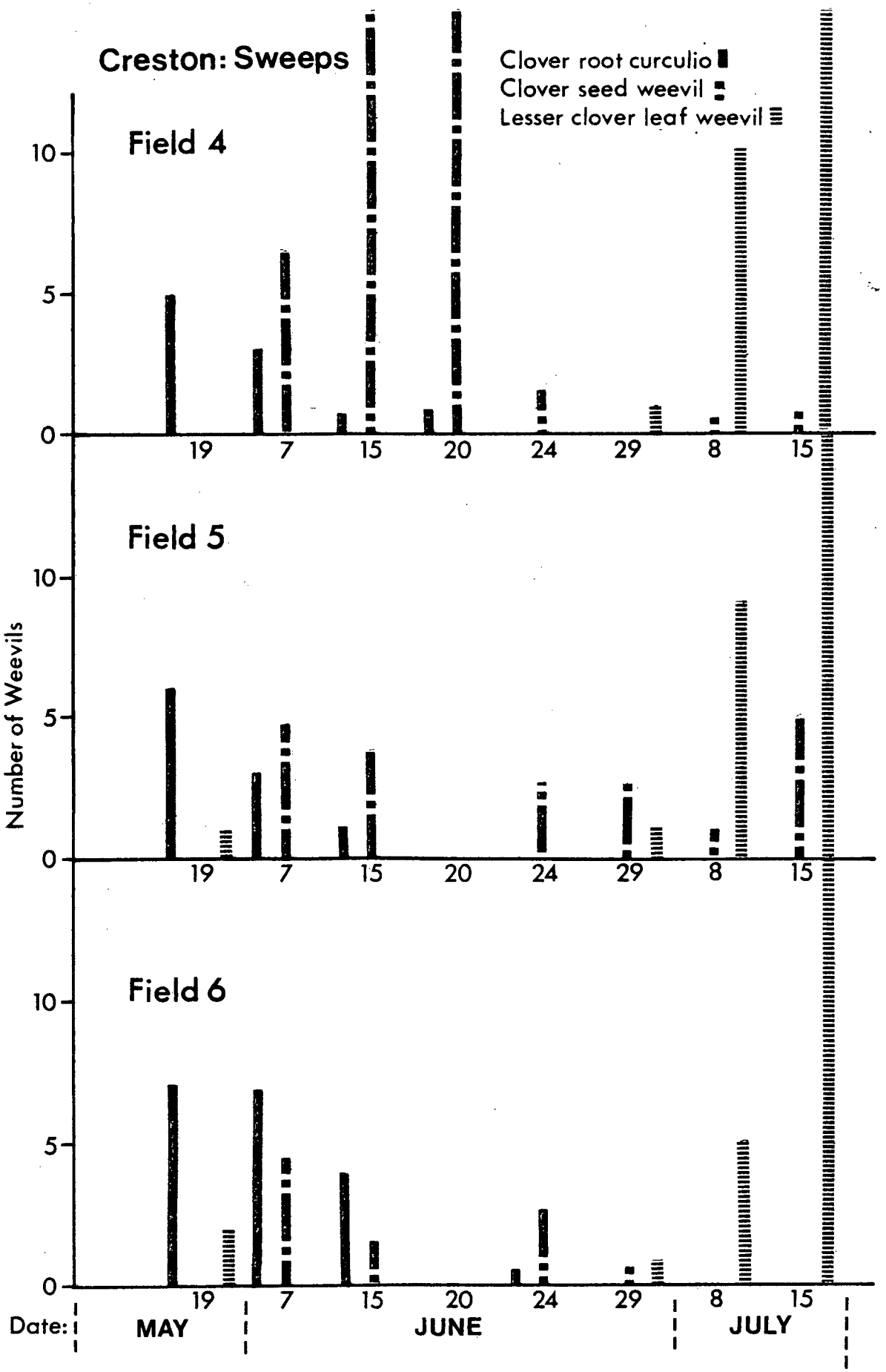
Field	Species	Date: May 19	June 7	June 15	June 20	June 24	June 29	July 8	July 15
1	A	14	0.5						
	B		5	7.5			0.8	0.4	0.4
	C	1				1	0.5	8	11
2	A	3							
	B		6.3	15.4		3.8	2.1	2.9	
	C					1	1.5	3	4
3	A	2	1						
	B		6.0	14.2	6.0	0.4	0.4		
	C						1	6	9
4	A	5	3	0.5	0.5				
	B		5.4	15.4	17.9	1.7		0.8	0.8
	C		1				1	10	18
5	A	6	3	1					
	B		4.6	3.5		2.5	2.5	0.8	5.0
	C	1					1	10	63
6	A	7	7	4					
	B		4.6	1.3		1.7	0.8		
	C	2		0.5			0.5	5	22

Weevil species - Clover root curculio - A
Clover seed weevil - B
Lesser clover leaf weevil - C

Figure 4.1.1

Weevils collected by means of sweeping the foliage in six fields in the Creston valley. The number of weevils is the average of four sites in each field converted to number of weevils for twenty sweeps. Collections were made on eight dates with a malathion spray control applied on the 22nd and 23rd day of June.


Creston: Sweeps
Field 1
Clover root curculio
Clover seed weevil
Lesser clover leaf weevil
Field 2
Field 3
Number of Weevils
0
5
10
19
7
15
20
24
29
8
15
Date:
MAY
JUNE
JULY


Creston: Sweeps
Clover root curculio
Clover seed weevil
Lesser clover leaf weevil
Field 4
Field 5
Field 6
Number of Weevils
0
5
10
19
7
15
20
24
29
8
15
Date:
MAY
JUNE
JULY

Adults of the three species of weevil essentially occur on three separate occasions within the sampled growing season. Larvae of the clover root curculio were observed to occur in June, while the adults from these larvae, emerged from pupation at the end of July, as sampled by pit fall traps. An aerial spray control was applied on the 22nd and 23rd of June using Malathion to control the clover seed weevil. Control appeared effective in reducing the clover seed weevil population. The presence of the clover seed weevil early in June in all fields should warrant concern when their feeding habits are taken into consideration.

4.1.1.3 Discussion

Sweeping appears to be an effective method of assessment of weevil activity. The starting date of May 19th was too late and should have been at least May the 1st. This would have given a better idea of the peak population of the clover root curculio. Also, a higher frequency of sweeps per week would have been desirable so that true peaks of adult weevil activity could have been obtained. This would facilitate planning of a more effective control programme. The sweeping should also take into account leaf feeders rather than just those on the flower parts.

4.1.2 Sticky Traps

4.1.2.1 Materials and Methods

Three fields were chosen as sites. These were fields 1, 5 and 6. This gave a rough representation of the study area from south to north. In each of the three fields, two locations for traps were chosen at random from four locations chosen earlier for other purposes. The sticky traps consisted of 9 inch by 11 inch yellow railroad board. "Stickum" was applied to one side

of the board on site. Two boards were placed on a stake with their traps three feet off the ground. One board faced south and one faced north. Collection dates and periods of exposure were: June 7th, June 24th, July 8th, July 19th and July 27th. When boards were collected they were wrapped in Saran wrap and placed in the freezer.

Assessment of the collections was based on a visual estimation only since it was virtually impossible to classify all organisms to genus, and to make an accurate count. It was then decided to make only a visual assessment and to classify to order only. However, Hymenopterans were counted and represented as average numbers per field. Thysanopteran numbers were noted on a scale of three, viz (a) few (10), (b) many (20), and (c) very many (30). Absence of a bar on the graph indicates that no organisms were observed.

4.1.2.2 Observations and Results

The results of the sticky traps are found in Figure 4.1.2.1. Organisms from the orders Hymenoptera and Thysanoptera occurred in noticeable numbers. Other organisms which occurred sporadically were: ladybird beetle (Hippodamia sp.), stink bugs (Pentatomidae), butterflies (Lepidoptera), weevils (Curculionidae) and flies (Diptera). The order Hymenoptera encompasses several wasps that are known to be parasitic and are considered to give some biological control. There is an increase in the wasp population up to June 24th and a drop after the spray applied on June 22nd. A rapid buildup was evident a month later. Thrips were present in June in field 5 only and then reappeared in large amounts in the latter half of July. Not all Hymenopterans and Thysanopterans are parasitic.

Table 4.1.2

Average number of organisms in groups collected by means of sticky traps at Creston; numbers presented for each group by field and date.

Field	Group	Date June 7	June 24	July 8	July 19	July 27
1	H		3		24	14
	T				20	30
5	H	1	15	2	16	36
	T	10			20	30
6	H	2.5	13	2	5	25
	T					30

Hymenoptera - H

Thysanoptera - T

Table 4.1.3.3

Average number of organisms in groups collected by means of pit fall traps at Creston; numbers presented for each group by field and date.

Field	Group	Date June 8	June 28	July 15	July 27
1	B	31	30	32	50
	S	16	8	54	41
	D	5	2	-	15
5	B	49	71	43	53
	S	35	60	53	24
	D	4	7	4	3
6	B	46	46	62	21
	S	5	33	15	15
	D	1	3	4	7

Beneficial - B
Scavenger - S
Destructive - D

Figure 4.1.2.1

Assessment of sticky traps at Creston; located in fields 1, 5 and 6, with two locations in each field and two boards at each location.

Graph date	Sampling period
June 7th	May 30th to June 7th
June 24th	June 7th to June 24th
July 8th	June 24th to July 8th
July 19th	July 8th to July 19th
July 27th	July 19th to July 27th

Numerical Assessment: for Thysanoptera

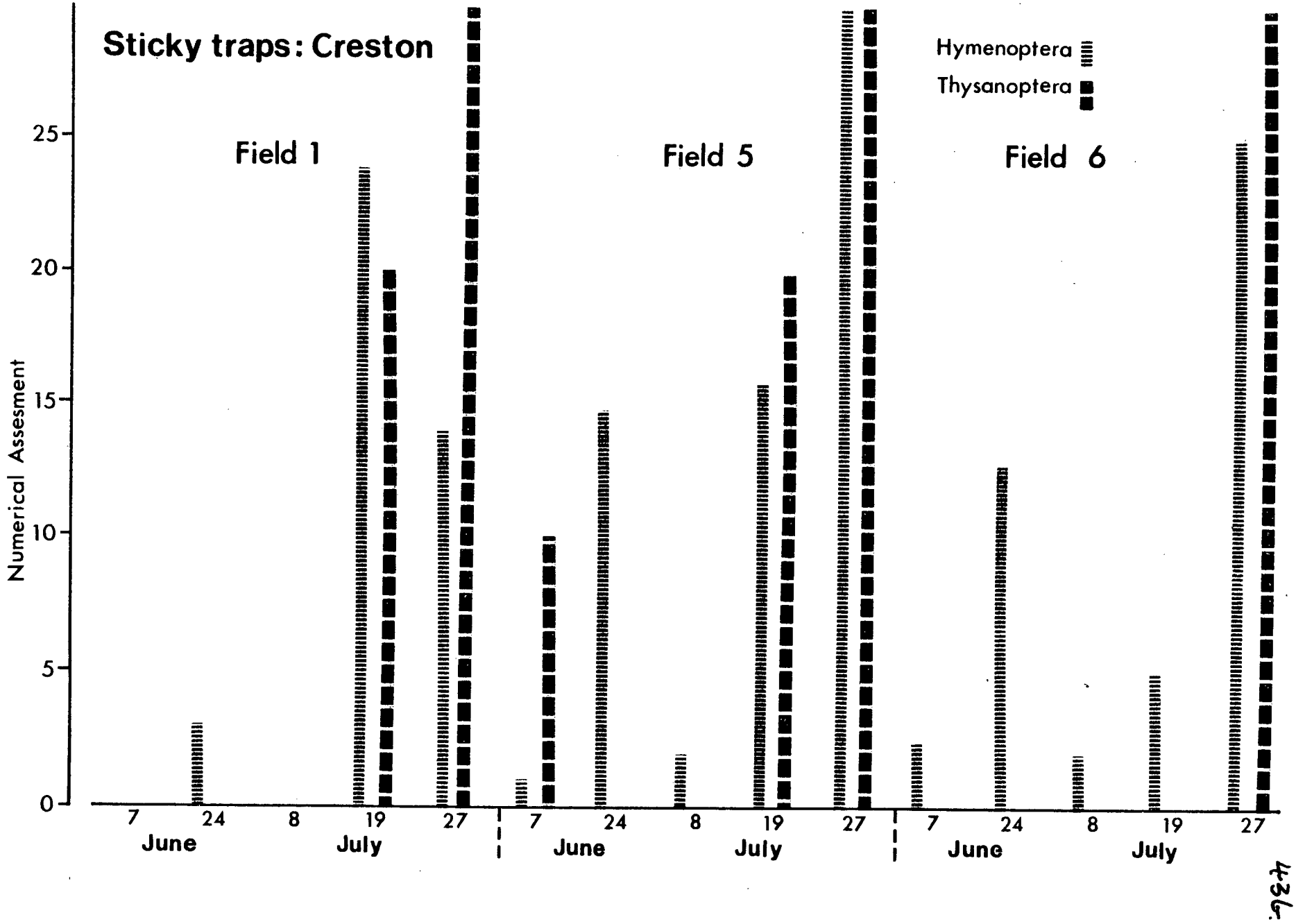
10 = Few
20 = Many
30 = Very Many

Numerical Assessment: for Hymenoptera

Average counts at two locations in three fields for five collection dates.

Figure 4.1.2.2

Insects caught on sticky traps in the Creston valley in three fields; two sites were located on each field and two traps were established at each site.


Sticky traps: Creston
Hymenoptera
Thysanoptera
Field 1
Field 5
Field 6
Numerical Assesment
0
5
10
15
20
25
7
24
8
19
27
June
July
436

4.1.2.3 Discussion

Sticky traps were useful to monitor flying insect. They have drawbacks, for instance, the "stickum" material is unpleasant to handle and if there is rain or dust it has a tendency to lose its effectiveness; birds perch on the boards and pick off a lot of the larger insects. Whether the boards faced north or south made little difference to the type or quantity of insects collected, and the traps were not useful in indicating the direction of insect migration on a local scale. More frequent collections would in all probability yield information that would be more valuable.

4.1.3 Pit Fall Traps

4.1.3.1 Materials and Methods

The same fields and locations were used for the pit fall traps as were used for the sticky traps. These traps were made using 3 1/2 inch wide Mason jars which were placed with the top of the jar level with the ground. Alcohol (70%), used as the killing and preserving medium, was added to each jar in the field. Two traps were placed at each location; one a meter north of a post, the other a meter south of the same post. Collection dates were June 8th (representing May 30th to June 8th), June 28th (representing June 8th to June 28th), July 8th (representing June 28th to July 8th), July 15th (representing July 8th to July 15th), July 27th (representing July 15th to July 27th). Samples as collected were placed in methaldehyde.

Samples were grouped according to "activity" and were tabulated as average numbers per field per collection.

4.1.3.2 Observations and Results

The results of the pit fall traps are found in Figure 4.1.3,1, and the organisms are grouped under the three headings of "Beneficial", "Scavenger", and "Destructive" (Tables 4.1.3.1 and 4.1.3.2). Such headings are self-

Table 4.1.3.1

Organisms collected by pit fall traps at Creston: Grouping of orders, families and/or genus by common name into three groups associated with their activities.

a) Beneficial - Predacious)

Taxon	Common name
- *ARANEIDA*	- Spiders
- *Staphylinidae*	- Rove beetles
- *Carabidae*	- Ground beetles
- *Histeridae*	- Hister beetles

b) Scavengers

Taxon	Common name
- *Silphidae*	- Burying beetles
- *Scarabaeidae*	- Scarab beetles
- *Nitidulidae*	- Sap beetles
- *Thysanura*	- Spring tails

c) Destructive

Taxon	Common name
- *Curculionidae*	- Weevils
- *Dermestidae*	- Skin beetles
- *Tenebrionidae*	- Darkling beetles
- *Elateridae*	- Click beetles
- *Chrysomelídae*	- Leaf beetles
- *Deroceras sp.*	- Slugs

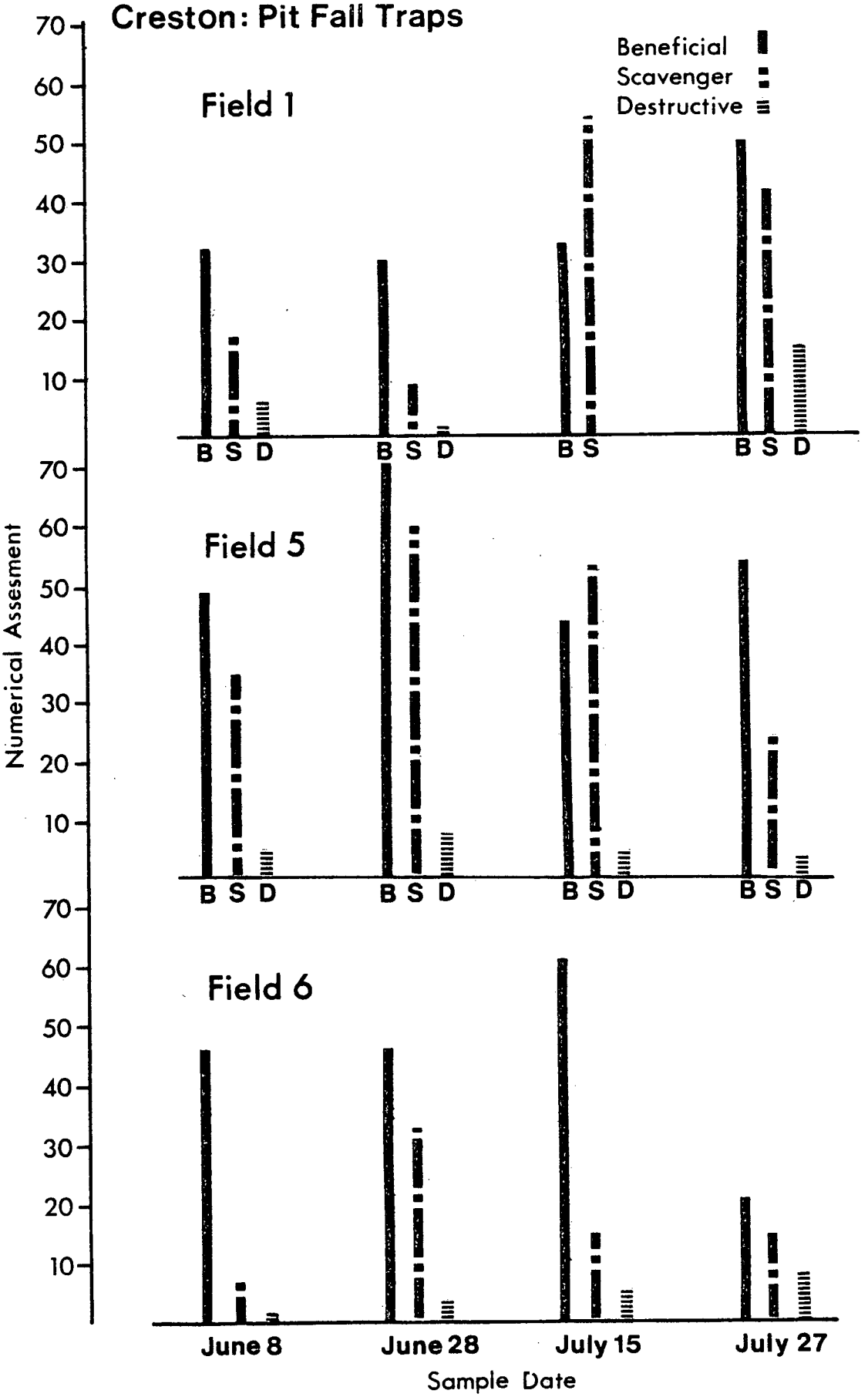
Table 4.1.3.2

Organisms collected in pit fall traps at Creston: samples taken on four separate dates from two locations in each of three fields.

	Dates of Collections			
	June 8	June 28	July 15	July 27
Field 1				
Beneficial	Spiders Ground beetles	Ground beetles Rove beetles	Spiders Ground beetles	Spiders Ground beetles Rove beetles Hister beetles
Scavengers	Sap beetles	Sap beetles Spring tails Burying beetles	Burying beetles Scarab beetles	Burying beetles Scarab beetles
Destructive	Weevils Darkling beetles	Weevils		Weevils
Field 5				
Beneficial	Spiders Ground beetles Rove beetles Hister beetles	Ground beetles Rove beetles Hister beetles	Ground beetles Rove beetles	Spiders Ground beetles Rove beetles
Scavengers	Burying beetles Scarab beetles	Burying beetles Spring tails Sap beetles	Burying beetles Scarab beetles Spring tails Sap beetles	Burying beetles Spring tails
Destructive	Weevils Slugs	Slugs Click beetles Skin beetles Leaf beetles	Click beetles	
Field 6				
Beneficial	Spiders Ground beetles Rove beetles	Spiders Ground beetles Hister beetles	Ground beetles Rove beetles	Spiders Ground beetles Rove beetles Hister beetles
Scavengers	Burying beetles Scarab beetles	Sap beetles Scarab beetles	Burying beetles Sap beetles	Scarab beetles
Destructive	Skin beetles	Skin beetles	Skin beetles Spring tails Slugs	Weevils

Figure 4.1.3

Graph showing insects collected in pit-fall traps in the Creston valley in three fields. Traps were located at two sites on each field and two traps were located at each site. Assessment is the average number of insects collected in each taxon for each date.


Creston: Pit Fall Traps
Beneficial
Scavenger
Destructive
Field 1
Field 5
Field 6
B S D
Numerical Assesment
70
60
50
40
30
20
10
June 8
June 28
July 15
July 27
Sample Date

explanatory and are used by Borror and Delong (1963).

A "biological control" appears to be in effect in all three fields and on all the dates. The spray control on June 22nd did not affect the "Beneficial" or "Scavenger" organisms to any great extent.

4.1.3.3 Discussion

It was hoped that the pit fall traps would yield more information on slug populations. A visual inspection of the foliage of the crop did indicate feeding by a rather large population of slugs. Samples of slugs collected were classified as the common slug Deroceras reticulatum (Muller). Pit fall traps proved to furnish useful information on the biological control in effect; more frequent collections would have been useful. The monitoring of slugs and snails would be better undertaken using baits as described by Crawford-Sidebotham (1972). Weevils collected on July 27th were those of the clover root curculio.

4.1.4 Soil Insect Sampling

4.1.4.1 Materials and Methods

A soil core of dimensions 10 centimeters deep by 10 centimeters diameter, giving a volume of 785 cubic centimeters was used. This is a commonly used device of the "plugger" type to give an essentially undisturbed cylinder of soil.

A) For root examination 4 dates were sampled with the plugger (May 30th, June 16th, July 14th and August 8th), and one date (September 25) an arbitrary quantity was sampled from the ploughed fields. A sample consisted of two plugs, and these plugs were washed carefully to avoid breakage and loss of roots. Damage assessment on the 30th of May was as to the presence or absence of root scoring. On the 16th June a numerical assessment was allocated ranging from 0 to 5 for all four locations on each of six fields. On the 14th July and 4th August only two samples were taken from each field.

B) For insect examination, sampling dates took place on the 5th August, 18th August and 25th September. The August samples were frozen, while the September samples were stored at 8°C. The method of extractions consisted of the use of sieves and dry extractions, with light and heat on the August samples and wet extractions on the September samples. In the dry and wet extractions plastic funnels and cheesecloth were used. In dry extractions insects were activated so that they migrate away from the light and heat and then collected at the base of the funnel. Time for extraction is approximately 4 days. The wet extraction procedure is much the same as the dry except that water is placed on the sample and aliquots are taken every half hour as the light and heat are increased.

C) Nematode examinations were undertaken at the Agriculture Canada Research Station, Vancouver. Samples to be examined consisted of 4 cores from each field (with plants in situ) placed in jiffy pots on the 5th August. The extraction of nematodes from the roots was by way of misting.

D) For assessment of pathogens (fungi and bacteria), plants were taken from fields 2, 3, 4, and 5 on the 25th September and stored in plastic bags at 8°C. These samples were subsequently examined by Dr. R. Copeman, Department of Plant Science, the University of B.C.

4.1.4.2 Observations and Results

A) Root damage ratings: The results are presented in Table 4.1.4.1. It is noteworthy that damage appears in almost all samples and that the roots deteriorate as the summer progresses. Moreover, damage begins before the end of May. Larvae fitting the description given by Davidson and Peairs (1960) of the clover root curculio were observed in the root zone of each core on dates May 30th and June 16th.

B) Soil insect examinations using sieves and dry extractions proved to be of little value; it appeared that most organisms perished in storage.

Table 4.1.4.1

An estimation of root damage by date to white clover plants in farmers' fields in Creston

Field	Location	Date collected 30/5	16/6	14/7	4/8
1 (Wyndell South)	1	+	3 (5)	3	4
	2	-	2 (7)		
	3	+	3 (-)	4	4
	4	+	2 (3)		
2 (Eastman)	1	-	3 (1)	3	4
	2	+	2 (3)		
	3	+	1 (11)		
	4	-	2 (5)	2	3
3 (Huscroft East)	1	+	3 (-)	3	4
	2	+	3 (-)		
	3	+	4 (-)		
	4	+	3 (1)	3	3
4 (Huscroft West)	1	+	3 (-)		
	2	+	3 (-)		
	3	-	0 (-)	3	2
	4	-	0 (-)	3	3
5 (Ogilvie)	1	-	1 (-)	4	3
	2	-	2 (2)		
	3	+	4 (1)		
	4	+	3 (7)	4	3
6 (Wyndell North)	1	+	1 (2)	4	5
	2	-	1 (3)		
	3	+	2 (4)	4	4
	4	-	3 (2)		

Damage Rating

a) For date - 30/5/77 visible insect injury +
no visible insect injury -

b) For dates 16/6, 14/7, and 4/8 ranking "damage" from 1 to 5 with 1 as very slight injury to 5 for extensive injury resulting in crown rot.

c) Numbers in brackets are numbers of larvae collected in soil and root cores.

C) Nematode examinations: The results are presented in Table 4.1.4.2. The occurrence of Pratylenchus spp. in field 2, may be of concern; it is to be noted that field 2 was at one time in hops. Other fields are "normal" (personal communication - Dr. McElroy, Agriculture Canada, Vancouver, B.C.).

D) Preliminary plant pathogen examinations: Observations in May revealed disorders of pathological nature in fields 3 and 4. Other fields appeared normal. On September 25th, after all fields had been ploughed, healthy plants were found in field 2, while in fields 3 and 4 after ploughing, all plants examined in the field were dying. Preliminary pathogen identification from fields 2, 3, 4 and 5 has indicated the presence of several Fusarium species of fungi in damaged roots. The Fusarium species that occurred predominantly in field 2 were not so predominant in fields 3, 4 and 5.

4.1.4.3 Discussion

It is evident from Table 4.1.4.1 that most of the root damage by the clover root curculio is very likely general throughout the valley and could have a bearing on the vigour, longevity and seed yield of white clover stands. The root damage observed on all roots fitted closely the description given by Kilpatrick and Dunn (1961). However, more samples and more sampling dates would be required to reveal the extent of damage throughout the area. It is evident from the absence of larvae in the July and August samples that most of the initial damage occurs in May and early June. It was observed that root washing was not essential, but the general examination of clover roots in the field would provide a good indication of the succession of infestation.

The presence of Fusarium spp. of fungi in the damaged white clover roots is in agreement with Kilpatrick and Dunn (1961) and also the work by O'Rourke (1969). These two papers indicate that Fusarium spp. are normal soil residents

Table 4.1.4.2

Nematode counts* obtained by extraction from soil-clover cores from Creston. Field sampling 5/8/77. Counts as number of nematodes per gram of air dry roots.

		Sampling area			
Pratylenchus (style bearing)		1	2	3	4
Lesion	Field 1	0	0	0	0
	Field 2	374	331	44	0
	Field 3	0	0	0	0
	Field 4	0	0	120	0
	Field 5	0	0	0	0
	Field 6	0	6	0	0
Ditylenchus (style bearing)					
Stem	Field 1	0	0	0	7
	Field 2	20	0	0	30
	Field 3	0	0	0	0
	Field 4	0	0	0	20
	Field 5	0	0	0	0
	Field 6	0	0	5	0
Aphelenchus (style bearing)					
Leaf	Field 1	24	21	50	26
	Field 2	7	15	0	0
	Field 3	0	160	103	20
	Field 4	0	86	14	0
	Field 5	23	0	0	0
	Field 6	0	6	32	0

*Made by the staff of the Research Station, Agriculture Canada, Vancouver, B.C.

but they enter the plant when it is under stress. I feel that the feeding of the clover root curculio on the Creston white clover is a major contributor to the entry of the pathogens isolated by Dr. Copeman.

4.2 Soil Sampling for Fertility

4.2.1 Materials and Methods

In soil sampling a one-half inch soil corer was employed. Cores were taken to plough depth. Subsampling at each field location was done on a "square grid" pattern. The dates for collection were May 30th and August 28th; 48 samples in all were collected.

Major available nutrients in the soils of the study areas were determined using the Morgan's Universal System. Soil reaction was determined using customary procedures by employing a pH meter.

4.2.2 Observations and Results

Availability of major nutrients was similar in all fields and sites within fields. Nitrate nitrogen averages were low at 8 kgs/ha for the 30th May sampling and at 7 kgs/ha for the 5th August sampling. Phosphorous readings were medium to high at 25 to 50 parts per million. Magnesium levels were very low at 12 parts per million, while calcium levels were very high at 250 parts per million. Soil reaction at all locations was mildly alkaline at pH 7.1 to 7.8.

4.2.3 Discussion

The quick test system is adequate for trouble shooting, but as a fertilizer recommendation it is not precise. The soil reaction and nutrient levels approximate those given for the area in the soil survey report by Wittneben and Sprout (1971). The mildly alkaline reaction of the soil coupled with the high level of calcium could have a bearing on the level of available phosphorous in the soil.

4.3 Phytomass Sampling

4.3.1 For Dry Matter Yields on 30-5-77

4.3.1.1 Materials and Methods

Sampling was carried out on the 30th May. A one-quarter meter quadrat was used and placed randomly twice at each location in each field. Clips of top growth were taken as close to the ground as possible. The material was then dried in the pot-hole drier at U.B.C. and weighed.

4.3.1.2 Observations and Results

The results are tabulated in Table 4.3.1.1 and graphed in Figure 4.3.1.1. It is to be seen that there is a fairly wide difference in yields between fields even at a date as early as May.

4.3.1.3 Discussion

More and larger samples would have given greater precision; the sheer volume of samples to store and transport and the labour in making plant separations would render the efforts of questionable worth.

4.3.2 For Dry Matter Yields on 5-8-77

4.3.2.1 Materials and Methods

Sampling was carried out on August 5th. The sampling procedure was the same as aforementioned in Section 4.3.1, except that three samples per location in each field were taken. Harvesting was timed to occur just prior to swathing the crop. The samples were air-dried at Creston. The material was transported to Vancouver and processed. Separation of grain stubble from the previous year's crop was done prior to the taking of total weight. The samples were weighed after standing at room temperature for a number of weeks.

4.3.2.2 Observations and Results

Plant top growth yields are given in Table 4.3.2.1 and in Figure 4.3.2.1. Again a substantial variation in yields between fields is recorded.

Table 4.3.1.1

Top weight sampling in six fields in Creston in May 1977.
(weights in gms. per 1/4 m^2 quadrat)

	kgs/ha	Mean	SD	CV%	Range	No. Samples
Field 1 Wyndell South	3640	91	16	18	62 - 113	8
Field 2 Eastman	2200	55	13	24	35 - 74	8
Field 3 Huscroft East	2200	55	9	16	37 - 63	8
Field 4 Huscroft West	2400	60	6	10	49 - 66	8
Field 5 Ogilvie	3400	85	17	20	58 - 116	8
Field 6 Wyndell North	3520	88	14	16	73 - 111	8

Table 4.3.2.1

Top weight sampling in six fields in Creston in August.
(weights in gms. per 1/4 m^2 quadrat)

	kgs/ha	Mean	SD	CV%	Range	No. Samples
Field 1 Wyndell South	6040	151	47	31	106 - 261	12
Field 2 Eastman	3640	91	16	18	65 - 122	12
Field 3 Huscroft East	5280	132	19	14	95 - 156	12
Field 4 Huscroft West	5280	132	51	39	68 - 263	12
Field 5 Ogilvie	6560	164	46	28	87 - 221	12
Field 6 Wyndell North	7320	183	50	27	109 - 248	12

Table 4.3.3.1

Seed weight in six fields in Creston, August 1977.
(weights in gms. per 1/4 m^2 quadrat)

	kgs/ha	Mean	SD	CV%	Range	No. Samples
Field 1 Wyndell South	672	16.8	5.3	31	11.2 - 30.8	12
Field 2 Eastman	468	11.7	1.6	13	9.6 - 15.1	12
Field 3 Huscroft East	972	24.3	4.5	18	16.9 - 29.4	12
Field 4 Huscroft West	756	18.9	8.8	47	6.9 - 28.3	12
Field 5 Ogilvie	468	11.7	5.6	48	5.3 - 21.1	12
Field 6 Wyndell North	480	12.0	4.0	33	6.7 - 16.8	12

Figure 4.3.1.1

The variation in weight of top growth in six fields in Creston, 30th May 1977 (1): Two quadrats from 4 replicates in each field, showing:

a) The arithmetic mean for each field ●
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars

Creston: weight of top growth (1)

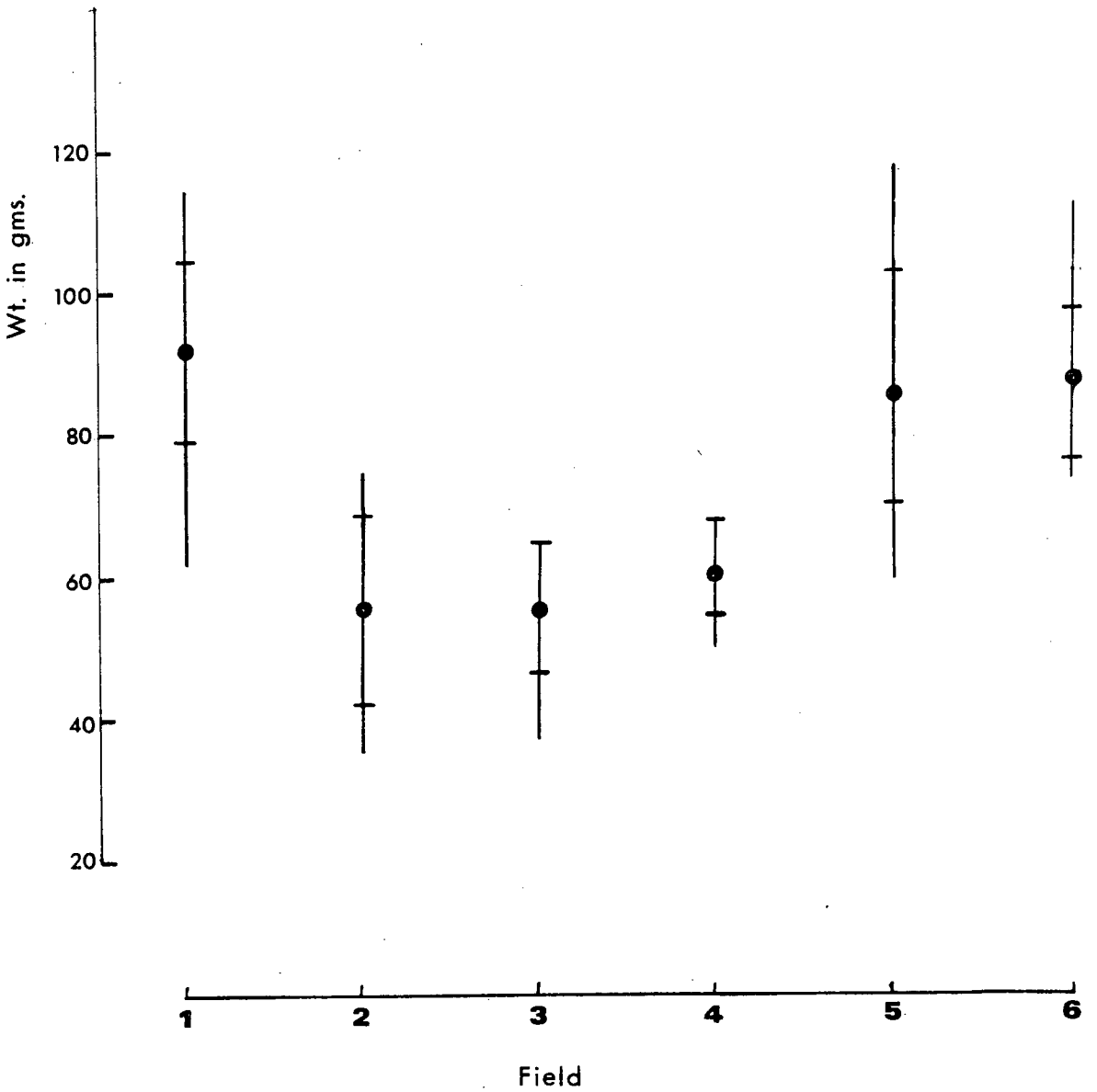
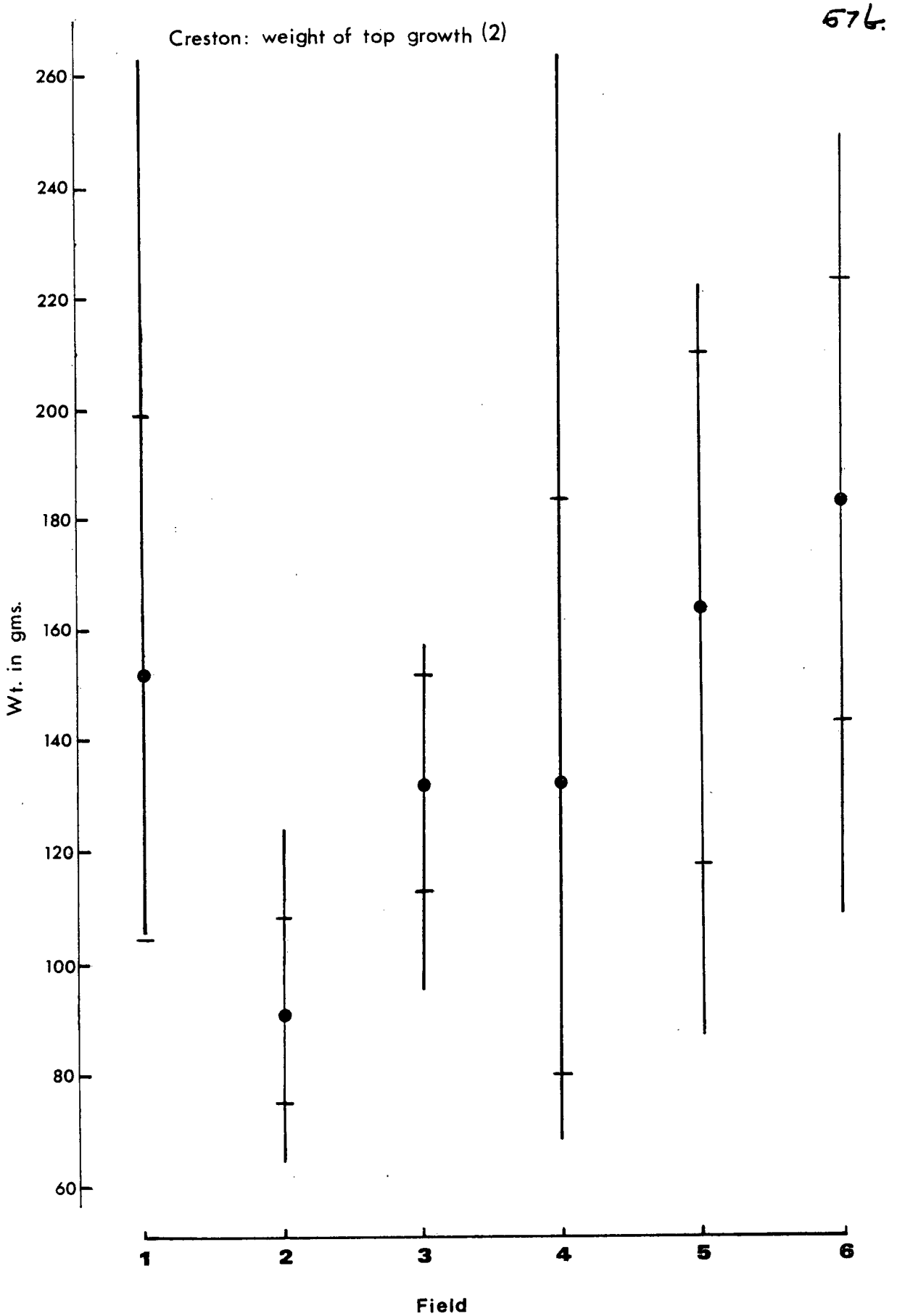


Figure 4.3.2.1

The variation in weight of top growth in six fields in Creston, August 1977 (2): Three quadrats from 4 replicates in each field showing -

a) The arithmetic mean for each field •
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars


Creston: weight of top growth (2)
260
240
220
200
180
160
140
120
100
80
60
Wt. in gms.
1
2
3
4
5
6
Field

4.3.2.3 Discussion

Harvesting of the material was carefully undertaken to keep loss to a minimum. The small quadrat was useful in this respect. Soil variability can partially explain the large standard deviations; it was further observed in one set within a single field that clover in certain areas of different soil texture would flower prematurely and not be very tall, whereas in other areas clover remained vegetative and grew vigorously. Disease, pests and other factors undoubtedly contributed to yield variability.

4.3.3 Seed and Inflorescence Yields on 5-8-77

4.3.3.1 Materials and Methods

Inflorescences were separated from the rest of the material harvested as per section 4.3.2. They were then counted and passed through the head thresher. The seed was then cleaned by passing through a series of sieves, hand winnowed, and finally weighed.

4.3.3.2 Observations and Results

Seed harvest and number of inflorescences per unit area are illustrated in Table 4.3.3.1 and 4.3.3.2 and Figures 4.3.3.1 and 4.3.3.2. Fields 2, 5 and 6 gave the lowest yields and fields 3 and 4 gave the highest yields, for both seed and inflorescences. The general trend from field to field for seed yield to approximate inflorescence number is evident. The average number of inflorescences per hectare was estimated to be 11.5 million; the average seed yields were estimated to range from 468 to 972 kgs per hectare (418-868 lbs/ac).

4.3.3.3 Discussion

It was anticipated that the small number and size of the samples would result in a rather large standard deviation. However it can be seen that increasing seed yield tends to relate to a moderate yield of standing crop; a heavy top growth tends to suppress number of heads and seed yield on a

Table 4.3.3.2

Number of heads per unit area in six fields in Creston taken in August 1977

	Mean	SD	CV%	Range	No. quadrats/ Sample
Field 1 Wyndell South	323	61	19	232 - 413	12
Field 2 Eastman	213	55	26	92 - 314	12
Field 3 Huscroft East	363	55	15	278 - 435	12
Field 4 Huscroft West	326	78	24	227 - 465	12
Field 5 Ogilvie	257	49	19	173 - 319	12
Field 6 Wyndell North	239	80	33	129 - 375	12

Table 4.3.3.3

Dependence of seed yield on number of inflorescences per unit area. Linear regression equations and correlation coefficients for six fields in Creston, August, 1977.

Field 1	Y = 0.98 + 0.05X	r = 0.56
Field 2	Y = 7.21 + 0.02X	r = 0.73 **
Field 3	Y = 8.89 + 0.04X	r = 0.52
Field 4	Y = -10.15 + 0.09X	r = 0.79**
Field 5	Y = -12.20 + 0.09X	r = 0.81**
Field 6	Y = 4.42 + 0.03X	r = 0.75**

r .01(10) = 0.7079**

Number of inflorescences - X; seed yield - Y

Number of samples per treatment 12

Figure 4.3.3.1

The variation in weight of seed from six fields in Creston, August 1977: Three quadrats from 4 replicates in each field showing -

a) The arithmetic mean for each field ●
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars

Creston: seed weight

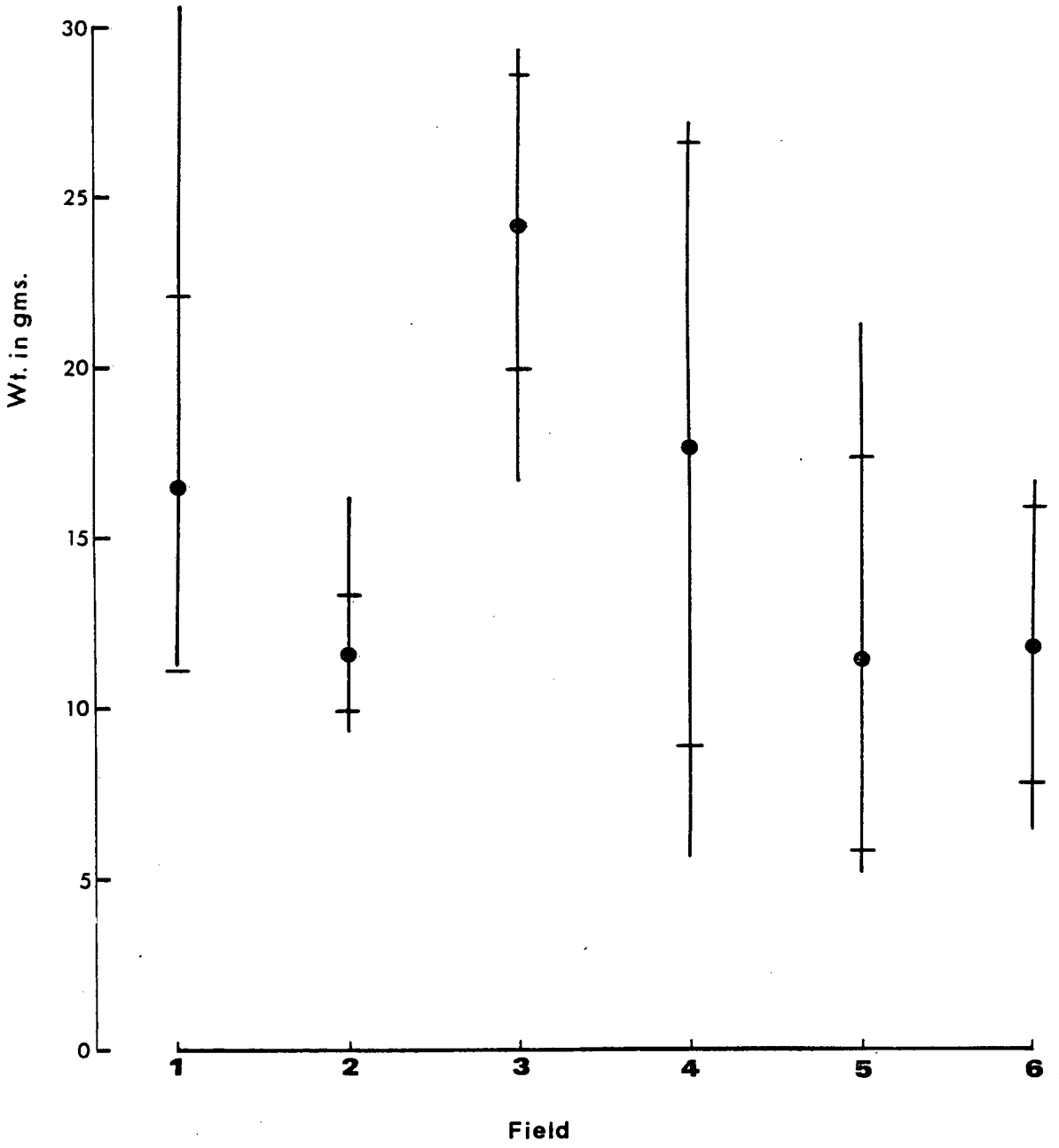
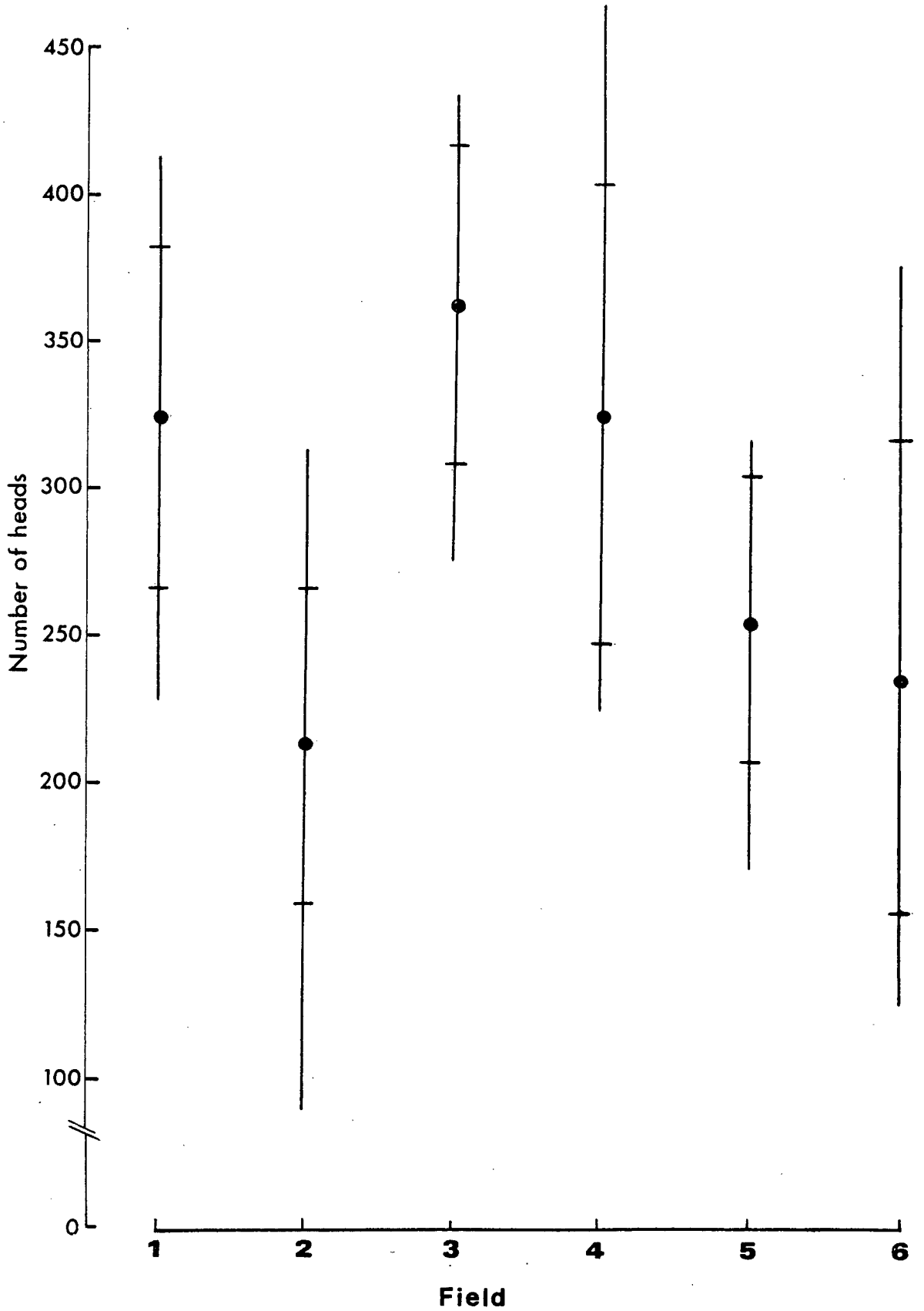


Figure 4.3.3.2

The variation in the number of heads per unit area in six fields in Creston, August 1977: Three quadrats from 4 replicates in each field showing -

a) The arithmetic mean for each field •
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars

CRESTON: Heads Per Unit Area



unit area. Table 4.3.3.3 lists the linear regression equations and correlation coefficients for inflorescence numbers and seed yield on a unit area. If seed yield is regarded as the dependent variable there is a strong suggestion that as numbers of inflorescences increase then seed yield also increases. Hawkins (1956) working with red clover finds a similar relationship. If my seed yields are compared to farmers' yields, the farmers are experiencing a seed loss while harvesting up to 50%.

4.3.4 Individual Head Examinations

4.3.4.1 Materials and Methods

Prior to harvest twenty-one inflorescences were taken from each sampling area in each field. Five heads were selected at random, rubbed to remove the seeds, and then winnowed. The seeds were then counted and examined for insect damage and other undesirable factors.

4.3.4.2 Observations and Results

It is notable that both the average number of seeds per head and standard deviations are similar for all fields except field 5; means lie between 144 to 161 seeds per head; insect damaged seeds ranged from 3% to 10% (Table 4.3.4.1 and Figure 4.3.4.1).

4.3.4.3 Discussion

The factor that caused the lowering in seeds per head in field 5 did not affect the number of heads per unit area and remains unexplained; perhaps it could be attributed to aberrant pollination or to erratic insect damage. Table 4.3.4.2 shows the "independence" of insect damaged seed to the influence of location.

4.3.5 Leaf Area, Petiole Length and Peduncle Length

4.3.5.1 Materials and Methods

A) Leaf Area: Four leaves and their petioles were collected at random

Table 4.3.4.1

Number of seeds per head in six fields in Creston, August 1977.

	Mean	SD	CV%	Range	No. heads/ Sample
Field 1 Wyndell South	151	73	48	13 - 259	20
Field 2 Eastman	161	94	58	31 - 376	20
Field 3 Huscroft East	160	71	44	58 - 325	20
Field 4 Huscroft West	144	94	65	36 - 347	20
Field 5 Ogilvie	97	59	61	37 - 243	20
Field 6 Wyndell North	161	82	51	31 - 311	20

Table 4.3.4.2

Contingency table for insect-damaged seed from individual seed heads from Creston field in August 1977. Classes are 'percentage of seeds damaged".

Field	Name	Class 0-5%	Class 5-100%	Total
1	Wyndell South	4 (6.10)	6 (3.90)	10
2	Eastman	9 (9.14)	6 (5.86)	15
3	Huscroft East	13 (12.19)	7 (7.81)	20
4	Huscroft West	11 (12.19)	9 (7.81)	20
5	Ogilvie	11 (12.19)	9 (7.81)	20
6	Wyndell North	16 (12.19)	4 (7.81)	20
Total:		64	41	105

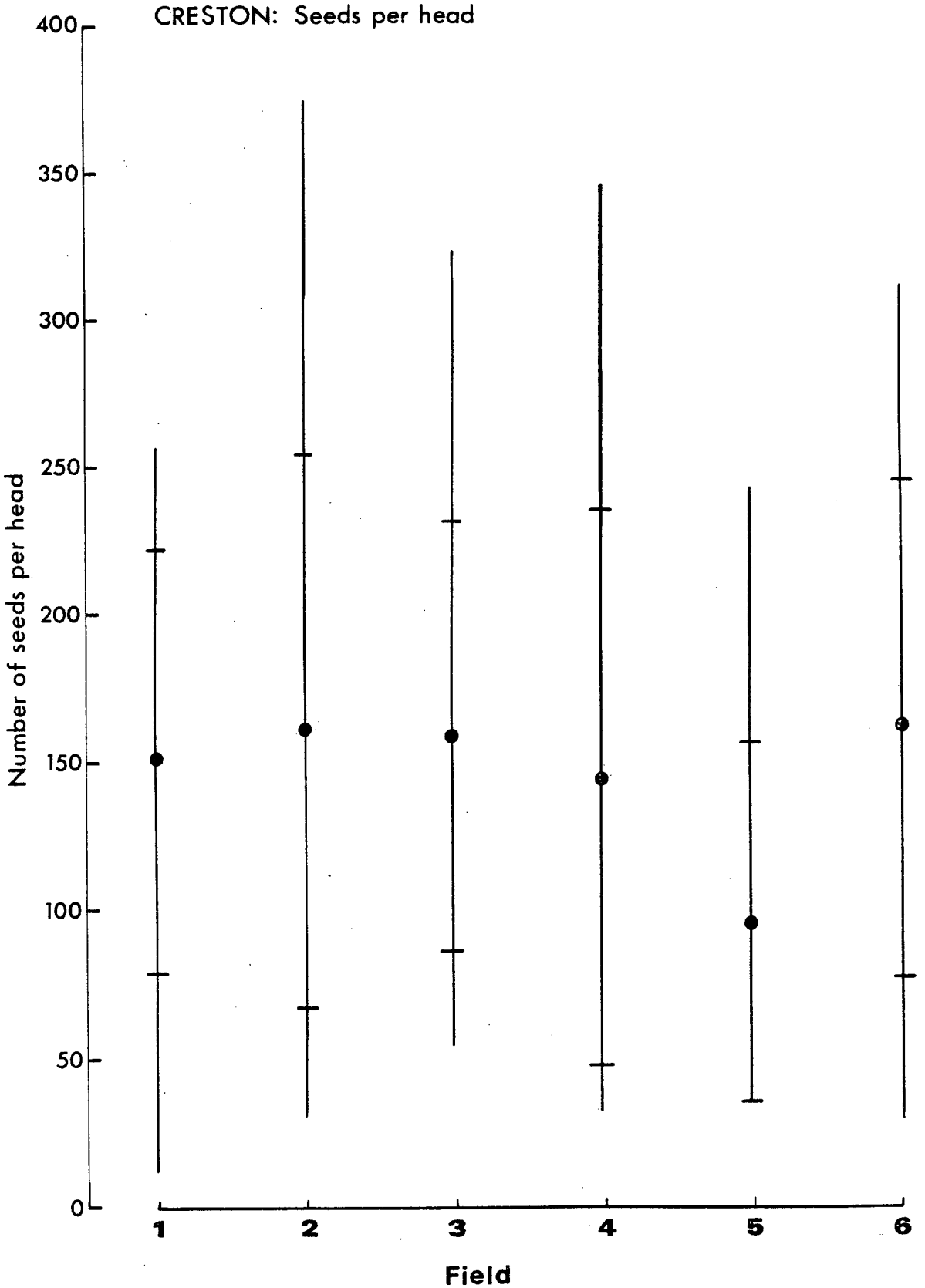
Expected values in brackets

Actual $x^2 = 5.72$ Expected x^2 (0.05)(5) = 11.07

Figure 4.3.4.1

The variation in number of seeds per head in six fields in Creston, August 1977: Five inflorescences taken from twenty-one inflorescences collected in the field from four replicates from each field showing -

a) The arithmetic mean for each field •
b) The simple range by vertical bars
c) $\pm$ one standard deviation from the mean by horizontal bars


CRESTON: Seeds per head
400
350
300
250
200
150
100
50
0
Number of seeds per head
1
2
3
4
5
6
Field

from each location in each field at the beginning of July. The leaf area was estimated in square centimeters using the method of Williams et al. (1964).

B) Petiole Length: Was measured on the same samples as in 4.3.5 A. Length was measured from the base of the trifoliate leaf to the base of the petiole where it joins the plant stem or stolon.

C) Peduncle Length: Samples were collected at the same time and from the same location. Length was measured from the base of the inflorescence to the point of attachment of the peduncle to the stem or stolon.

4.3.5.2 Observations and Results

A) Leaf Area: A summary of results of the leaf area measurements is found in Table 4.3.5.1 and Figure 4.3.5.1. Leaf area appears to be similar in all fields except 5 and 6, where the upper limit of the range was noticeably higher. By comparing the data with those given in Figure 4.3.2.1 it is evident that a general increase in leaf area follows an increase in top growth weight under field conditions in Creston. Mean leaf area under field conditions in the Creston Valley is consistently smaller than single plantings at U.B.C.

B) Petiole Length: The results of the measurements for length of leaf petiole are found in Table 4.3.5.2 and Figure 4.3.5.2. The means are similar from field to field.

C) Inflorescence Peduncle: The results of the measurements for the length of inflorescence peduncle are found in Table 4.3.5.3 and in Figure 4.3.5.3. Means are similar from field to field.

4.3.5.3 Discussion

The method of leaf area estimation by comparing our leaves with those of leaves from samples of known area proved to be rapid and relatively reliable.

Table 4.3.5.1

Average leaf areas in centimeters for six fields in Creston in July 1977.

	No.	Mean (cm^2)	SD	CV%	Range	No. leaves per sample
Field 1 Wyndell South		9	4	44	4 - 15.8	16
Field 2 Eastman		8	2	25	6.3 - 15.8	16
Field 3 Huscroft East		9	3	33	5 - 12.6	16
Field 4 Huscroft West		9	3	33	6.3 - 15.8	16
Field 5 Ogilvie		12	4	33	6.3 - 20	16
Field 6 Wyndell North		13	5	38	6.3 - 20	16

Table 4.3.5.2

Average leaf petiole lengths in centimeters for six fields in Creston in July 1977.

	Mean	SD	CV%	Range	No. petioles per sample
Field 1 Wyndell South	19	5	26	9 - 25	16
Field 2 Eastman	22	3	14	16 - 27	16
Field 3 Huscroft East	18	4	22	11 - 23	16
Field 4 Huscroft West	20	5	25	14 - 29	16
Field 5 Ogilvie	19	5	26	11 - 27	16
Field 6 Wyndell North	21	3	14	14 - 26	16

Table 4.3.5.3

Average (inflorescence) peduncle lengths in centimeters for six fields in Creston, July 1977.

	Mean	SD	CV%	Range	No. peduncles per sample
Field 1 Wyndell South	32	4	13	26 - 39	16
Field 2 Eastman	37	4	11	32 - 44	16
Field 3 Huscroft East	34	4	12	27 - 44	16
Field 4 Huscroft West	36	5	14	29 - 45	16
Field 5 Ogilvie	38	5	13	29 - 48	16
Field 6 Wyndell North	37	5	14	29 - 47	16

Figure 4.3.5.1.

The variation in leaf area in six fields in Creston, July 1977: Four leaves randomly taken from four replicates from each field, showing:

a) The arithmetic mean for each field •
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars

CRESTON: Leaf Area

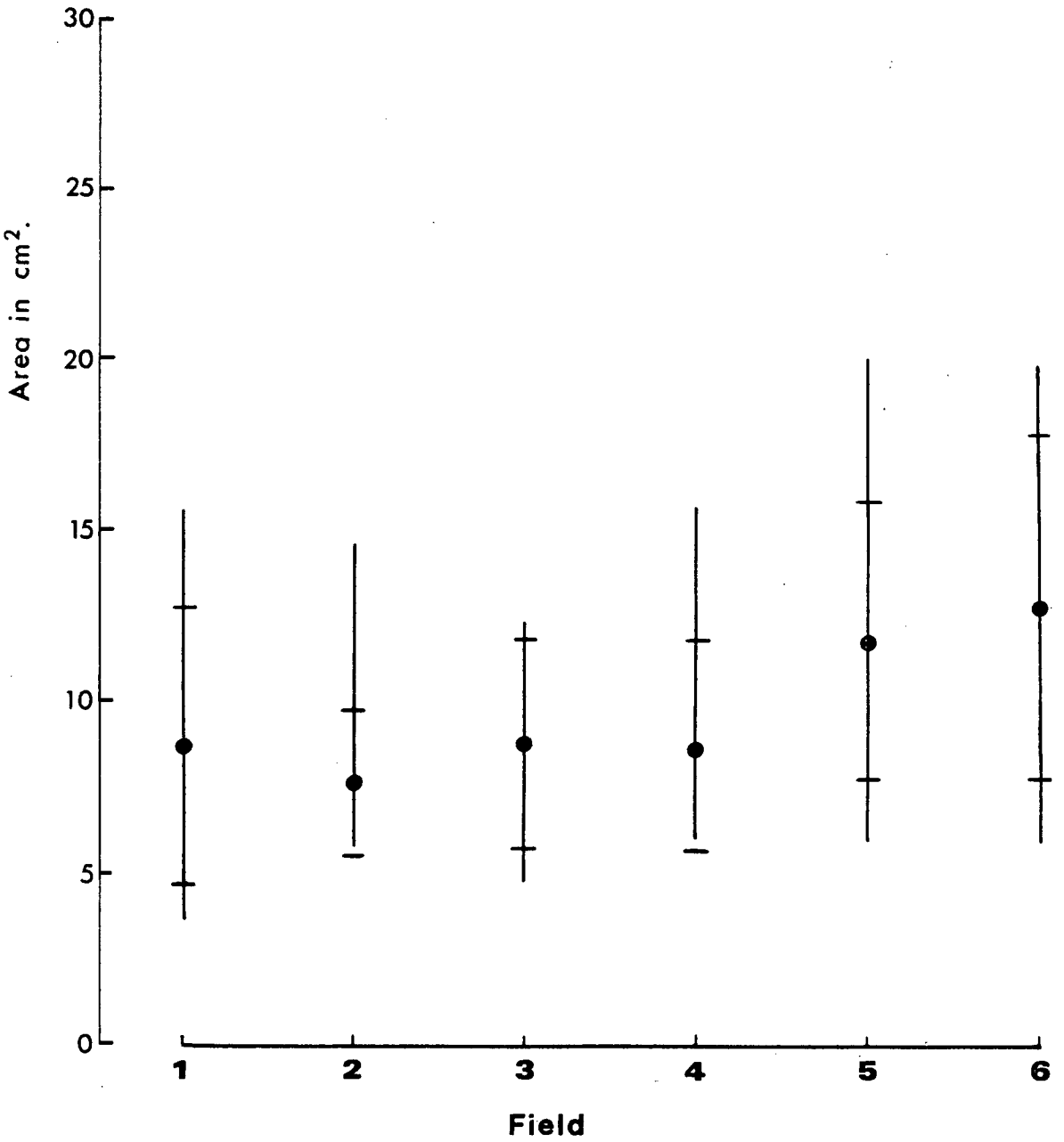


Figure 4.3.5.2

The variation in leaf petiole length for six fields in Creston, July 1977: Four petioles randomly taken from replicates from each field showing:

a) The arithmetic mean for each field •
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars

CRESTON: Leaf Petiole

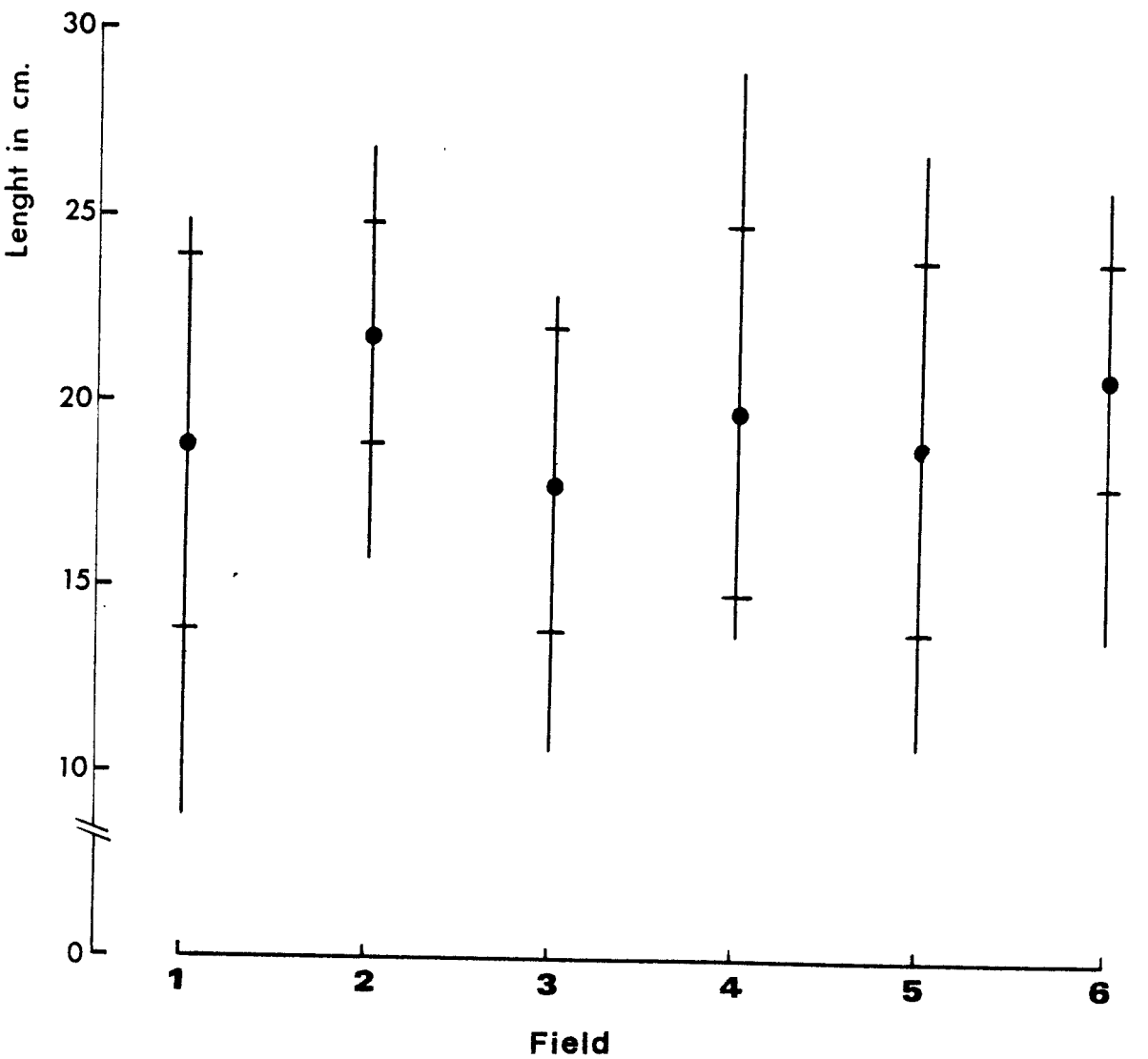
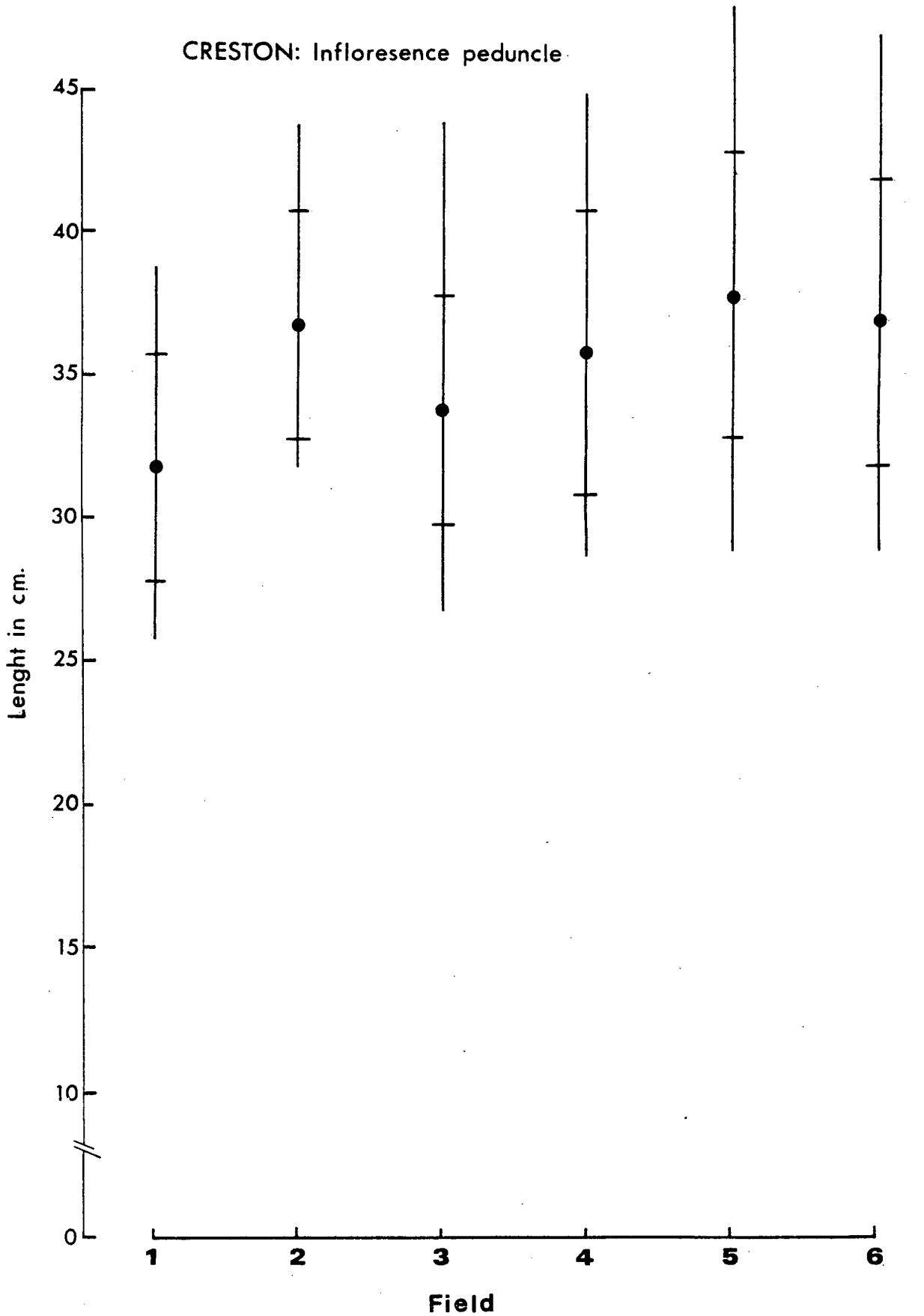


Figure 4.3.5.3

The variation in inflorescence peduncle length for six fields in the Creston valley, July 1977: Four peduncles randomly drawn from four replicates from each field, showing:

a) The arithmetic mean for each field •
b) The simple range by vertical bar
c) one standard deviation from the mean by horizontal bars


CRESTON: Infloresence peduncle
45
40
35
30
25
20
15
10
0
Lenght in cm.
1
2
3
4
5
6
Field

The comparison of petiole and peduncle lengths may afford some measure of assessing a kind of hindrance to the activity of pollinators. By comparing Figures 4.3.5.2 and 4.3.5.3, peduncle length is on an average 15.8 cms longer than petiole length. Examination of Table 4.3.5.4 shows little relationship between leaf area and petiole length, or between petiole length and peduncle length.

4.3.6 Swath Sampling for Standing Crop and Seed Weight

4.3.6.1 Materials and Methods

Line transects were laid along randomly chosen swaths in fields 2, 3 and 6, immediately after the crop had been swathed by the farmers. Ten samples along a transect were gathered from each field; each sample was a one meter length of swath and as wide as the width of swath (a sample thus consisted of more than three square meters of standing crop). The material gathered was air-dried, weighed and then threshed in the stationary thresher at U.B.C. Seed was cleaned with the aid of hand sieves and winnowing. Seed was then weighed.

4.3.6.2 Observations and Results

Standing crop yields are given in Table 4.3.6.1 and Figure 4.3.6.1. There appears to be a wide range in yields between fields. For field 6 there appears a wide range of yields within the field itself. Seed weight measurements are given in Table 4.3.6.2 and Figure 4.3.6.2. Seed weights follow closely standing crop yields in fields 2 and 3; however in field 6 seed yields are lower than in the other two fields despite the relatively large yield of standing crop.

4.3.6.3 Discussion

By measuring the yields in the swath I obtained an approximation of yields the farmer is getting from his combine. However, threshing and seed

Table 4.3.6.1

Estimates of standing crop yields obtained by sampling swaths in three Creston fields, August 1977.

	Air dry yield kgs/ha	Mean	SD	CV%	Range
Field 2 Eastman	2760	69	7	11	55 - 80
Field 3 Huscroft East	3480	87	12	14	64 - 107
Field 6 Wyndell North	5120	128	32	25	83 - 189

Number of samples from swaths in each of three fields - 10.

Table 4.3.6.2

Estimates of field seed yield obtained by sampling swaths in three Creston fields, August 1977

	Air dry yield kgs/ha	Mean	SD	CV%	Range
Field 2 Eastman	324	8.1	1.2	15	6.5 - 10.4
Field 3 Huscroft East	440	11.0	2.7	24	7.2 - 16.0
Field 6 Wyndell North	364	9.1	1.7	19	7.0 - 13.1

Number of samples from swaths in each of three fields - 10.

Sample weights in gms. air dry weight per 1/4 m^2.

Figure 4.3.6.1

The variation in standing crop in three Creston fields, August 1977: Ten samples from a swath in each field showing:

a) The arithmetic mean for each field •
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars

Creston: weight of top growth from swaths.

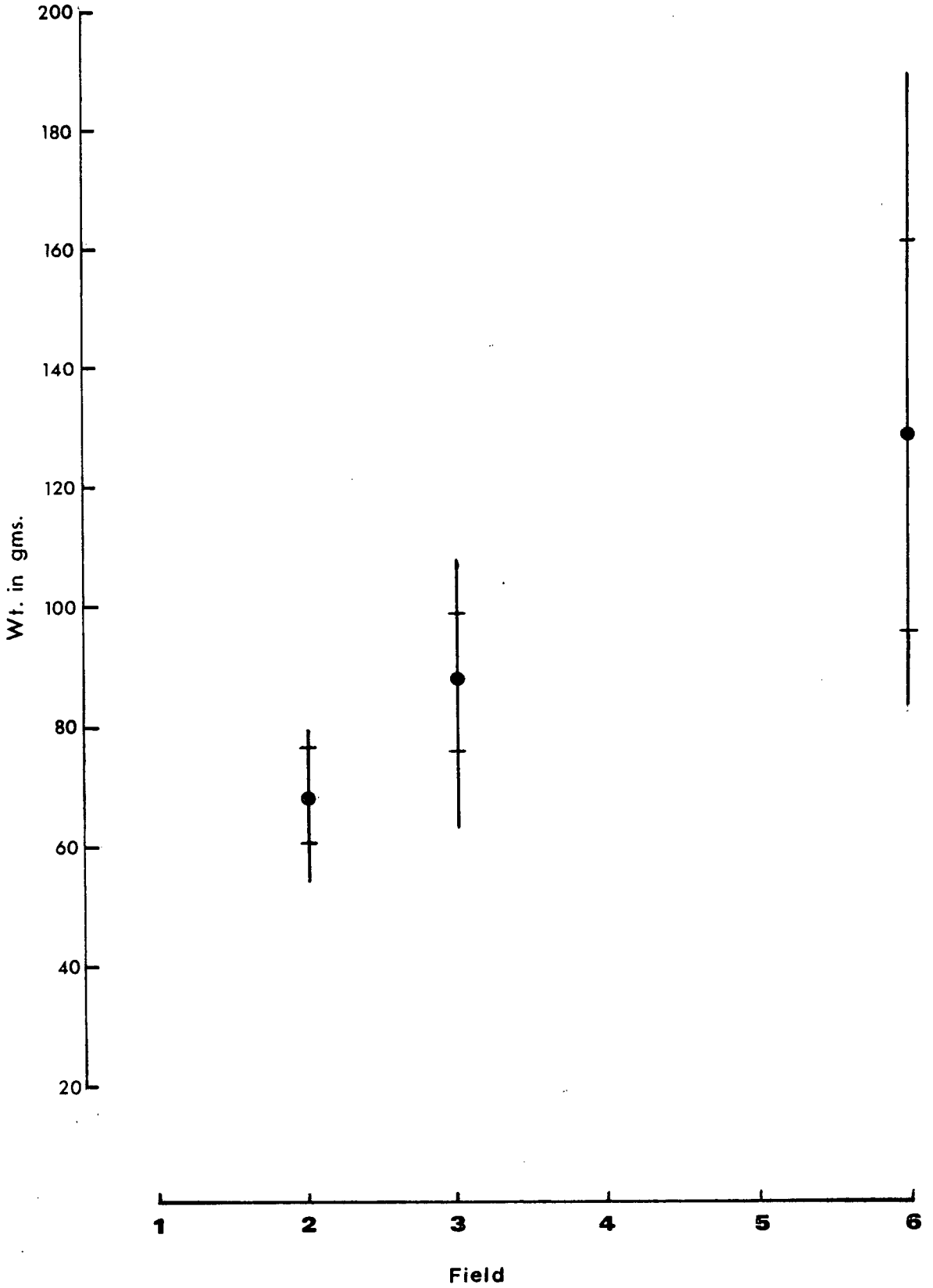
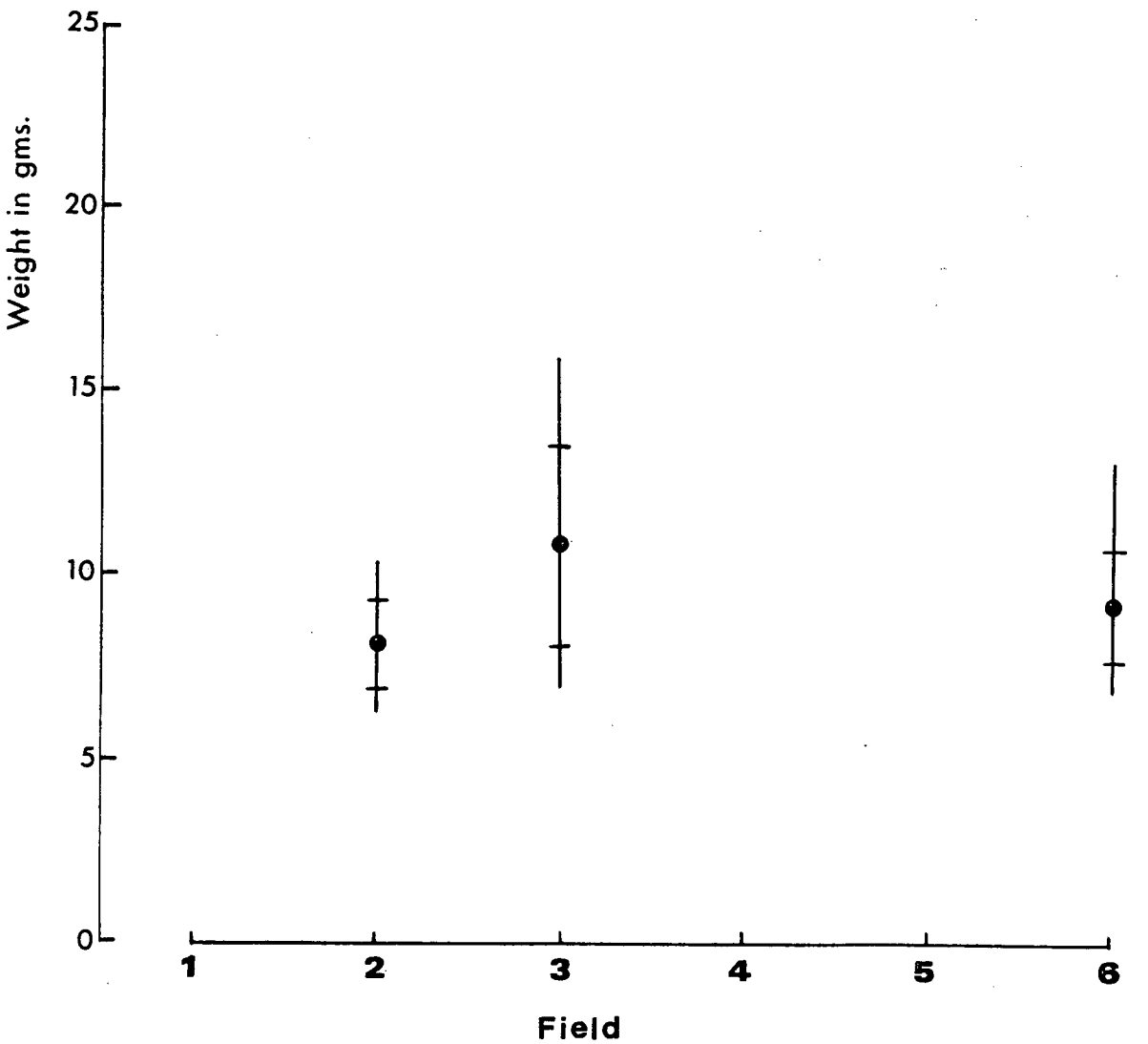


Figure 4.3.6.2

The variation in the weight of seed from three fields in Creston, August 1977: Ten samples from a swath in each field showing:

a) The arithmetic mean for each field •
b) The simple range by vertical bars
c) ± one standard deviation from the mean by horizontal bars

CRESTON: Seed Weight From Swath



cleaning methods differ. It was noticed even at time of my sampling, many heads were lost and considerable shattering of seed pods took place; therefore the yields presented in Table 4.3.6.2 are lower than true yields in the standing crop as measured by quadrat but show similar trends.

4.4 Sampling for Nitrogen Fixing Activity

4.4.1 Materials and Methods

Two sampling dates were chosen using the acetylene reduction technique for determining nitrogen fixation rates in the Creston white clover seed fields. The technique was based on that used by Halliday and Pate (1976). For each field four plant-soil cores were taken with the 10 cm. dia. plugger to make two samples per field. The roots were hand-washed with care and the excess water was removed from the roots with paper towels. Each sample (2 cores) was placed in an assay chamber. The assay chambers were constructed from two liter acid bottles. The bottoms of the acid bottles had been removed and the edge ground on plate glass to make an airtight seal when stop cock grease was applied. The top of the chamber was fitted with a serum bottle stopper through which syringe needles could pass. Fifty millilitres of acetylene were introduced to give a concentration of 0.03 atm. Incubation lasted for four hours and five millilitre samples of "gas" were withdrawn and placed in seven millilitre vacutainer tubes.

Total plant nitrogen was obtained by use of the method of Chapman and Pate (1961). Samples from the standing crop as described in sections 4.3.1 and 4.3.2 were used for determining total plant N. A check on the accuracy and precision of the procedure was performed on standard samples of known composition obtained from Canada Department of Agriculture, Ottawa.

4.4.2 Observations and Results

Samples from the acetylene reduction trials have yet to be analyzed for

ethylene. The results of the total plant nitrogen fixed are given in Table 4.4.1. It appears that most activity in fixation of atmospheric nitrogen for white clover under Creston conditions, in the crop's second year, is in the early part of the summer. It should be observed that in some cases there is a net loss in N in the latter part of the growing season.

4.4.3 Discussion

The use of glass bottles as assay chambers under field conditions at Creston proved to be clumsy and slow. Chambers that were more flexible which could be taken onto the field would have been more practical. Plastic bags as outlined by Lee et al. (1977) would have been far more practical.

4.5 Mowing Trials

4.5.1 Materials and Methods

Field 6 was chosen for this trial and two locations in the field were selected. At both locations on the 16th June, 3 strips 3 feet wide and 10 meters long were mown as close to the ground as possible. Spaced between mown strips were unmown strips one meter wide and half a meter wide respectively. Harvesting took place on the 5th August and 3 samples of each treatment, both cut and uncut, were taken. Samples were air-dried, weighed and threshed.

4.5.2 Observations and Results

Total weight of the cut areas was on an average 18% lower than the uncut areas. Blossom numbers appeared fewer on the cut area. Although all the samples were threshed, the seed was found to be impossible to separate from the concomitant material.

4.5.3 Discussion

Mowing in the middle of June was about a month too late. The crop was in the process of blooming and the total result was a lowering of the top growth yields and numbers of blossoms. If mowing is to be undertaken to

Table 4.4.1

Yield of N* in kgs/ha for standing crops in 6 Creston fields.

Field	Location		Date: 30-5-77	Date: 5-8-77**
1	WS	1	109.4	190.6
		2	110.9	106.1
		3	100.2	106.9
		4	125.3	137.6
2	E	1	43.5	74.1
		2	60.2	71.6
		3	60.2	117.4
		4	49.2	66.5
3	HE	1	77.0	135.0
		2	71.2	143.4
		3	49.7	139.1
		4	70.6	106.1
4	HW	1	68.3	116.0
		2	33.9	116.7
		3	76.5	183.7
		4	67.8	81.4
5	O	1	139.1	211.2
		2	121.3	167.8
		3	90.5	78.1
		4	109.3	141.3
6	WN	1	122.3	285.1
		2	103.8	91.0
		3	144.9	156.6
		4	113.8	125.1

* Kjeldahl determination

** Total nitrogen includes all top growth including seed. The seed nitrogen taken from standard tables by Miller (1958).

reduce vegetative growth in favour of seed production it should be done no later than the end of May in the Creston Valley.

4.6 Coated Seed Trial

The coating of clover and other seeds to improve establishment has been in existence for several years. The method used for the Creston trials was developed in Australia and New Zealand as a means of inoculating white clover seed. The expectation is that greater numbers of the rhizobia will be retained on the coated seed and that their survival is insured up to the time of planting. The process is no longer confined to legumes and is becoming a popular means of applying plant nutrients to the seeds of many crop species.

4.6.1 Materials and Methods

The trial was undertaken on the Mulligan farm. The seed used was of Creston origin and the coating process was done by Cel Pril Industries Inc., Manteca, California. On the 16th June the trial was laid out and seeded on a three by three Latin square design; each ultimate plot was three meters by three meters. Treatments were a) coated seed, b) standard inoculation, c) not inoculated (see trial at U.B.C. section 4.11).

4.6.2 Observations and Results

Rainfall after seeding was insufficient to produce a good establishment; consequently the trial was abandoned.

4.7 Fertilizer Trial

4.7.1 Materials and Methods

The trial was undertaken on the Mulligan farm. Seed for this trial was of Creston origin. On the 16th of June the trial was laid out in a split plot design with three replications. Each plot was two meters by two meters. The trial was also seeded on the 16th June. The following were the treatments

and in each replication, each treatment had an untreated adjacent plot:

a) Nitrogen at 100 kgs per hectare

b) Sulfur at 10 kgs per hectare

c) Boron at 2 kgs per hectare

d) Molybdenum at 1 kg per hectare

e) Calcium at 70 kgs per hectare

f) Nitrogen at 65 kgs per hectare

Phosphorous at 80 kgs per hectare

(P_2O_5)

Potassium at 50 kgs per hectare

(K_2O)

4.7.2 Observations and Results

Rainfall after seeding was insufficient to produce a stand; consequently the trial was abandoned.

4.8 Sampling for Cyanogenesis in Creston White Clover

4.8.1 Materials and Methods

On the 1st July all fields were sampled for cyanogenesis. At 09:00 hours 5 samples each of 150 mg. of leaf tissue, from each location in each field, were placed in 10 mm x 120 mm test tubes. Samples were bruised, 10 drops of chloroform added, a strip of picrate paper was placed in the tube and held in place by a rubber stopper. Samples were incubated for 24 hours at 22°C.

4.8.2 Observations and Results

No reaction was observed after 24 hours in any sample from Creston.

4.8.3 Discussion

This would then agree with Daday's (1954 b and c) observations on the decrease in numbers of white clover plants giving a cyanogenic reaction

where there is a decrease in the average January isotherm.

B. Studies Undertaken at the University of B.C.

4.9 Strain Evaluation Using Groups of Single Plants

4.9.1 General Comments on Experimental Design and Layout

Seeds were obtained from Buckerfields Ltd., Vancouver, B.C, the Central Experimental Farm, Agriculture Canada, Ottawa, and the State University of Oregon, Corvallis, Oregon. Twenty separate strains or lots were gathered; Table 4.9.1.1 gives the origins and 1000 seed weights. Seed lots from Creston are all designated as Canada No. 1 seed. Seed lots used in my trials came from lots harvested on individual farms; seed for grower plantings may well have come from other growers in the Creston area but it is unlikely that much seed in recent years has come from outside of the Creston area.

Seeds were planted as individuals in the greenhouse on March 4, 1977. On the 26th April they were moved to the cold frames for hardening off. In May the field site was prepared at U.B.C. and a basic application of commercial fertilizer was applied. The rates of 350 kgs/ha 0-20-0 (70 kgs/ha P_2O_5) and 142 kgs/ha 0-0-60 (85 kgs/ha K_2O) were used.

Plants were established in the field on the 5th and 6th May at a spacing of 65 cms between plants and 1 meter between rows of 5 plants. The twenty strains were at random in each of four blocks; each strain therefore was represented by five plants in ultimate plots and in four blocks to give a total representation of 20 individual plants per strain. Measurements and observations were made on individual plants.

4.9.2 Variation in Flowering by Date

4.9.2.1 Materials and Methods

The assessment of flowering was done by counting the number of inflor-

Table 4.9.1.1

Weight of seed from twenty strains of white clover planted at U.B.C.: weight in grams for 1000 seeds for each strain.

Strain	Origin	
A (Piper)	Creston	0.7774 grams
B (Ordek)	Creston	0.6020 grams
C (O.C. Ranch)	Creston	0.7639 grams
D (Huscroft)	Creston	0.7553 grams
E (Moon)	Creston	0.7417 grams
G (Stager)	Creston	0.7662 grams
H (Staples)	Creston	0.7586 grams
K (Ogilvie)	Creston	0.8005 grams
L (Sutcliffe)	Creston	0.7314 grams
M (Barbian)	Netherlands	0.6232 grams
O (Pertina)	Netherlands	0.7379 grams
P (Tohoku)	Japan	0.4799 grams
R (Kivi)	Germany	0.6782 grams
S (Daenok)	Denmark	0.7959 grams
U (Pajberg)	Denmark	0.6658 grams
T (L51-6-40)	Louisiana	0.5708 grams
V (L75-614-S1)	Louisiana	0.6217 grams
W (L51-6LAWC-50)	Louisiana	0.6142 grams
X (L081-007-1344)	Louisiana	0.5999 grams
Y (L081-007-2675)	Louisiana	0.6002 grams

escences that had at least one floret in full bloom on each plant. Observation of flowering took place on three dates: June 8th, June 22nd and July 19th.

4.9.2.2 Observations and Results

The F-test in the analysis of variation suggest differences between strains and between dates. The observations are listed in Tables 4.9.2.1 4.9.2.2 and 4.9.2.3 and Figures 4.9.2.1, 4.9.2.2 and 4.9.2.3. Table 4.9.2.1 gives the mean number of heads in flower per plant by strain and the percentage of plants without blossoms by date. The strains are ranked; a strain of course will not necessarily have the same rank on each date. Table 4.9.2.2 displays the occurrence of the same strains within a rank through the two following dates: June 22nd and July 19th. A prominent feature is that the early blossoming plants and strains tend to bloom more profusely throughout the season; plants and strains producing few or no blossoms early in the season tend strongly to continue to remain vegetative and poor bloomers.

Tables 4.9.2.1, 4.9.2.2 and 4.9.2.3 illustrate the flowering response at U.B.C. for the twenty strains. All of the strains of Creston origin rank within the free flowering twelve strains through the flowering season.

4.9.2.3 Discussion

Flowering of the white clover plant is a response to a combination of temperature and day length. Crowder (1960) observed that strains of white clover from the more northern or southern latitudes would not flower freely under his conditions in Colombia. On the other hand my trials revealed that the two strains of warmer latitudes did not flower as freely as those from the cooler latitudes. Free flowering strains are important for seed production and the Creston strains, compared to the remaining strains, are considered to be good seed producers.

Table 4.9.2.1

Twenty strains grown at U.B.C. ranked according to average blossom number per plant and percentage of plants without blossoms for three dates

Date 8-6	Rank: 1-5					Rank: 6 - 10					Rank: 11-15					Rank: 16-20				
Strain	A	U	C	O	G	M	B	L	S	D	R	K	H	P	E	W	V	Y	X	T
Mean Blossom #/plant	.95	.85	.75	.50	.45	.45	.35	.35	.35	.33	.20	.20	.15	.10	.05	.05	.05	.00	.00	.00
% No Blossoms	60	75	65	70	85	70	80	85	80	73	70	85	95	90	95	95	95	100	100	100
Date 22-6																				
Strain	B	D	U	E	H	P	L	S	G	A	R	K	C	W	M	O	Y	V	X	T
Mean Blossom #/plant	3.25	2.95	2.40	2.35	2.20	2.0	1.65	1.65	1.5	1.4	1.00	0.70	0.70	0.60	0.50	0.50	0.35	0.05	0.00	0.00
% No. Blossoms	20	27	50	40	65	25	65	45	60	70	65	70	60	80	85	80	80	95	100	100
Date 19-7																				
Strain	B	U	H	L	W	A	D	Y	E	G	K	C	X	P	M	R	S	T	V	O
Mean Blossom #/plant	28.55	24.25	23.70	22.30	21.30	20.95	20.33	20.05	19.75	16.15	15.50	13.95	13.65	12.9	12.85	12.7	12.20	11.70	11.50	6.40
% No Blossoms	0	5	10	5	20	10	10	5	15	30	15	20	25	10	10	35	25	5	30	25

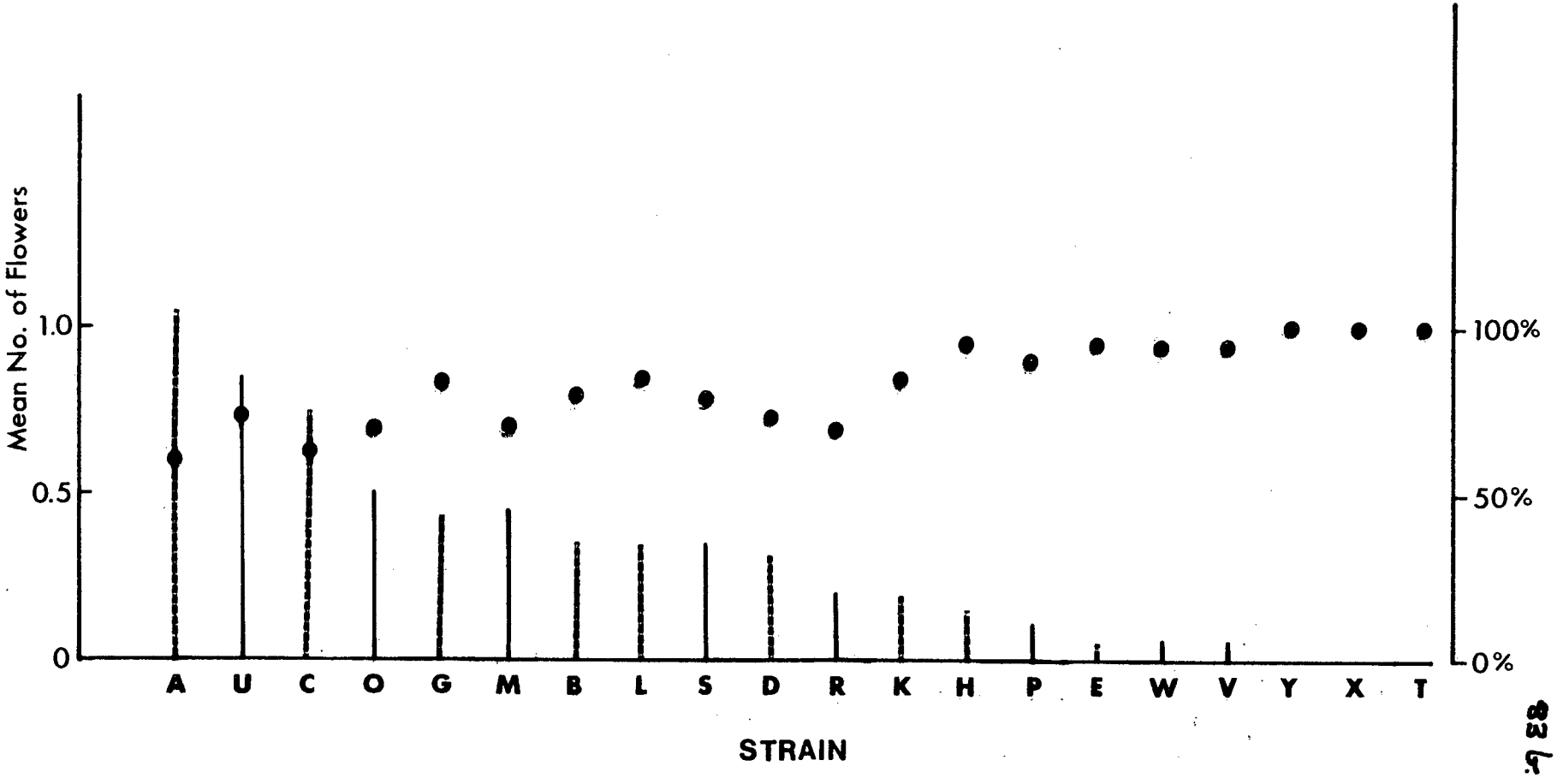
Figure 4.9.2.1

The response in flowering by twenty strains of white clover on the 8-6-77; the twenty strains at random in each of four blocks; each strain represented by five plants in ultimate plots; ultimate plots located at random within each block.

The Creston strains represented by a hatched vertical line.

The other non Creston strains represented by a solid vertical line.

U.B.C.- Mean No. Of Flowers Per Plant On 8-6-77
Strains Of Creston Origin |
Percentage Of Plants With No Inflorescence ●



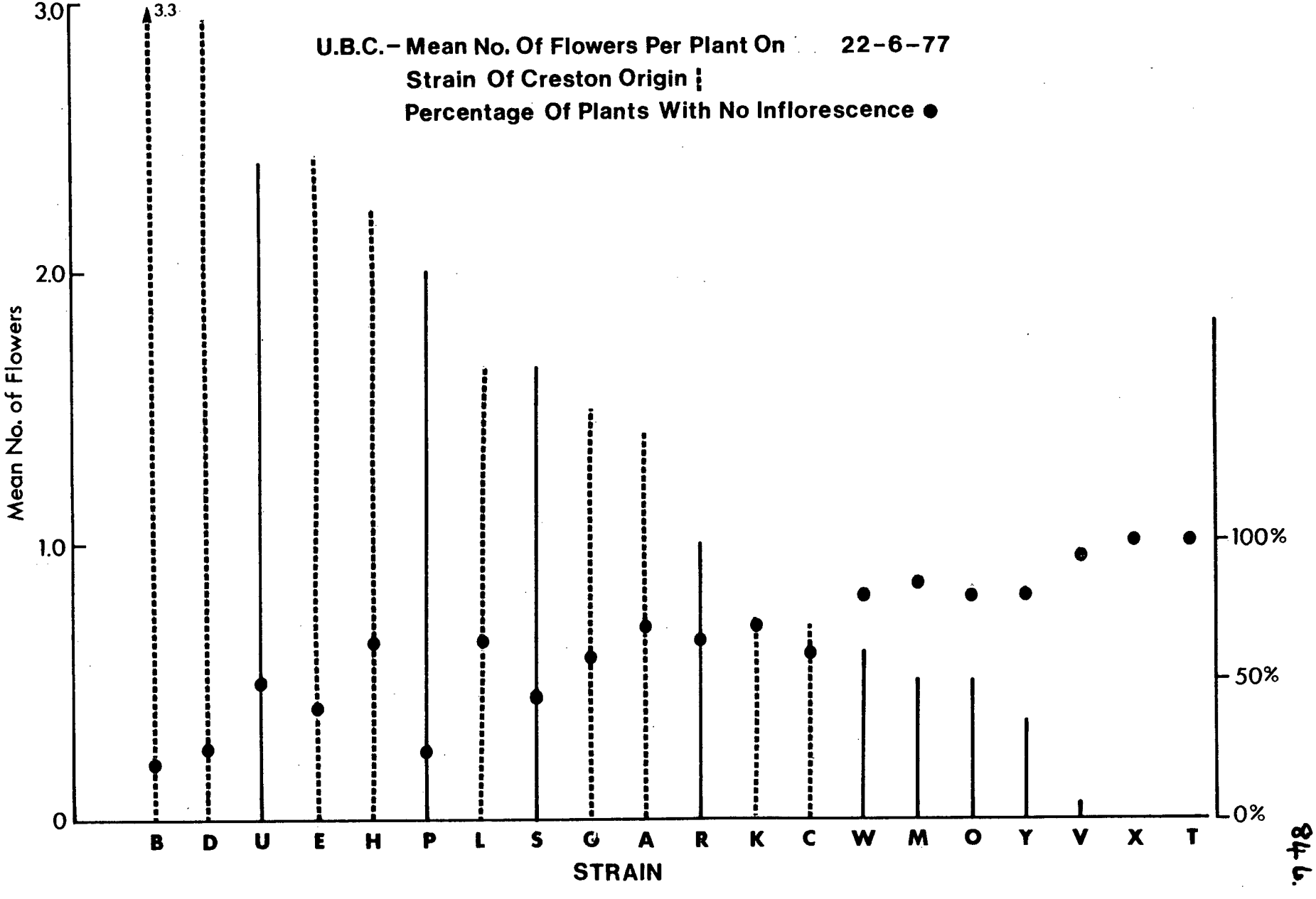
B.C.

Figure 4.9.2.2

The response in flowering by twenty strains of white clover on the 22-6-77; the twenty strains at random in each of four blocks; each strain represented by five plants in ultimate plots; ultimate plots located at random within each block.

The Creston strains represented by a hatched vertical line.

The other non-Creston strains represented by a solid vertical line.


U.B.C.– Mean No. Of Flowers Per Plant On 22-6-77
Strain Of Creston Origin ¦
Percentage Of Plants With No Inflorescence ●
Mean No. of Flowers
3.0
2.0
1.0
0
3.3
100%
50%
0%
B D U E H P L S G A R K C W M O Y V X T
STRAIN

84.6

Figure 4.9.2.3

The response in flowering by twenty strains of white clover on the 19-7-77; the twenty strains at random in each of four blocks; each strain represented by five plants in ultimate plots; ultimate plots located at random within each block.

The Creston strains represented by a hatched vertical line.

The other non-Creston strains represented by a solid vertical line.

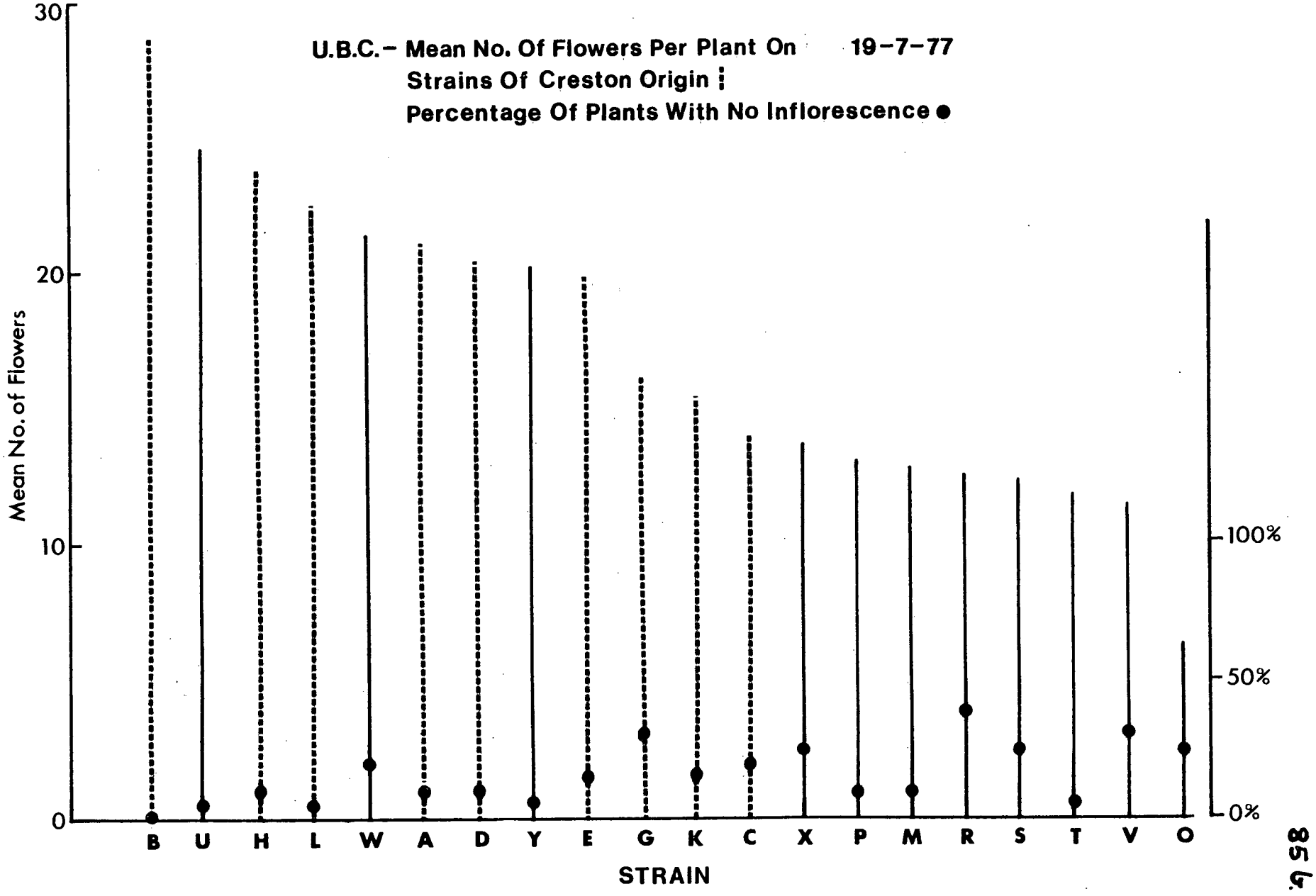

U.B.C.– Mean No. Of Flowers Per Plant On 19-7-77
Strains Of Creston Origin ¦
Percentage Of Plants With No Inflorescence ●
Mean No. of Flowers
30
20
10
0
100%
50%
0%
B U H L W A D Y E G K C X P M R S T V O
STRAIN

Table 4.9.2.2

Relationship of earliness in flowering to the number of inflorescences produced; occurrence of number of strains within ranks for two dates.

		Date 19/7			
	Rank	1-5	6-10	11-15	16-20
	1- 5	3	2	0	0
Date	6-10	1	2	1	1
22/6	11-15	1	0	3	1
	16-20	0	1	1	3

Ranks correspond to order within a scale of twenty.

4.9.3 Variation in Leaf Area

4.9.3.1 Materials and Methods

Collections of leaves and petioles were made on July 20th and October 13th. Collections were made by taking the third open leaf and petiole from the end of a stolon and another leaf and petiole from the centre of the plant. These samples were considered new and old respectively. The trifoliate leaf area estimates were made using premeasured leaves as outlined by Williams et al. (1964). Area estimated was in square centimeters.

4.9.3.2 Observations and Results

The results from the samples taken on two dates are found in Tables 4.9.3.1 and 4.9.3.2. The results are also portrayed in Figures 4.9.3.1 and 4.9.3.2. The F-test in the analysis of variation suggests significant differences between strains and between dates for leaf area.

4.9.3.3 Discussion

Mature leaves produced later in the season are smaller than those produced earlier. These results are similar to those reported by Beinhart (1963). In the Creston strains there is as great a range in variability as in other strains; the leaf area means for Creston strains show that the Creston white clover strains are indeed intermediate. Strain "M" from the Netherlands is more of a wild type. Comparative information is provided in contingency tables. The large Chi-square values suggest that leaf area differences are not random differences under the conditions of this trial but are associated by strain. King (1961) found difference in leaf area to be governed by environmental factors.

Another intresting feature is the increase in leaf area, by the Louisiana strains, for the fall grown material. This could be a factor in carbohydrate storage.

Table 4.9.3.1

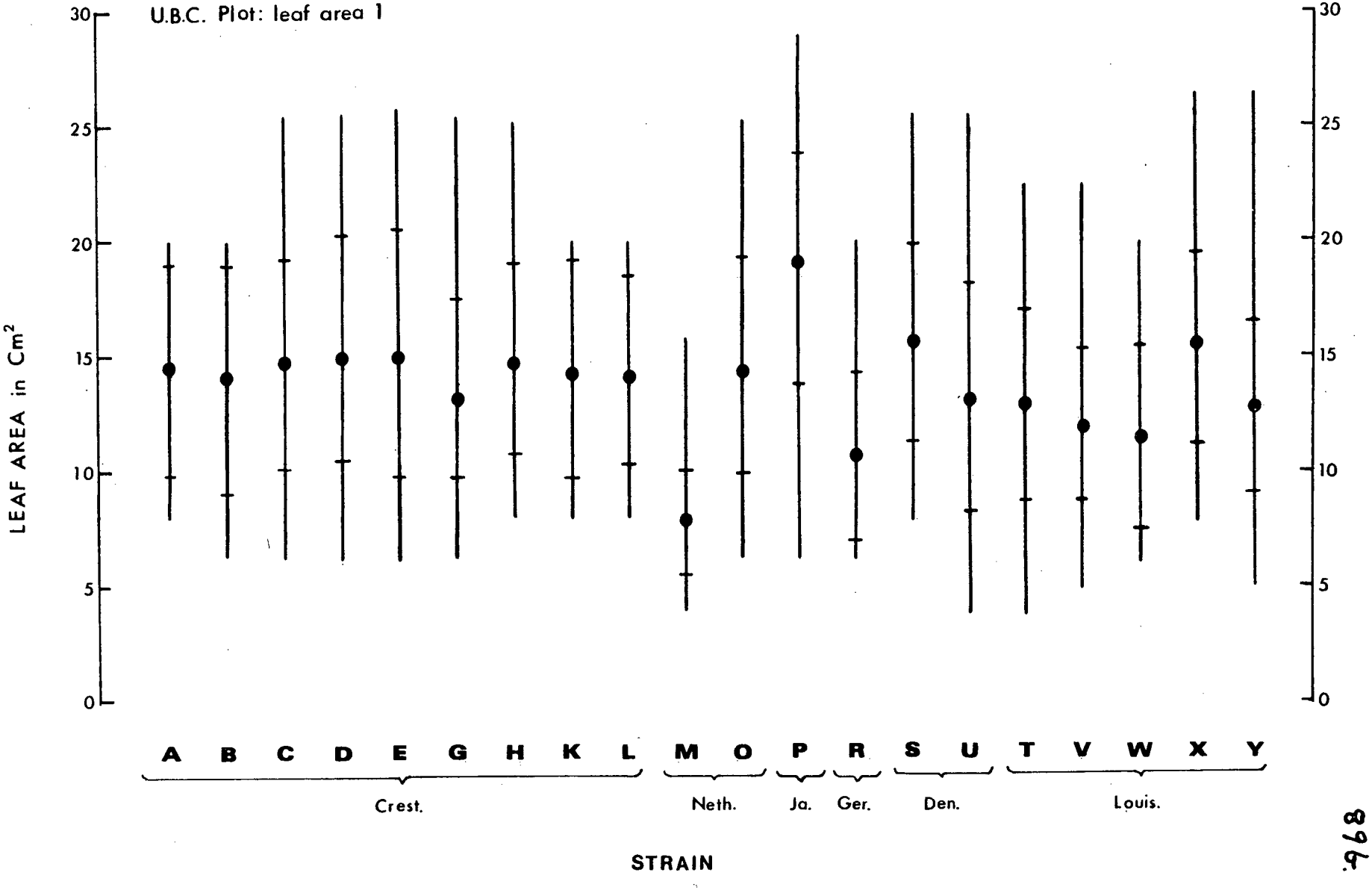
Leaf area in square centimeters for individual plants in twenty strains grown in a uniform nursery at U.B.C.
Sampling date 20-7-77(1)

Strain	Source	No. leaves per sample	Mean	SD	CV%	Range
A	Creston	40	14.5	4.4	30	8 - 20
B	Creston	40	14.0	5.0	36	6.3 - 20
C	Creston	40	14.7	4.6	31	6.3 - 25.1
D	Creston	30	14.9	5.2	35	6.3 - 25.1
E	Creston	38	15.0	5.3	35	6.3 - 25.1
G	Creston	40	13.0	4.7	36	6.3 - 25.1
H	Creston	40	14.9	4.1	28	8 - 25.1
K	Creston	40	14.7	4.5	31	8 - 20
L	Creston	40	14.2	4.0	28	8 - 20
M	Netherlands	40	7.7	2.3	30	4 - 15.8
O	Netherlands	40	14.7	4.8	33	6.3 - 25.1
P	Japan	40	18.0	5.3	29	6.3 - 29
R	Germany	40	10.8	3.8	35	6.3 - 20
S	Denmark	40	15.6	4.3	28	8 - 25.1
U	Denmark	40	13.1	4.9	37	5 - 25.1
T	Louisiana	40	12.9	4.1	32	4 - 20
V	Louisiana	40	12.0	3.2	27	5 - 20
W	Louisiana	40	11.4	4.1	36	6.3 - 20
X	Louisiana	40	15.3	4.1	27	8 - 25.1
Y	Louisiana	40	12.9	3.8	29	6.3 - 25.1

Figure 4.9.3.1

The variation in leaf area on the 20-7-77 (1) for twenty strains at random in each of four blocks; each strain represented by five plants in ultimate plots; ultimate plots located at random within each block, showing:

a) The arithmetic mean for each strain •
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars


U.B.C. Plot: leaf area 1
LEAF AREA in Cm²
30
25
20
15
10
5
0
A B C D E G H K L M O P R S U T V W X Y
Crest.
Neth.
Ja.
Ger.
Den.
Louis.
STRAIN

968

Table 4.9.3.2

Leaf area in square centimeters for individual plants in twenty strains of white clover grown in a uniform nursery at U.B.C.

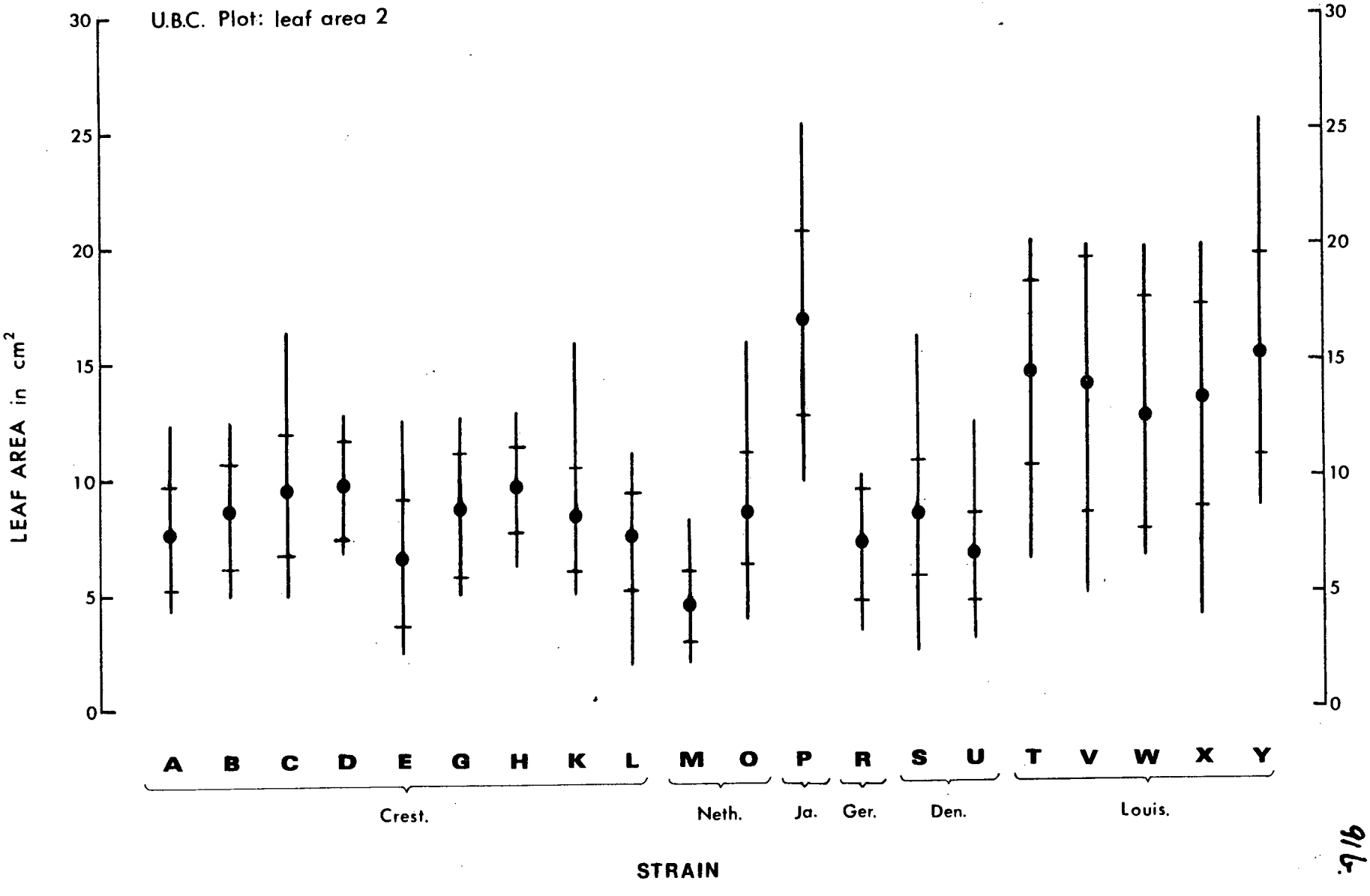
Sampling date 13-10-77(2)

Strain	Source	No. leaves per sample	Mean	SD	CV%	Range
A	Creston	20	7.5	2.3	31	4 - 12.6
B	Creston	20	8.4	2.3	27	5 - 12.6
C	Creston	20	9.3	2.8	30	5 - 15.8
D	Creston	10	9.6	2.0	21	6.3 - 12.6
E	Creston	18	6.4	2.7	42	2.5 - 12.6
G	Creston	20	8.2	2.5	30	5 - 12.6
H	Creston	20	9.5	1.9	20	6.3 - 12.6
K	Creston	18	8.1	2.7	33	6.3 - 15.8
L	Creston	20	7.2	2.2	31	2 - 10
M	Netherlands	20	4.5	1.5	33	2 - 8
O	Netherlands	20	8.2	3.0	37	4 - 15.8
P	Japan	20	16.7	4.0	24	10 - 25.1
R	Germany	18	7.1	2.5	35	3.2 - 10
S	Denmark	18	8.2	2.6	32	6.3 - 15.8
U	Denmark	20	6.5	2.0	31	3.2 - 12.6
T	Louisiana	20	14.6	4.7	32	6.3 - 20
V	Louisiana	20	14.0	5.6	40	5 - 20
W	Louisiana	20	12.8	5.0	39	6.3 - 20
X	Louisiana	20	13.1	4.4	34	4 - 20
Y	Louisiana	20	15.2	4.4	29	10 - 25.1

Figure 4.9.3.2

The variation in leaf area on the 13-10-77 (2) for twenty strains at random in each of four blocks; each strain represented by five plants in ultimate plots; ultimate plots located at random within each block, showing:

a) The arithmetic mean for each strain •
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars


U.B.C. Plot: leaf area 2
LEAF AREA in cm²
30
25
20
15
10
5
0
A B C D E G H K L M O P R S U T V W X Y
Crest.
Neth.
Ja.
Ger.
Den.
Louis.
STRAIN

916.

Table 4.9.3.3

Contingency table and Chi-square values for average leaf area for twenty strains grown at U.B.C.; sampled on the 20-7-77.

Leaf Area (cm^2)	Strain																				Total
	Creston									Netherlands		Ja	Ge	Denmark		Louisiana					
	A	B	C	D	E	G	H	K	L	M	O	P	R	S	U	T	V	W	X	Y	
0 - 4	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	4
5 - 9	5	9	5	3	4	11	2	6	5	29	4	1	16	1	7	5	6	14	2	4	139
10 - 14	15	11	14	14	14	15	18	12	15	8	14	10	18	15	17	20	26	17	15	24	312
15 - 19	19	18	20	10	17	12	19	20	20	1	20	21	6	22	15	13	8	9	21	11	302
20 -	1	2	1	3	3	2	1	2	0	0	2	8	0	2	1	0	0	0	2	1	31
Total	40	40	40	30	38	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	788

Actual $\chi^2 = 257.18^{**}$ Expected $\chi^2_{.05(76)} = 97.34$

Table 4.9.3.4

Contingency table and Chi-square values for average leaf area for twenty strains grown at U.B.C.; sampled on the 13-10-77.

Leaf Area (cm^2)	Strain																				Total
	Creston									Netherlands		Ja	Ge	Denmark		Louisiana					
	A	B	C	D	E	G	H	K	L	M	O	P	R	S	U	T	V	W	X	Y	
0 - 4	2	1	1	0	4	0	0	0	2	10	1	0	4	0	1	0	0	0	1	0	27
5 - 9	15	11	12	4	10	13	9	11	15	10	13	0	14	9	18	2	4	4	1	0	175
10 - 14	3	8	6	6	4	7	11	7	3	0	5	7	0	8	1	9	9	9	12	10	125
15 - 19	0	0	1	0	0	0	0	0	0	0	1	12	0	1	0	9	5	7	6	8	50
20 -	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	2	5
Total	20	20	20	10	18	20	20	18	20	20	20	20	18	18	20	20	20	20	20	20	382

Actual $\chi^2 = 317.05^{**}$ Expected $\chi^2_{0.05(76)} = 97.34$

The method by Williams et al. (1964) for estimations of leaf area proved to be a practical and quick method of assessing leaf area in the field. Although the method is designed to allow for leaf shape, the greatest difficulty appeared when abnormally long leaves were encountered.

4.9.4 Variation in Petiole Length

4.9.4.1 Materials and Methods

The same samples taken for leaf area served for the measurement of petiole length. The petiole lengths were measured in centimeters from the base of the petiole at the junction of the stipules and petiole to the junction of the leaf pedicels.

4.9.4.2 Observations and Results

The F-test in the analysis of variation suggest differences between strains and between dates for petiole length. The results of petiole length measurements are shown in Table 4.9.4.1 and 4.9.4.2. It is again noteworthy that length decreases in the fall. The Louisiana strains again have the longer petioles.

Tables 4.9.4.3 and 4.9.4.4 relate leaf area and petiole length for both fully grown leaves and petioles to leaf area and petiole length for younger leaves and petioles for two dates of observation. The correlation coefficients illustrate that the differences in growth of leaves and petioles are relatively independent within the intermediate form of white clover.

4.9.4.3 Discussion

The work done by Denne (1966) and by Brougham (1966) traces the expansion and history of area and length of leaves and petioles in the white clover canopy. Continual growth makes for unreliable measurements (or confounding) when samples are not correlated with age; the information in Tables 4.9.4.3 and 4.9.4.4 supports these observations.

Table 4.9.4.1

Avg. petiole length in centimeters for individual plants in twenty strains grown in a uniform nursery at U.B.C., sampling date 20-7-77

Strain	Source	No. petioles per sample	Mean	SD	CV%	Range
A	Creston	40	21.75	5.26	24	13-30
B	Creston	40	23.18	4.13	18	14-31
C	Creston	40	20.58	5.33	26	12-34
D	Creston	30	20.97	6.23	30	11-33
E	Creston	38	20.76	5.12	25	8-31
G	Creston	40	20.75	5.07	24	10-30
H	Creston	40	22.03	5.46	25	8-31
K	Creston	40	22.25	5.25	24	12-35
L	Creston	40	23.18	5.53	24	15-34
M	Netherlands	40	17.75	4.68	26	9-26
O	Netherlands	40	19.88	4.36	22	13-28
P	Japan	40	23.48	6.50	28	14-40
R	Germany	40	20.33	5.44	27	11-33
S	Denmark	40	20.15	4.69	23	10-30
U	Denmark	40	19.83	5.99	30	9-34
T	Louisiana	40	23.28	4.91	21	15-34
U	Louisiana	40	21.30	5.89	28	9-35
W	Louisiana	40	21.63	5.84	27	13-34
X	Louisiana	40	22.75	5.37	24	11-34
Y	Louisiana	40	23.50	5.46	23	13-36

Table 4.9.4.2.

Avg. petiole length in centimeters for individual plants in twenty strains grown in a uniform nursery at U.B.C., sampling date 13-10-77

Strain	Source	No. petioles per sample	Mean	SD	CV%	Range
A	Creston	20	14.45	3.41	24	7-21
B	Creston	20	13.85	4.18	30	6-21
C	Creston	20	15.78	5.24	33	7-25
D	Creston	10	14.50	2.80	19	11-20
E	Creston	18	11.40	4.60	40	4-21
G	Creston	20	13.95	4.11	29	6-22
H	Creston	20	16.85	3.76	22	10-23
K	Creston	18	15.78	4.35	28	9-24
L	Creston	20	13.55	3.38	25	7-20
M	Netherlands	20	10.40	3.39	33	5-17
O	Netherlands	20	13.55	4.89	36	5-23
P	Japan	20	20.05	5.75	29	12-29
R	Germany	18	12.39	4.80	39	6-24
S	Denmark	18	13.39	2.57	19	9-19
U	Denmark	20	11.00	3.34	30	7-19
T	Louisiana	20	17.90	5.19	29	11-25
U	Louisiana	20	17.90	5.59	31	10-28
W	Louisiana	20	17.85	5.71	32	8-28
X	Louisiana	20	18.30	6.61	36	7-29
Y	Louisiana	20	21.30	5.03	24	13-30

Table 4.9.4.3

Relationship of leaf area to petiole length; measurements taken on 20-7-77 for twenty strains grown in a uniform nursery at U.B.C.

Strain	Origin	Fully grown leaves and petioles: Linear Regression Equation	Fully grown leaves and petioles: Correlation Coefficient	Younger leaves and petioles: Linear Regression Equation	Younger leaves and petioles: Correlation Coefficient
A	Creston	Y = 19.86 + 0.29X	r = 0.34	Y = 10.89 + 0.51X	r = 0.68*
B	Creston	Y = 24.21 + 0.08X	r = 0.09	Y = 22.32 - 0.09X	r = -0.07
C	Creston	Y = 14.62 + 0.59X	r = 0.30	Y = 8.14 + 0.61X	r = 0.79**
D	Creston	Y = ----------	r = ----	Y = ----------	r = ----
E	Creston	Y = 16.12 + 0.49X	r = 0.57	Y = 8.39 + 0.54X	r = 0.58
G	Creston	Y = 21.95 + 0.12X	r = 0.13	Y = 18.07 + 0.01X	r = 0.01
H	Creston	Y = 23.32 + 0.10X	r = 0.14	Y = 8.46 + 0.64X	r = 0.60
K	Creston	Y = 20.04 + 0.39X	r = 0.29	Y = 9.55 + 0.62X	r = 0.69*
L	Creston	Y = 21.66 + 0.25X	r = 0.25	Y = 18.04 + 0.11	r = 0.18
M	Netherlands	Y = 17.39 + 0.42X	r = 0.21	Y = 10.49 + 0.51	r = 0.34
O	Netherlands	Y = 16.40 + 0.47X	r = 0.77**	Y = 15.92 + 0.01	r = 0.04
P	Japan	Y = 26.32 + 0.14X	r = 0.17	Y = 20.64 - 0.01	r = -0.01
R	Germany	Y = 17.43 + 0.54X	r = 0.63	Y = 16.21 + 0.11	r = 0.18
S	Denmark	Y = 18.22 + 0.38X	r = 0.35	Y = 16.09 + 0.12	r = 0.42
U	Denmark	Y = 9.70 + 0.76X	r = 0.75*	Y = 10.78 + 0.40	r = 0.55
T	Louisiana	Y = 15.84 + 0.68X	r = 0.55	Y = 15.19 + 0.35	r = 0.50
V	Louisiana	Y = 22.88 + 0.11X	r = 0.11	Y = 23.59 - 0.28	r = 0.33
W	Louisiana	Y = 24.46 + 0.11X	r = 0.09	Y = 12.89 + 0.45	r = 0.64*
X	Louisiana	Y = 23.64 + 0.12X	r = 0.13	Y = 9.77 + 0.60	r = 0.52
Y	Louisiana	Y = 28.03 - 0.002X	r = -0.002	Y = 26.04 - 0.58	r = 0.39

Leaf area - X; Petiole length - Y; Sample size - 10 observations per treatment

r.05(8) = 0.6319* r.01(8) = 0.7646**

Table 4.9.4.4

Relationship of leaf area to petiole length; measurements taken the 13-10-77 for twenty strains grown in a uniform nursery at U.B.C.

Strain	Origin	Fully grown leaves and petioles		Younger leaves and petioles	
		Regression equation	Correlation Coefficient	Regression equation	Correlation Coefficient
A	Creston	Y = 9.60 + 0.92X	r = 0.72*	Y = 5.18 + 0.96X	r = 0.80**
B	Creston	Y = 5.61 + 1.37X	r = 0.77**	Y = 3.84 + 0.84X	r = 0.71*
C	Creston	Y = 13.61 + 0.59X	r = 0.41	Y = 9.25 + 0.34X	r = 0.27
D	Creston				
E	Creston	Y = 4.75 + 1.39X	r = 0.77**	Y = 3.25 + 0.94X	r = 0.76*
G	Creston	Y = 8.36 + 0.94X	r = 0.74*	Y = 4.57 + 0.89X	r = 0.55
H	Creston	Y = 12.53 + 0.75X	r = 0.49	Y = 13.50 + 0.05X	r = 0.50
K	Creston	Y = 7.30 + 1.25X	r = 0.71	Y = 11.41 + 0.30X	r = 0.24
L	Creston	Y = 7.87 + 1.03X	r = 0.84**	Y = 4.42 + 1.02X	r = 0.69*
M	Netherlands	Y = 6.72 + 1.30X	r = 0.65*	Y = 3.40 + 1.08X	r = 0.81**
O	Netherlands	Y = 9.22 + 0.75X	r = 0.60	Y = 0.15 + 1.40X	r = 0.71*
P	Japan	Y = 25.25 - 0.04X	r = -0.05	Y = 6.76 + 0.47X	r = 0.41
R	Germany	Y = 4.95 + 1.42X	r = 0.81**	Y = 4.28 + 0.75X	r = 0.90**
S	Denmark	Y = 15.47 - 0.04X	r = -0.06	Y = 8.91 + 0.33X	r = 0.52
U	Denmark	Y = 9.00 + 0.61X	r = 0.46	Y = 8.79 - 0.01X	r = -0.02
T	Louisiana	Y = 13.73 + 0.57X	r = 0.77**	Y = 16.97 - 0.16X	r = -0.15
V	Louisiana	Y = 10.82 + 0.79X	r = 0.60	Y = 13.93 + 0.08X	r = 0.12
W	Louisiana	Y = 20.49 + 0.10X	r = 0.08	Y = 14.72 - 0.05X	r = -0.08
X	Louisiana	Y = 12.90 + 0.74X	r = 0.43	Y = 7.69 + 0.51X	r = 0.43
Y	Louisiana	Y = 20.46 + 0.38X	r = 0.21	Y = 16.56 + 0.05X	r = 0.07

Leaf area - X; Petiole length - Y; Sample size - 10 observations per treatment

r.05(8) = .6319* r.01(8) = .7646**

4.9.5 Variation in Leaf Shape and Markings

4.9.5.1 Materials and Methods

Observation of leaf shapes and markings took place in the latter part of July 1977. Leaves were classified according to 4 shape categories (Oval, Round, Heart-shaped, Round incised). The inverted "V" marks were grouped as present or absent as used by Cahn and Harper (1976). The classification of the inverted "V" mark was grouped into three categories: broken and faint, complete and faint, large and intense. The presence and absence of anthocyanin flecks were noted as per Carnahan et al. (1955) and Corkill (1971).

4.9.5.2 Observations and Results

Leaf shape:- Observations on leaf shape for the twenty different strains is given in Table 4.9.5.1. Examination of the distribution suggests that round leaves and round incised leaves are the most abundant.

Inverted "V" mark:- The two methods used and data assessment of the studies on the inverted "V" mark are given in Tables 4.9.5.2 and 4.9.5.3. The F-test in the analysis of variation suggests significance between strain but not among block means. In Table 4.9.5.2 the ratio of inverted "V" marks to no "V" marks is given; the Creston and Netherlands strains had ratios of 3:1 or 4:1 with less intense marks, while the Japanese, German and Louisiana strains had ratios from 19:1. to 20:0. The two Denmark strains at ratios of 12:1 were intermediate. Table 4.9.5.3 is a contingency table displaying leaf markings in four categories. The distributions are obviously not random.

Anthocyanin flecking:- The ratios for the presence and absence of anthocyanin flecking are given in Table 4.9.5.4. There appears to be a difference between strains, but it is not strongly indicated

Table 4.9.5.1

Contingency table and Chi-square values for white clover leaf shapes on twenty strains grown in a uniform nursery at U.B.C.

Leaf Shape	Strain A	B	C	D	E	G	H	K	L	M	O	P	R	S	U	T	V	W	X	Y	Total
1	1	0	0	0	0	0	0	0	0	1	0	6	0	0	0	2	0	1	0	1	12
2	6	4	6	11	8	2	3	7	7	4	6	2	7	9	6	6	4	3	5	10	116
3	2	5	1	0	1	4	7	2	2	4	3	7	3	1	3	0	1	0	2	0	48
4	6	6	8	4	5	9	5	5	6	6	6	0	4	4	6	7	10	11	8	4	120
Total	15	15	15	15	14	15	15	14	15	15	15	15	14	14	15	15	15	15	15	15	296

Actual $\chi^2 = 136.13^{**}$ Expected $\chi^2_{.05(57)} = 75.61$

Leaf Shape

1 = Oval — Ovate or obovate

2 = Round — Orbiculate

3 = Heart shaped — Obcordate

4 = Round incised — Orbiculate notched

Table 4.9.5.2

Ratio of water marks to no water marks for twenty strains of white clover grown at U.B.C.

Strain	Origin	Water mark	No water mark	Pooled
A	Creston	15	5	
B	Creston	18	2	
C	Creston	16	4	
D	Creston	12	3	
E	Creston	16	3	4:1
G	Creston	16	4	
H	Creston	14	6	
K	Creston	17	3	
L	Creston	13	7	
M	Netherlands	18	2	
O	Netherlands	12	8	3:1
P	Japan	20	0	20:0
R	Germany	19	1	19:1
S	Denmark	18	2	
U	Denmark	14	6	12:1
T	Louisiana	19	1	
V	Louisiana	20	0	
W	Louisiana	20	0	19:1
X	Louisiana	20	0	
Y	Louisiana	20	0	

Table 4.9.5.3

Contingency table and Chi-square values for four classes of leaf markings in 20 strains of white clover grown in a uniform nursery at U.B.C.

																					Total
No mark	5	2	4	3	3	4	6	3	7	2	8	0	1	2	6	1	0	0	0	0	57
Broken and faint	5	6	3	5	3	4	4	8	3	5	2	0	2	8	4	0	0	0	0	0	62
Complete and faint	7	4	9	2	8	12	6	5	5	7	4	3	5	7	3	4	2	1	2	1	97
Large and intense	3	8	4	5	5	0	4	4	5	6	6	17	12	3	7	15	18	19	18	19	178
Total	20	20	20	15	19	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	394
	----------Creston----------									Netherlands		Ja	Ge	Denmark		-------Louisiana---------				
	A	B	C	D	E	G	H	K	L	M	O	P	R	S	U	T	V	W	X	Y	

Actual $\chi^2 = 156.64$ Expected $\chi^2_{.05(57)} = 75.61$

Table 4.9.5.4

Ratio of anthocyanin flecking to no flecking for twenty strains of white clover grown at U.B.C.

Strain	Origin	Flecking	No Flecking	Pooled
A	Creston	12	8	
B	Creston	4	16	
C	Creston	6	14	
D	Creston	4	11	
E	Creston	7	12	1:2
G	Creston	6	14	
H	Creston	9	11	
K	Creston	9	11	
L	Creston	6	14	
M	Netherlands	10	10	1:1
O	Netherlands	10	10	
P	Japan	2	18	1:9
R	Germany	8	12	2:3
S	Denmark	4	16	1:3
U	Denmark	7	13	
T	Louisiana	5	15	
V	Louisiana	5	15	
W	Louisiana	4	16	1:3
X	Louisiana	8	12	
Y	Louisiana	3	17	

(Table 4.9.5.5). The presence or absence of anthocyanin leaf flecks is only a random association with the presence or absence of the inverted "V" leaf mark (Table 4.9.5.6).

4.9.5.3 Discussion

Leaf shape was not an easy parameter to study because of the multiplicity of intermediate shapes. However it is nonetheless an important biological one since leaves are the primary organs of photosynthesis. On the other hand the inverted "V" mark and anthocyanin flecking appear to be less significant biologically. They are so difficult to classify that "presence" or "absence" were the only reliable categories. They are nonetheless useful markings for field identification.

4.9.6 Variation in Plant Height

4.9.6.1 Materials and Methods

Plant height was measured in late September by placing a ruler in the centre of the plant and noting the height of the canopy. Individual leaves that were held higher than the canopy were ignored.

4.9.6.2 Observations and Results

Plant height measurements are found in Table 4.9.6.1 and in Figure 4.9.6.1. Creston strains show a wide range of variation, although the Louisiana strains were consistently the tallest. The F-tests in the analysis of variation suggested that the difference between strain means were highly significant. Plant height measurements follow close to those of petiole length.

4.9.6.3 Discussion

Only one measurement of plant height was taken. This was about two weeks prior to the petiole length measurements but again the Creston strains are intermediate while the Louisiana strains are tall for this time of year.

Table 4.9.5.5

Contingency table and Chi-square values for anthocyanin flecking in 20 strains of white clover grown in a uniform nursery at U.B.C.

	Strain																				
	A	B	C	D	E	G	H	K	L	M	O	P	R	S	U	T	V	W	X	Y	Total
Presence	8	4	6	4	7	6	9	9	6	10	10	2	8	4	7	5	5	4	8	3	129
Absence	12	16	14	11	12	14	11	11	14	10	10	18	12	16	13	15	15	16	12	17	265
Total	20	20	20	15	19	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	394

Actual $\chi^2 = 23.41$ Expected $\chi^2_{.05(19)} = 30.14$

Table 4.9.5.6

Contingency table for white clover leaf markings and anthocyanin leaf flecking on twenty strains grown in a uniform nursery at U.B.C.

Anthocyanin flecking	Inverted "V" Leaf Mark Absent	Present	Total
Absent	36 (38.34)	229 (226.66)	265
Present	21 (18.66)	108 (110.34)	129
Total	57	337	394

Expected values in brackets.

Actual $X^2 = 0.50$ Expected X^2 .05(1) = 3.84

Table 4.9.6.1

Plant height in centimeters for individual plants in twenty strains grown in a uniform nursery at U.B.C.

Strain	Source	No. plants per sample	Mean	SD	CV%	Range
A	Creston	20	17.0	3.7	22	12 - 25
B	Creston	20	16.0	3.5	22	9 - 22
C	Creston	20	16.6	5.1	31	6 - 25
D	Creston	15	15.7	2.4	15	12 - 21
E	Creston	19	14.0	4.3	31	5 - 21
G	Creston	20	14.6	3.5	24	6 - 19
H	Creston	20	16.9	3.5	21	11 - 22
K	Creston	20	17.5	2.7	15	12 - 22
L	Creston	20	14.7	5.0	34	8 - 30
M	Netherlands	20	11.5	2.9	25	7 - 17
O	Netherlands	20	13.8	2.9	21	6 - 18
P	Japan	20	21.6	5.5	25	11 - 31
R	Germany	20	13.3	4.2	32	5 - 23
S	Denmark	20	13.2	3.2	24	6 - 19
U	Denmark	20	12.9	3.8	29	4 - 18
T	Louisiana	20	22.9	2.6	11	19 - 28
V	Louisiana	20	23.8	4.1	17	14 - 30
W	Louisiana	20	22.7	4.4	19	9 - 29
X	Louisiana	20	21.4	5.3	25	7 - 29
Y	Louisiana	20	23.3	4.7	20	9 - 33

Figure 4.9.6.1

The variation in plant height for twenty strains at random in each of four blocks; each strain represented by five plants in ultimate plots; ultimate plots located at random within each block, showing:

a) The arithmetic mean for each strain •
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars

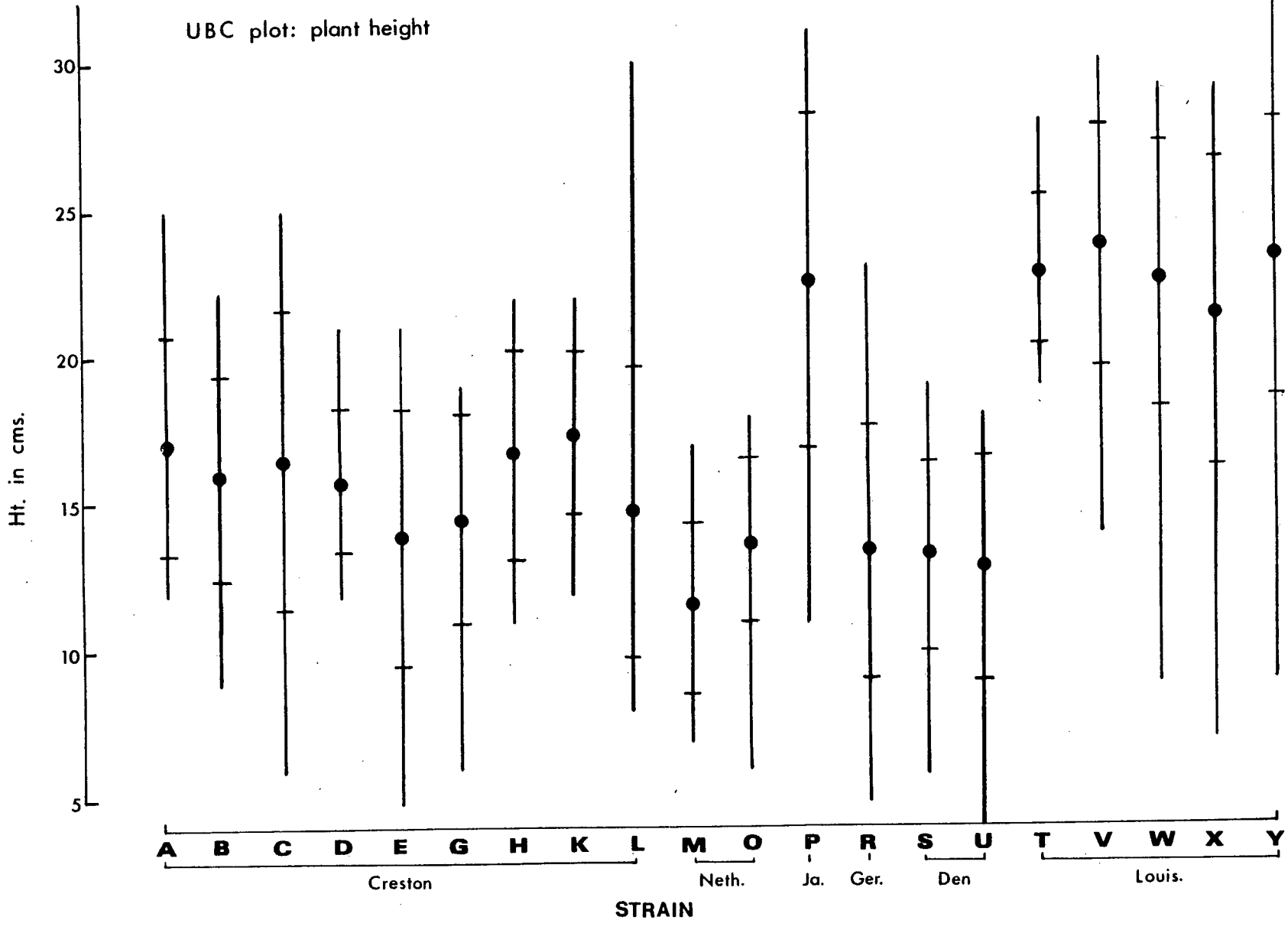

UBC plot: plant height
Ht. in cms.
30
25
20
15
10
5
A B C D E G H K L M O P R S U T V W X Y
Creston
Neth.
Ja.
Ger.
Den
Louis.
STRAIN

Fig. 108

4.9.7 Variation in Cyanogenesis

4.9.7.1 Materials and Methods

In the latter half of September, all plants were sampled for cyanogenesis. The method used was by means of the picrate paper test, using a set of standards. Picrate paper was prepared as outlined by Nowosad and MacVicar (1940) and stored in an airtight container. A standard cyanide solution was prepared by dissolving 241 mg of potassium cyanide in one litre of distilled water. At 09:00 hours, samples of about 150 mg of fresh plant tissue from each plant were placed in 10 mm x 120 mm test tubes, samples were bruised and 10 drops of chloroform added. A strip of picrate paper was placed in the tube and held in place by a rubber stopper. A set of standards were made up by placing a range of measured quantities of the cyanide solution in test tubes to give a range of values from 0 µg to 80 µg. A strip of picrate paper was placed in each of these tubes and held in place with a rubber stopper. All samples were incubated at 30°C for twenty hours. Picrate papers were then compared with the standards and a relative rating of the level of reaction for each plant was established.

4.9.7.2 Observations and Results

There were some variations in HCN levels from block to block because only one block could be done on a given day. The differences between strains were very marked. All Creston and Netherlands strains gave a negative reaction while all Louisiana strains gave a highly positive reaction. The Japanese strain Tohoku was negative while one Danish strain Pajberg Milka and a German strain Kivi gave occasional positive reactions.

4.9.7.3 Discussion

The method of making a set of standards gave a reasonable approximation of the amount of release by each plant of hydrogen cyanide. For the purpose

of this study, positive or negative reactions were sufficient.

4.9.8 Variation in Plant Weight

4.9.8.1 Materials and Methods

On two dates, August 18th and October 19th, growth was cut; the material was thoroughly dried in a pot hole drier. Weights were recorded in gms of air-dry material.

4.9.8.2 Observations and Results

The data obtained from the sampling on the two dates are given in Tables 4.9.8.1 and 4.9.8.2 and Figures 4.9.8.1 and 4.9.8.2. The F-test was insignificant for weight between strains for the first date (August 18th) and this is illustrated in Figure 4.9.8.1. The second date (October 19th) shows the lower weights for all strains but it is noteworthy that the Louisiana strains are heavier producers in late summer and fall than the Creston strains; leaf area responds likewise.

4.9.8.3 Discussion

There appears to be a relationship between leaf area and plant weight, however, it is noteworthy that smaller leaved plants are more persistent.

4.10 Variation Within and Between Clones

4.10.1 Comment on Trial Layout

On the 1st September 1976 plants were collected from four areas in each of five fields in the Creston area. The plants originated from plantings made in 1975 and 1976 and are designated by the name of the farmer owning a field, and by year of planting. These plants were grown in the greenhouse at U.B.C. and in December 1976 cuttings were made from individual plants. Three cuttings from a single runner made up a set of three genetically "identical" plants. Each set was not necessarily related to the

Table 4.9.8.1

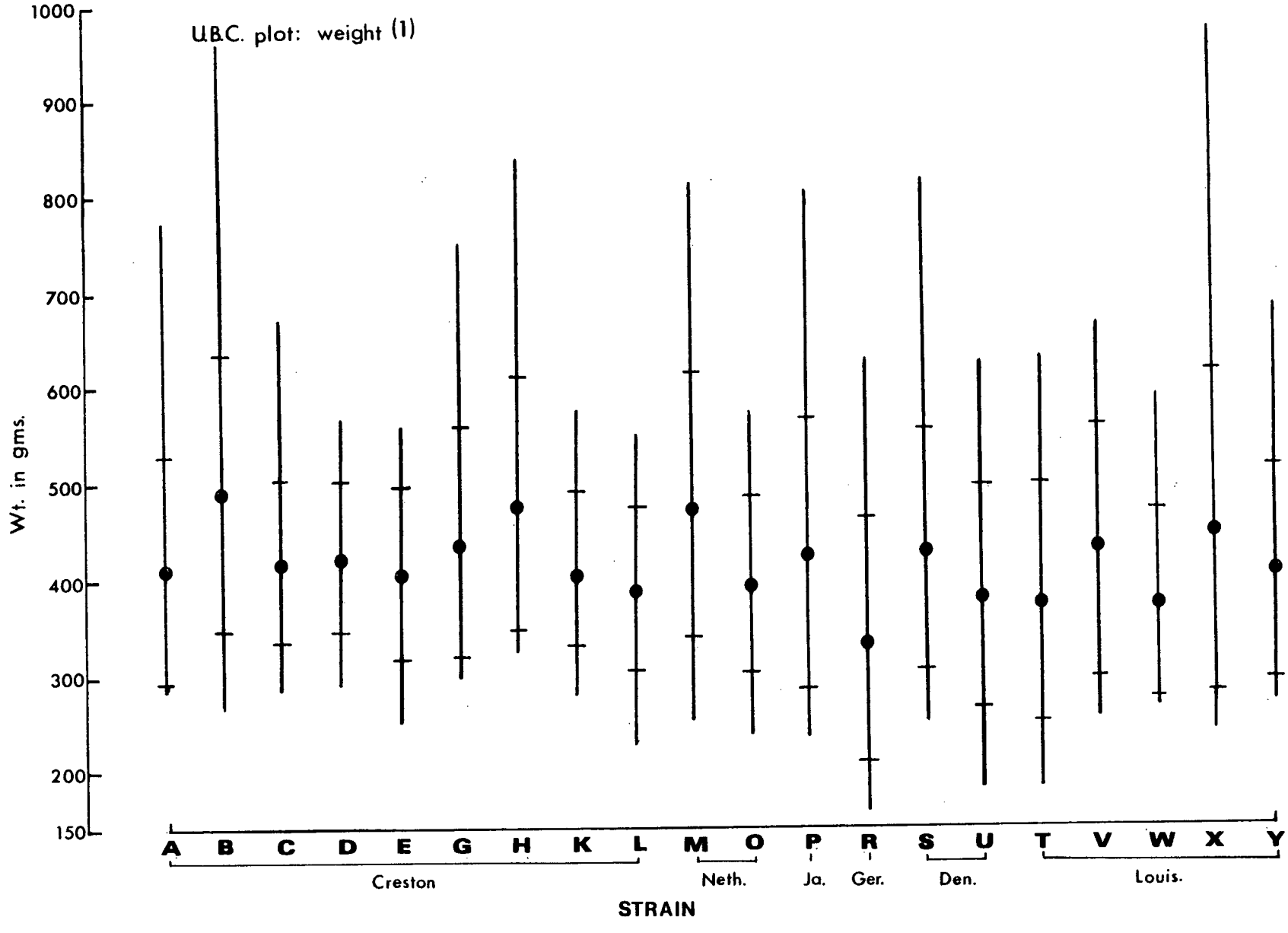
Individual plant weights in grams for twenty strains grown in a uniform nursery at U.B.C. Sampling date 18-8-77(1)

Strain	Source	No. plants per sample	Mean	SD	CV%	Range
A	Creston	20	409	115	28	282 - 772
B	Creston	20	492	146	30	270 - 961
C	Creston	20	419	81	19	282 - 664
D	Creston	15	421	79	19	289 - 567
E	Creston	19	405	89	22	255 - 559
G	Creston	20	435	120	28	300 - 748
H	Creston	20	476	132	28	313 - 838
K	Creston	20	406	81	20	280 - 574
L	Creston	20	384	91	24	223 - 548
M	Netherlands	20	478	140	29	257 - 817
O	Netherlands	20	392	91	23	241 - 577
P	Japan	20	424	140	33	235 - 803
R	Germany	20	328	126	38	158 - 622
S	Denmark	20	430	128	30	259 - 819
U	Denmark	20	379	120	32	181 - 622
T	Louisiana	20	374	126	34	183 - 630
V	Louisiana	20	434	131	30	260 - 666
W	Louisiana	20	376	95	25	271 - 584
X	Louisiana	20	445	173	39	241 - 975
Y	Louisiana	20	409	110	27	273 - 684

Figure 4.9.8.1

The variation in standing crop weight from the harvest on the 18-8-77 (1) of white clover plants from the twenty strains at random in each of four blocks; each strain represented by five plants in ultimate plots; ultimate plots located at random within each block, showing:

a) The arithmetic mean for each strain •
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars


U.B.C. plot: weight (1)
Wt. in gms.
1000
900
800
700
600
500
400
300
200
150
A B C D E G H K L M O P R S U T V W X Y
Creston
Neth.
Ja.
Ger.
Den.
Louis.
STRAIN

1126

Table 4.9.8.2

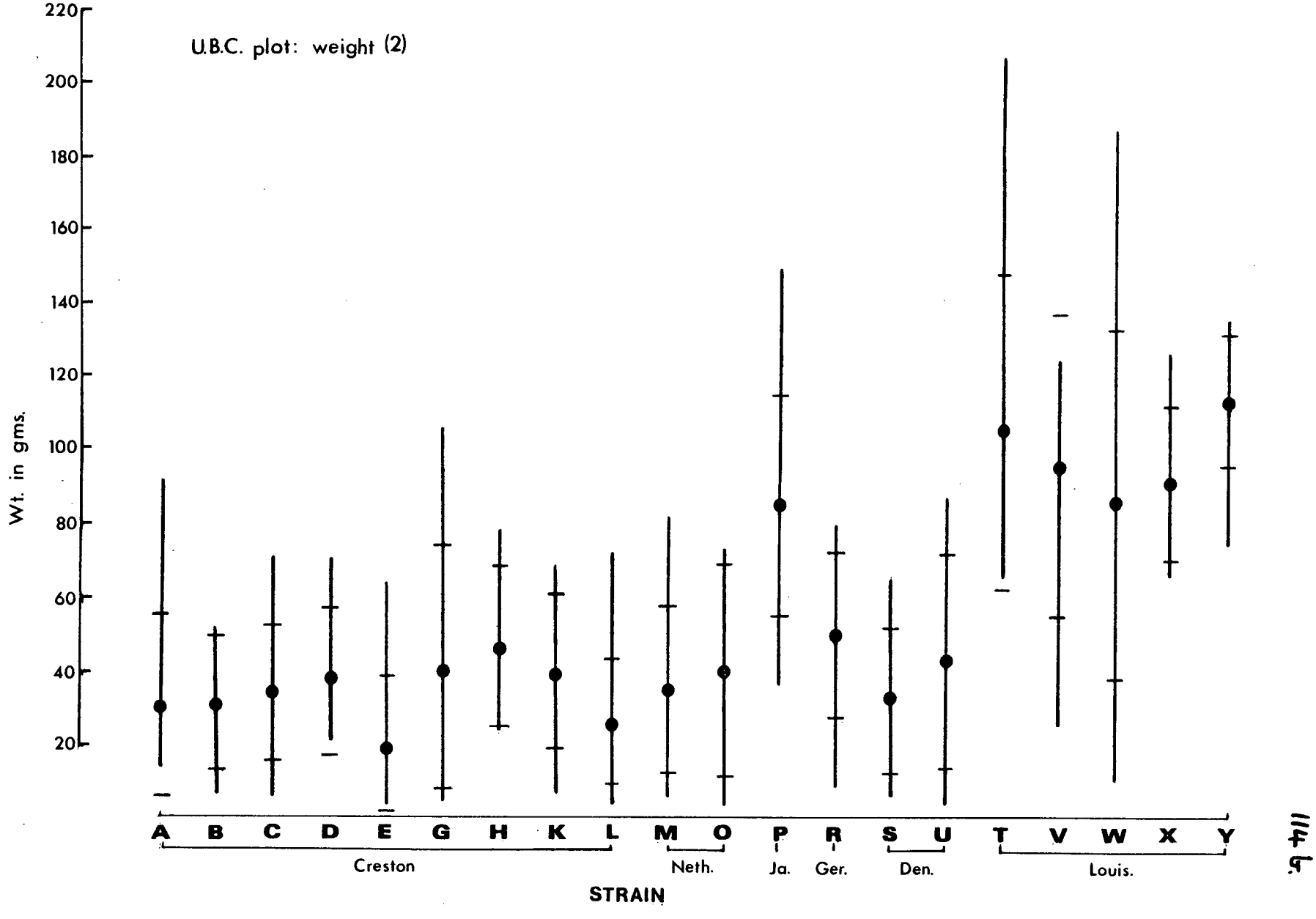
Individual plant weights in grams for twenty strains grown in a uniform nursery at U.B.C. Sampling date 19-10-77(2)

Strain	Source	No. plants per sample	Mean	SD	CV%	Range
A	Creston	10	30	25	83	14 - 93
B	Creston	10	31	18	58	8 - 52
C	Creston	10	34	19	56	6 - 72
D	Creston	5	37	20	54	21 - 71
E	Creston	9	19	19	100	5 - 64
G	Creston	10	41	33	80	6 - 108
H	Creston	10	46	22	48	23 - 77
K	Creston	9	40	21	53	9 - 68
L	Creston	10	25	18	72	4 - 72
M	Netherlands	10	35	22	63	7 - 82
O	Netherlands	10	40	28	70	3 - 73
P	Japan	10	85	30	35	37 - 148
R	Germany	9	50	22	44	9 - 79
S	Denmark	9	32	20	63	6 - 64
U	Denmark	10	43	29	67	4 - 86
T	Louisiana	10	105	43	41	66 - 206
V	Louisiana	10	96	40	42	25 - 125
W	Louisiana	10	85	47	55	10 - 186
X	Louisiana	10	91	22	24	67 - 126
Y	Louisiana	10	113	19	17	75 - 135

Figure 4.9.8.2

The variation in standing crop weight from harvest on the 19-10-77 (2) of white clover plants from twenty strains at random in each of four blocks; each strain represented by five plants in ultimate plots; ultimate plots located at random within each block, showing:

a) The arithmetic mean for each strain ●
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars


U.B.C. plot: weight (2)
Wt. in gms.
220
200
180
160
140
120
100
80
60
40
20
A B C D E G H K L M O P R S U T V W X Y
Creston
Neth.
Ja.
Ger.
Den.
Louis.
STRAIN

other sets from the same area of the field. The cuttings were planted in unsterilized Creston soil and placed in the misting chamber. On April 1st, 1977, these plants were moved to the cold frames for hardening. On June 14th the plants were set out in Totem Field, U.B.C. in a randomized design with four blocks.

The main reason for the clonal propagation was to obtain plants that were genetically identical so that we could compare the variation caused by non-genetic factors such as variation in soil, root damage and other unidentifiable factors.

4.10.2 Variation in Leaf Area

4.10.2.1 Materials and Methods

Two samples were taken from each plant on each of two dates (July 20th and October 20th). A sample consisted of a trifoliate leaf and its petiole. One sample was taken from the third open leaf from the end of a stolon (young leaf). The other sample was taken from the centre of the plant. Leaf area was estimated in square centimeters by use of the method outlined by Williams et al. (1964).

4.10.2.0 Observations and Results

The results of these observations are listed in Tables 4.10.2.1 and 4.10.2.2. and Figures 4.10.2.1 and 4.10.2.2. Considerable modification in leaf area is evident from the late planting. The F-test in the analysis of variation suggested differences between strains and between the two sampling dates; plants of the 1976 planting showed a small reduction in mean leaf area.

Tables 4.10.2.3 and 4.10.2.4 show the variability within each clone. This variability is associated mainly with error in sampling and other non-inherited factors. If we compare the average coefficients of variation to

Table 4.10.2.1

Area representation of individual leaves (in square centimeters) for five strains of white clover grown in a uniform nursery at U.B.C., sampled 20-7-77(1).

Strain	Source	Mean(cm^2)	SD(cm^2)	CV%	Range
1	Staples 1975	7.68	1.65	21	4 - 12.6
2	Ogilvie 1975	7.88	2.45	31	5 - 15.8
3	Mulligan 1976	5.79	1.51	26	3.2 - 10
4	Eastman 1975	7.14	2.85	40	2 - 12.6
5	Eastman 1976	6.94	2.65	38	3.2 - 15.8

Number of leaves for each treatment - 48

Table 4.10.2.2

Area representation of individual leaves (in square centimeters) for five strains of white clover grown in a uniform nursery at U.B.C., sampled 20-10-77(2).

Strain	Source	Mean(cm^2)	SD(cm^2)	CV%	Range
1	Staples 1975	7.10	2.13	30	2.5 - 12.6
2	Ogilvie 1975	7.30	4.10	55	1.6 - 20
3	Mulligan 1976	6.81	2.6	38	2.5 - 12.6
4	Eastman 1975	6.59	2.8	42	2 - 12.6
5	Eastman 1976	5.01	1.62	32	1.3 - 10

Number of leaves for each treatment - 48

Figure 4.10.2.1

The variation in leaf area on the 20-7-77 (1); plants were grown from cuttings and established in a uniform nursery. The five strains are at random in each of four blocks; each strain is represented by nine plants in the ultimate plots; the ultimate plots are located at random within each block, showing:

a) The arithmetic mean for each strain ●
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars

U.B.C. Clones: Leaf Area (1)

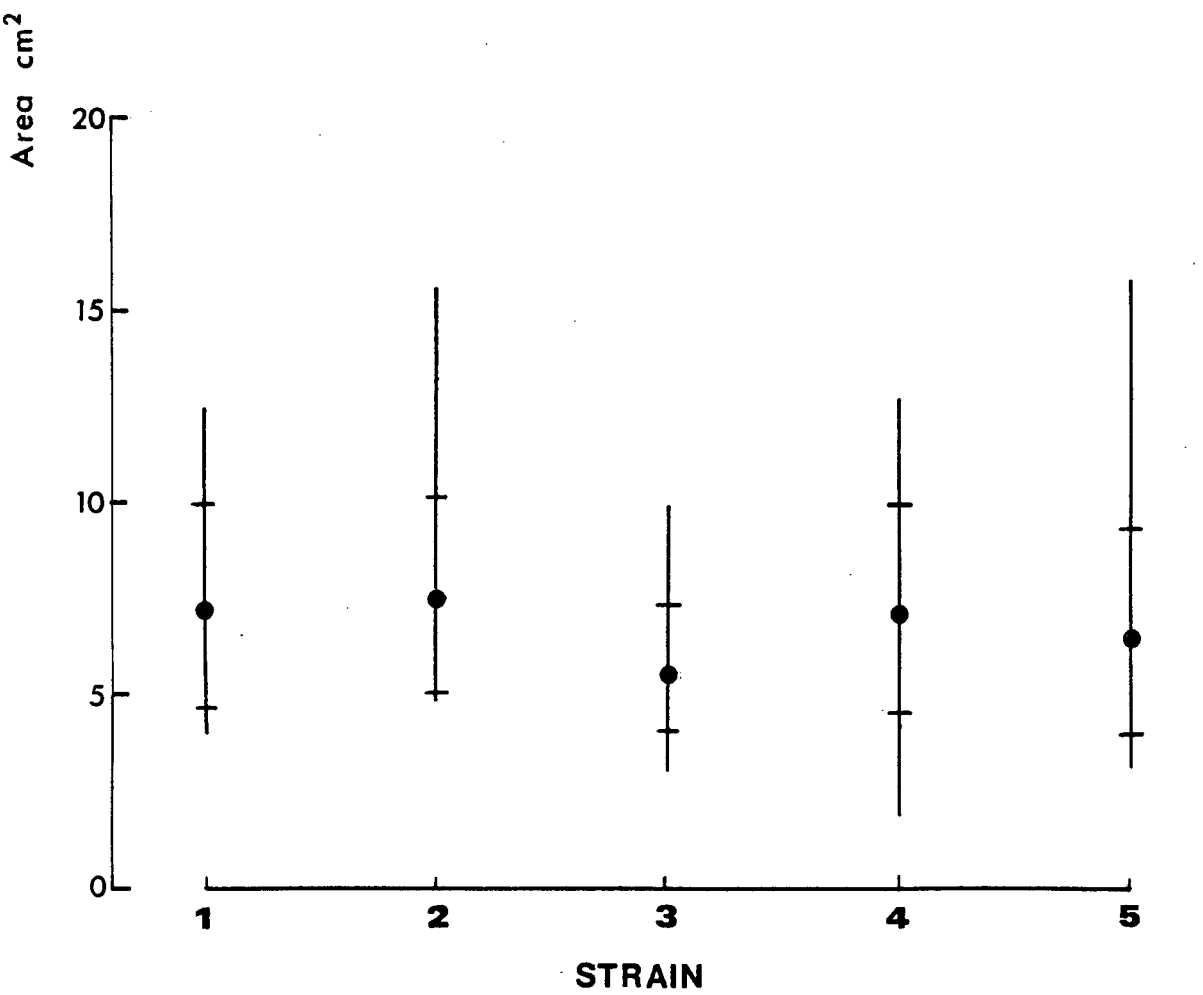


Figure 4.10.2.2.

The variation in leaf area on the 20-10-77 (2); plants were grown from cuttings and established in a uniform nursery. The five strains are at random in each of four blocks; each strain is represented by nine plants in the ultimate plots; the ultimate plots are located at random with each block.

a) The arithmetic mean for each strain ●
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars

U.B.C. Clones: Leaf Area (2)

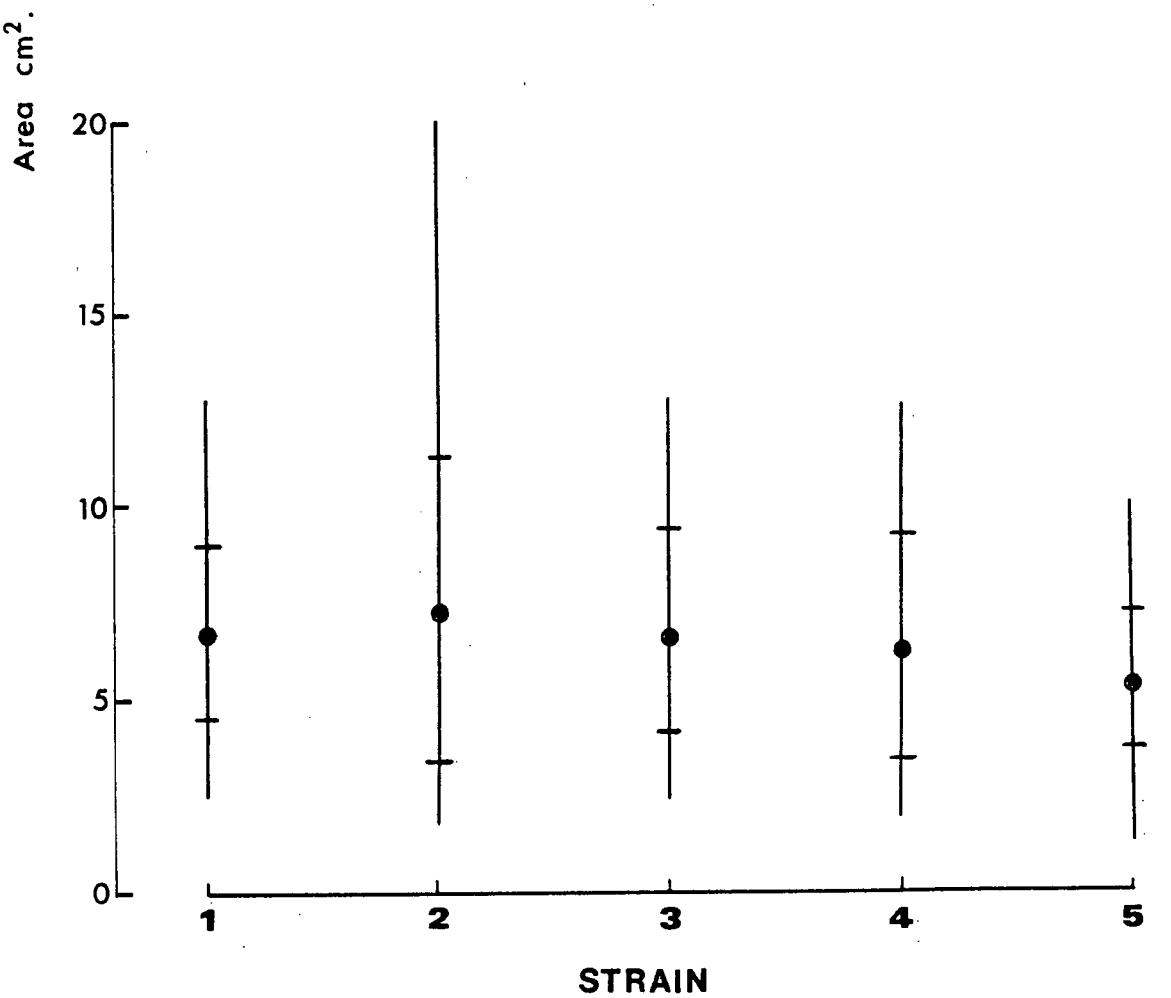


Table 4.10.2.3

Mean leaf area variability within clones of five strains of white clover grown in a uniform nursery at U.B.C.; three plants in each clone, eight clones in each strain; average of the mean and the standard deviation coefficient for eight clones in each strain; collection date 20-7-77.

a - old leaf

b - young leaf

Strain	Source	Mean	SD	CV%
1	Staples 1975	a - 7.86	1.06	13
		b - 7.49	1.48	20
2	Ogilvie 1975	a - 8.06	1.46	18
		b - 7.69	1.75	23
3	Mulligan 1976	a - 5.74	0.78	14
		b - 5.84	1.56	27
4	Eastman 1975	a - 8.10	2.19	27
		b - 6.27	1.21	19
5	Eastman 1976	a - 6.31	1.23	19
		b - 7.57	1.32	17

Table 4.10.2.4

Mean leaf area variability within clones of five strains of white clover grown in a uniform nursery at U.B.C.; three plants in each clone, eight clones in each strain; average of the mean and the standard deviation for eight clones in each strain; collection date 20-10-77.

a - old leaf

b - young leaf

Strain	Source	Mean	SD	CV%
1	Staples 1975	a - 6.88	1.33	19
		b - 7.33	1.34	18
2	Ogilvie 1975	a - 6.41	1.65	26
		b - 8.20	1.96	24
3	Mulligan 1976	a - 6.02	1.01	17
		b - 7.59	1.16	15
4	Eastman 1975	a - 6.50	1.34	21
		b - 6.86	1.29	19
5	Eastman 1976	a - 5.05	1.02	20
		b - 4.98	1.68	34

to those in Tables 4.10.2.1, 4.10.2.2, 4.9.3.1 and 4.9.3.2 we see that about 30-60 percent variation can be attributed to genetic factors.

4.10.3 Variation in Petiole Length

4.10.3.1 Materials and Methods

The same samples taken for leaf area also served for petiole length measurements. Petiole lengths were measured in centimeters from the base of the petiole at the junction of the stipules and petiole to the junction of the leaf pedicels.

4.10.3.2 Observations and Results

The results of observations on petiole length are given in Tables 4.10.3.1 and 4.10.3.2. The F-test in the analysis of variation suggests a difference between strains both for young petioles and old petioles on both dates of observation (July 20th and October 20th).

Table 4.10.3.3, the relationship of leaf area to petiole length for the two sampling dates. Strain 4 is the only strain showing a highly significant relationship of leaf area to petiole length for both dates.

4.10.4 Variation in Plant Weight

4.10.4.1 Materials and Methods

On September 1st all plants were harvested, dried in the pot hole drier, and weighed.

4.10.4.2 Observations and Results

The F-test in the analysis of variation suggests differences between strains. The air-dry weights of material harvested are presented in Table 4.10.4.1 and Figure 4.10.4.1. Strain three only shows any abnormal yield. Table 4.10.4.2 gives the average variation that exists between plants within a clone for the five strains and comparing the coefficients of variation to those in Tables 4.10.4.1 and 4.9.8.1 we see that 75 to 30 percent variation in plant weight is due to genetic factors.

Table 4.10.3.1

Petiole length in centimeters for five strains of white clover grown in a uniform nursery at U.B.C., sampled 20-7-77.

Strain	Source	Mean	S.D.	CV %	Range
1	Staples 1975	13.71	3.68	27	7 - 21
2	Ogilvie 1975	12.98	4.26	33	6 - 21
3	Mulligan 1976	8.94	2.49	28	5 - 15
4	Eastman 1975	13.48	3.93	29	6 - 22
5	Eastman 1976	11.42	3.44	30	6 - 20

Number of samples for each treatment - 48.

Table 4.10.3.2

Petiole length in centimeters for five strains of white clover grown in a uniform nursery at U.B.C., sampled 20-10-77.

Strain	Source	Mean	S.D.	CV %	Range
1	Staples 1975	15.58	3.49	22	8 - 23
2	Ogilvie 1975	14.42	5.13	36	6 - 25
3	Mulligan 1976	13.31	3.63	27	6 - 19
4	Eastman 1975	13.42	4.88	36	5 - 22
5	Eastman 1976	11.54	3.46	30	4 - 18

Number of samples for each treatment - 48.

Table 4.10.3.3

Linear regression equations and correlation coefficients for white clover clonal material for two dates grown in a uniform nursery at U.B.C. Plants were vegetatively propagated from material of Creston origin. Five treatments, each treatment contained eight groups with three genetically identical plants in each group.

Treatment No.	Name	Leaf area and petiole length (Date 20-7-77) Regression Equation	Leaf area and petiole length (Date 20-7-77) Correlation Coefficient	Leaf area and petiole length (Date 20-10-77) Regression Equation	Leaf area and petiole length (Date 20-10-77) Correlation Coefficient
1	Staples 1975	Y= 6.38+0.98X	r=0.43*	Y=13.37+0.21X	r=0.13
2	Ogilvie 1975	Y= 8.73+0.53X	r=0.34	Y= 8.46+0.82X	r=0.65**
3	Mulligan 1976	Y= 8.03+0.22X	r=0.12	Y=13.78-0.001X	r=0.0007
4	Eastman 1975	Y= 6.64+0.96X	r=0.69**	Y= 4.88+1.28X	r=0.74**
5	Eastman 1976	Y=11.24+0.02X	r=0.02	Y=11.90-0.07X	r=-0.03

Leaf area - X; Petiole Length - Y: Leaf area - X; Petiole Length - Y

Sample size - 24 observations per treatment.

r.05(22) = 0.4143*

r.01(22) = 0.5268**

Table 4.10.4.1

Top weight in grams for five strains of white clover grown in a uniform nursery at U.B.C.

Strain	Source	Mean	SD	CV %	Range
1	Staples 1975	139.6	48.3	35	71.3-244.8
2	Ogilvie 1975	146.7	37.8	26	81.0-218.7
3	Mulligan 1976	90.6	17.7	20	60.2-133.3
4	Eastman 1975	129.1	50.3	39	75.3-260.7
5	Eastman 1976	123.2	30.3	25	80.8-186.0

Number of samples for each treatment - 24.

Table 4.10.5.1

Height in centimeters for five strains of white clover grown in a uniform nursery at U.B.C.

Strain	Source	Mean	SD	CV %	Range
1	Staples 1975	17.4	2.8	16	7-22
2	Ogilvie 1975	18.7	6.2	33	9-29
3	Mulligan 1976	15.4	2.9	19	9-19
4	Eastman 1975	15.5	4.9	32	6-23
5	Eastman 1976	14.9	3.2	22	8-20

Number of samples for each treatment - 24.

Figure 4.10.4.1

The variation in top weight for plants grown from cuttings in a uniform nursery; the five strains at random in each of four blocks; each strain represented by nine plants in ultimate plots; ultimate plots located at random within each block, showing:

a) The arithmetic mean for each strain ●
b) The simple range by vertical bar
c) ± one standard deviation from the mean by horizontal bars

U.B.C. Clones: Top weight

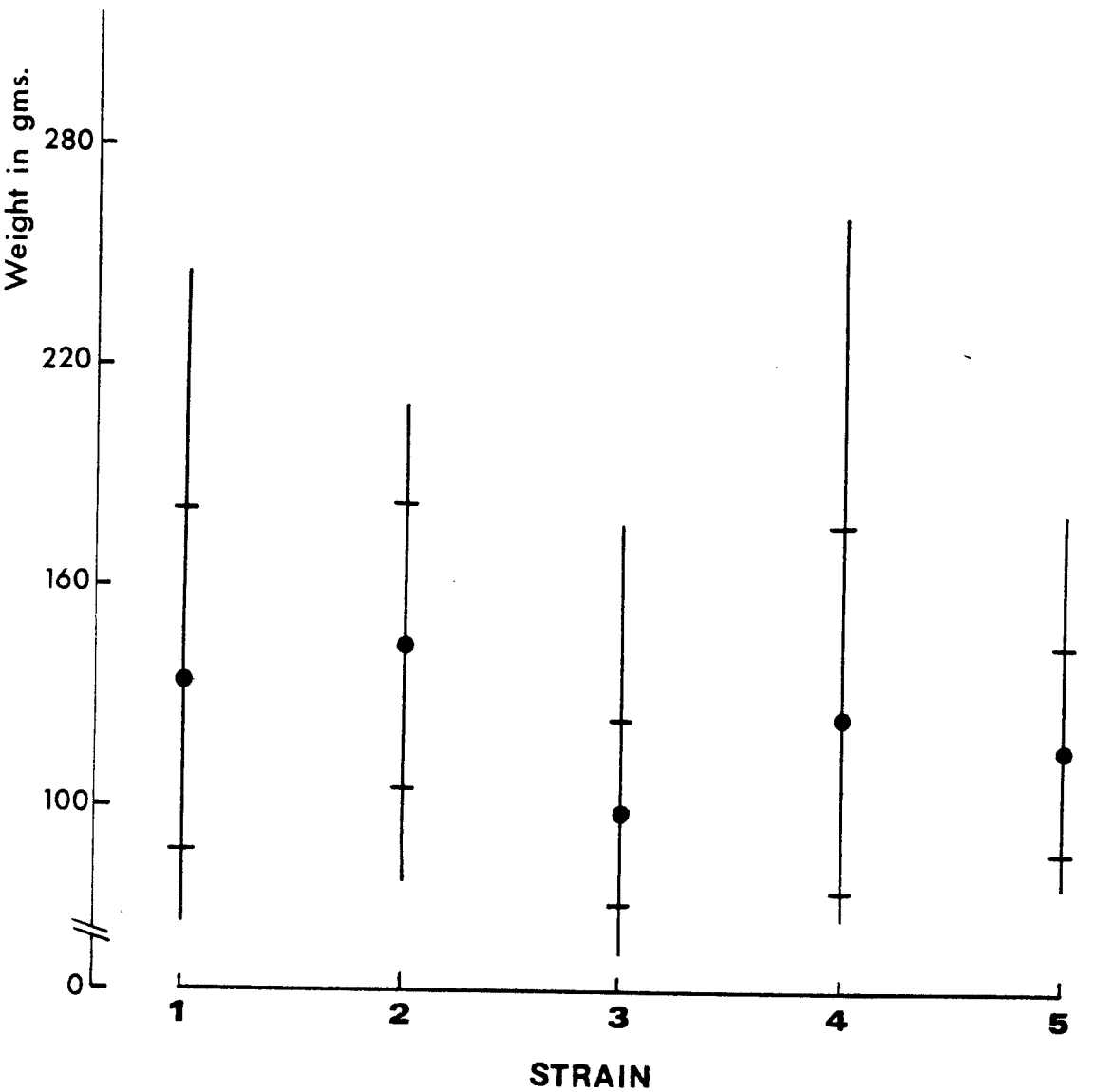


Table 4.10.4.2

Mean, weight and standard deviation of top growth within clones from five strains of white clover grown in a uniform nursery at U.B.C.; three plants in each clone, eight clones in each strain.

Strain	Source	Mean	S.D.	CV %
1	Staples 1975	139.63	15.91	11
2	Ogilvie 1975	146.67	10.13	7
3	Mulligan 1976	90.62	14.40	16
4	Eastman 1975	129.11	14.29	11
5	Eastman 1976	123.19	14.65	12

Table 4.10.5.2

Mean, plant height and standard deviation within clones from five strains of white clover grown in a uniform nursery at U.B.C.: three plants in each clone, eight clones in each strain.

Strain	Source	Mean	S.D.	CV %
1	Staples 1975	17.42	1.75	10
2	Ogilvie 1975	18.67	2.24	12
3	Mulligan 1976	15.38	1.31	9
4	Eastman 1975	15.54	1.48	10
5	Eastman 1976	14.92	1.80	12

4.10.5 Variation in plant height

4.10.5.1 Materials and methods

Plant height was measured in early September. The measurement was made in centimeters by measuring the distance from the ground to the upper surface of the plant canopy. Single leaves above the canopy level were ignored.

4.10.5.2 Observations and results

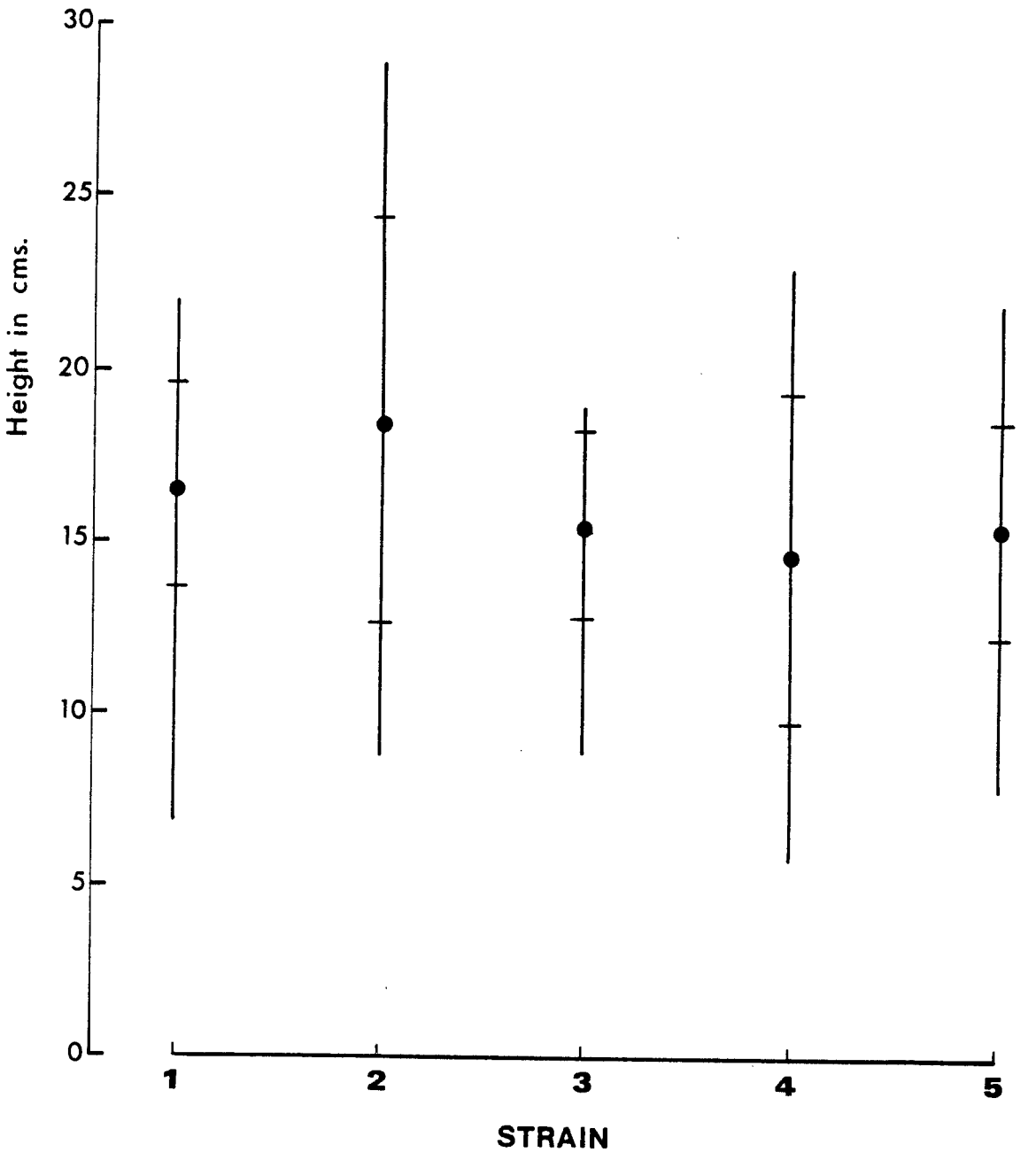
The F test in the analysis of variation suggests differences between strains. The results are presented in Table 4.10.5.1 and Figure 4.10.5.1. Table 4.10.5.2 gives average variation that exists between plants within a clone for the 5 strains. By comparing the coeffcient of variation in Tables 4.9.6.1, 4.10.5.1 to 4.10.5.2 we observe that 54 percent of the variation in plant height is attributed to genetic factors.

Figure 4.10.5.1

The variation in height of white clover plants grown from cuttings in a uniform nursery; the five strains at random in each of four blocks; each strain represented by nine plants in ultimate plots; ultimate plots located at random within each block, showing:

a) The arithmetic mean for each strain ●
b) The simple range by vertical bar
c) one standard deviation from the mean by horizontal bars

U.B.C. Clones: Plant Height



4.10.6 Variation in Leaf Markings

4.10.6.1 Materials and Methods

The leaf markings observed were the inverted "V" marks and the anthocyanin flecking marks. The grouping of the inverted "V" leaf mark and that for flecking was by means of the simple observation of presence and absence.

4.10.6.2 Observations and Results

Results of counts of the inverted "V" mark and anthocyanin flecks are found in Table 4.10.6.1, the presence of the leaf mark generally being the one in greater abundance. Anthocyanin flecks were more frequently absent than present. It was noted in the field that all plants from each clone had similar inverted "V" leaf marks and leaf flecks. Although plants were collected at random, it is interesting to note the difference in ratios of leaf marks to no leaf marks in each strain. Table 4.10.6.2 gives the contingency table for the presence and absence of the two sets of leaf markings.

4.11 Coated Clover Seed Trial

4.11.1 Materials and Methods

The trial was undertaken on the Totem Field at U.B.C. The seed used was of Creston origin and the coating process was done by Cel Pril Industries Inc., Manteca, California. On June 23rd, the trial was laid out and seeded on a three by three Latin square design, each plot being three meters by three meters. Treatments were a) coated seed, b) standard inoculation, c) not inoculated.

4.11.2 Observations and Results

On September 23rd one meter square quadrats were clipped from each plot, air-dried in the pot hole drier and weighed to the nearest ounce. No significant difference was observed between any of the treatments.

Table 4.10.6.1

Counts of leaf marks for five strains of white clover grown as clonal material in a uniform nursery at U.B.C. Plants were vegetatively propagated from material of Creston origin. Five treatments, each treatment contained eight groups with three genetically identical plants in each group.

Strain	Plants with inverted Leaf mark Present	Plants with inverted Leaf mark Absent
1	9 (6)	15 (18)
2	12 (0)	12 (24)
3	24 (0)	0 (24)
4	15 (6)	9 (18)
5	18 (9)	12 (21)

Plants outside of brackets - inverted "V" mark.

Plants inside of brackets - anthocyanin flecks.

Table 4.10.6.2

Contingency table for inverted leaf marks and anthocyanin flecking on clonal material grown in a uniform nursery at U.B.C. Plants were vegetatively propagated from material of Creston origin. Five treatments, each treatment contained eight groups with three genetically identical plants in each group.

Anthocyanin leaf flecks	Inverted "V" Leaf Marks Absent	Present	Total
Absent	39 (40)	66 (65)	105
Present	9 (8)	12 (13)	21
Total	48	78	126

X^2 actual = 0.26 X^2 expected .05(1) = 3.84

4.11.3 Discussion

The coating of white clover seed under U.B.C. field conditions did not give any appreciable increases in establishment or yield to that of uncoated seed, dusted or not dusted with humus base rhizobial culture.

4.12 Acetylene Reduction Trials

4.12.1 Materials and Methods

An ethylene analyzer (portable gas chromatograph) was constructed in the Department of Plant Science at U.B.C. Specifications and design were followed closely to those outlined by Mallard et al. (1977). Gas chromatographic and troubleshooting procedures were consulted from the text by McNair and Bonelli (1969). The Taguchi gas sensor model No. T GS-812 was obtained from Southwest Technical Products Corp., 219 West Rhapsody, San Antonio, Texas. The column packed with Poropak R and Poropak N (mesh size 80-100) was ordered prepacked from Chromatographic Specialists Ltd., P.O. Box 758, Brockville, Ontario. The length and diameter of the column was 44 cm x 3.18 mm (Mallard et al. 1977). Electrical power for the analyzer was provided by a 12-volt car battery. The carrier gas was nitrogen at 20 ml/min.

4.12.2 Observations and Results

It was found that good separation of ethylene from acetylene could be obtained from a carrier gas flow rate of 20 ml per minute. However tailing of the acetylene peak proved problematic. The carrier gas was then changed to compressed air and good separation of ethylene from acetylene was obtained with no tailing of the acetylene peak. Sensitivity appeared good, with 1 part per million being detected when a sample of 1 ml was introduced. When 0.1 ml was injected, 10 parts per million were detected under laboratory conditions.

The column is unheated and relies on the ambient air temperature. However moisture could become problematic if samples are contaminated, rendering the column ineffective.

14.12.3 Discussion

It is considered that the unit is sufficiently accurate to detect nitrogenase activity as early as one hour after the beginning of incubation of samples *in situ* in soil.

5. GENERAL DISCUSSION AND CONCLUSIONS

The information provided in Table 1.1 shows that the present hectarage in white clover that is grown in the Creston Valley of British Columbia is a quarter of that of a decade ago. Likewise, the yield of seed per hectare has declined, while the price of common white clover seed has doubled in the same time. The general trend for some markets of white clover seed, especially in the European section, is to close their doors on common seed and open them more avidly to certified seed. The returns to growers for producing certified seed are lucrative, and in general there is a 15% increase in seed prices for certified over common seed. In the highly competitive markets for white clover seed on this continent and elsewhere two things appear to be in demand: a) a statement of seed origin (reflecting the climate and management system under which it is produced), b) a name, along with the usual high standards of freedom from disease and weed seeds that accompany a certificate.

The use and interest shown by some phases of agriculture in white clover has diminished over the years as the use of artificial nitrogen has increased. With the rising prices of fertilizer nitrogen the world over, in turn a result of the rising cost of petroleum products, a general interest in legumes and their nitrogen fixing ability is again apparent. It is unlikely that white clover will be used in very high yielding grass pastures, but its application in other situations is increasing (e.g., range rehabilitation, revegetation of mining spoil banks, roadsides and other applications). Changes in marketing policy, together with changes in the agricultural and non-agricultural applications are reasons for the Creston valley seed industry to reassess its role.

The seeking out of the causes for the decline in seed yield and persistence of the white clover crop in the Creston valley is one of the primary objectives of this study. Although the scope of the study was broad, areas such as those of disease could have been more fully explored.

The investigation into possible insect pests revealed that three species of weevil appear in large enough populations to have a bearing on the apparent decrease in seed yield. The cumulative injury by these three weevil species must be of significance. Such effects as lower floret fertility and seed damage by the adults and larvae of the clover seed weevil are apparent (see Table 4.3.4.2). Poor development of seeds may well result from interrupted translocation as a result of larval feeding on the roots (see Table 4.1.4.1). The action of the clover root curculio larvae leads to a weakening of the stand, weed infestation, poor seed filling, pathogen entry, plant stress and and loss of plant persistence. The lack of persistence in the white clover seed crop, apparently related to weevil incidence, renders it impossible for the farmer to keep the crop longer than one seed harvest. The long-term consequences of this will lead to a natural selection of plants for a biennial habit. This may well be of importance to the seed trade because white clover is normally a long-lived perennial in agricultural practice. The trade is not likely to be much interested in stressing lasting persistence.

The sampling methods also revealed other organisms, such as slugs, nematodes and rodents. These organisms should not be overlooked for the damage they too can cause. Although control by chemical means is possible, their presence in the crop was observed to be sporadic, both in time and location. I feel that they are not the principal causes of the general seed yield decline but are contributory.

Soil fertility and reaction tests indicated that the nutrients and hydrogen ion concentrations were not limiting plant growth. However, the high soil reaction and calcium content lead me to believe that there is a general need by Creston white clover for extra phosphorus fertilizer. The calcium ions combine with the applied phosphate fertilizer to form principally dicalcium phosphate which is a relatively unavailable form to plants. In

adjacent alfalfa crops, boron deficiency was noted on several occasions; it was not noted in white clover fields.

The sampling of seed and phytomas revealed that there is a large variation in yield both within and between fields. Some general trends were observed.

One of these trends that the heavy top growth of white clover does not necessarily mean heavier seed yield. In other areas where white clover is grown for seed, notably Britain, defoliation of the crop in early summer is practiced. Leguminous crops, other than white clover, benefit from an early summer cutting, which stimulates reproductive growth as opposed to vegetative growth, and which helps create a more open stand. It was observed that fifty percent of the vegetative growth at Creston takes place prior to the end of May. A similar practice of vegetative removal early in the season in the Creston valley may prove beneficial, but allowance in time must be made for a carbohydrate build-up for seed production. Too late a cutting will lead to poor seed production.

Floral bud initiation is stimulated by this practice of opening up the stands and it was observed at Creston that the number of inflorescences was strongly associated with seed yield. Legumes, as previously mentioned, fix their own nitrogen, and consequently a high nitrogen level is maintained in the soil. This widens the nigrogen-phosphorus ratio. This ratio widening creates a tendency for the white clover plant to remain in a vegetative state, as the phosphorus-nitrogen-carbon ratios remain wide. However, by the application of phosphorus to the seed crop, this ratio is narrowed and a balance maintained for reproductive growth. By using phosphorus for this purpose we are able to manipulate a crop into reproductive growth without losing its genetic capacity for vegetative growth.

The potential seed yields as measured by the quadrat method were almost always larger than those obtained by the grower. In some cases growers yields

were almost half that of the potential yield. The explanation for this being that considerable shattering and seed head loss occurs at time of harvest. Plant stress can result in early maturity, head and seed loss at harvest.

I feel that pollination of the white clover seed crop at Creston is not a problem. The recommendations in effect in other countries lead to the conclusion that each field in the Creston valley had an adequate number of pollinator hives. Beehives are routinely placed in each crop of white clover. In every case measurements of peduncle length were greater than petiole length, ensuring adequate exposure of inflorescences for bees. In the field it was observed that inflorescences were always well above the foliage. It may well be that it is important to have inflorescences well exposed above the canopy for good pollination. However, it was observed that long stubble remaining after the previous grain harvest and even weeds of low stature such as dandelion and of course tall weeds such as perennial sow thistle appeared to stand well above the crop. Such obstructions could lead to poor bee activity on the white clover. The clipping of weeds in field 6 could be responsible for the high number of seeds per head, while in field 5, where there was a heavy infestation of dandelions, we have a lower seed count per inflorescence. A cutting of top growth in early summer would likely diminish tall weeds. The malathion spray coming on the 22nd of June was observed to deter but not halt bee activity for about two days. This spray comes when the crop is in full bloom and a small drop in seed yield may be apparent.

Total plant nitrogen (Table 4.4.1) demonstrated that the majority of nitrogen fixation occurred prior to the end of May. In some cases a deficit in plant nitrogen occurs and this could be a result of the feeding by the larva of the clover root curculio on the plant nodules and roots.

The Kjeldahl nitrogen determination is not nearly as sensitive as the acetylene-reduction technique in measuring the ability of a plant-system to

"fix" atmospheric nitrogen. The Kjeldahl analysis is also destructive and consequently does not allow for repeated observations. It took me a long time to adapt the acetylene-reduction technique to my conditions. With the help of Mr. Ilmars Derics we constructed a portable field unit (similar to Mallard et al. 1977) for measuring the production of ethylene. The use of the acetylene-reduction technique and field measurement of ethylene produced could be a very useful measure of the effect by the clover root curculio on the white clover system.

The observations carried out at U.B.C. were made largely on single plants.

The date of flowering and number of inforescences produced are important to forage seed production. It is in the interests of the grower that a plant produces the maximum amount of seed and this is one reason why seed production is often to be carried out in an area other than its area of greatest use for forage. As documented, white clover initiates reproductive growth in response to daylength and temperature. White clover is a long day species and different strains respond in their flowering to different combinations of temperature and daylength. It appears from one year's information that the Creston strains are well adapted to the latitude for seed production.

Leaf area and petiole length in white clover are important in helping to categorize strains as to their various forage uses. To elaborate, Ladino clover, which has a large leaf area and petiole length, is used for short-term hay and grazing pastures where the ability to withstand heavy grazing is not essential. However, the wild white clovers with their small leaf area and petiole length are suited to areas of low fertility and have the ability to persist almost indefinitely under heavy grazing. In my trial on twenty strains I found that the Japanese strain "Tohoku" is of the large-leaved intermediate white clover while the Dutch strain "Barbian" is small-leaved.

Leaf markings of the inverted "V" type and the anthocyanin flecking found on white clover plants in my trial varied considerably, but little distinction could be made between strains. However, the Louisiana strains stood out by their consistent presence of the intense "V" leaf mark. These are strains from warmer climates. The anthocyanin marks, which are expressed to a greater extent under cooler temperatures, show again that the strains from the warmer climates favour their absence. The contingency table (Table 4.9.5.6) shows the independent inheritance, of the two leaf marks, from each other.

The measure of cyanogenesis, because of the negative reactions in the strains from the Creston valley, appears to be of little use. However, if the Danish strain "Pajberg milka or Kivi" were being grown, then the test could be of use due to its occasional cyanogenic reaction. Again, as has been documented in the literature, strains grown in warmer climates (viz. in our trials Louisiana strains) were cyanogenic.

Plant weight did not separate out strains in the first cut; however, in the second harvest plant weight was useful in separating out strains that have a good fall recovery after defoliation. This was well displayed by the Louisiana strains which had a heavier weight on the second cut. It is felt that this good regrowth and cyanogenic reaction of strains of southern origin can lead to a poor persistence in the more northern latitudes. The Creston strains, with fall dormancy and smaller leaves, should display a greater degree of hardiness.

The purpose of planting the clonal material was to compare the non-inherited variability with that of the plants produced from seed. The measurements of leaf area, petiole length, plant height and weight were compared by using the coefficient of variation. It was observed that there exists substantial non-inheritable variation within the clonal and seed material attributable to sampling error, soil conditions or root damage.

Recommendations for further work:

a) There should be more intensive monitoring of the insect populations, especially the three major species of weevil, with a view to determining the most effective controls. This would also include the problem of not affecting the pollinators.

b) Biological nitrogen fixation studies should be carried out to determine the ability of the Creston white clover to fix atmospheric nitrogen under field conditions.

c) There should be the development, by individual plant selection, from the present Creston white clover stocks of named land strains. Domestically, two needs are apparent and two separate strains should be considered. First, an intermediate type, suited to a hay and grazing system, with moderate persistence. Second, a small-leaved type that is suited to heavy grazing and poor edaphic and climatic conditions; this small-leaved type is greatly needed for the range and non-agricultural rehabilitation projects of this province.

d) The Creston valley is ideally suited to the production of white clover for seed; however, the management of the white clover crop needs revision. First, the practice of early defoliation in the growing season of the first clover seed harvest needs consideration. Second, the soils of the Creston valley are calcareous and the carryover of available phosphatic fertilizer may be insufficient for optimum reproductive growth. This would warrant a spring application of superphosphate or triple superphosphate to the clover seed crop. These two management techniques would greatly assist the growers in producing larger marketable quantities of seed.

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Figure 7.1 The Creston valley in south-eastern B.C. is suitable for the production of high quality white clover seed.

Figure 7.2(a) White clover ranks as one of the world's more important forage plants....its application in other situations is increasing (viz. range rehabilitation).

Figure 7.2(b) Open grassland

Figure 7.2(c) Forested range

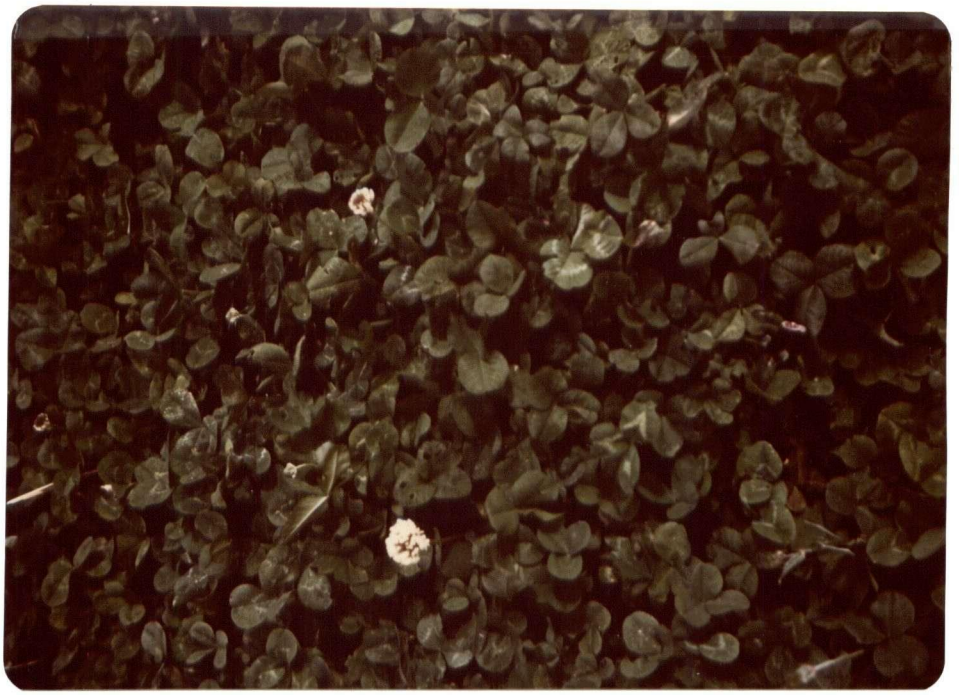

Figure 7.3(a) ....fifty percent of the vegetative growth of white clover, on the Creston flats, takes place prior to the end of May.

Figure 7.3(b) Creston strains, with less fall recovery in to growth, should display a greater persistence.

Figure 7.4(a)

The action of the clover root curculio larvae leads to a weakening of the stand, weed infestation..... pathogen entry and loss of plant persistence.

Figure 7.4(b)

Other organisms such as slugs, nematodes and rodents...their presence...observed to be sporadic.

Figure 7.4(c) ....even weeds of low stature such as dandelions.

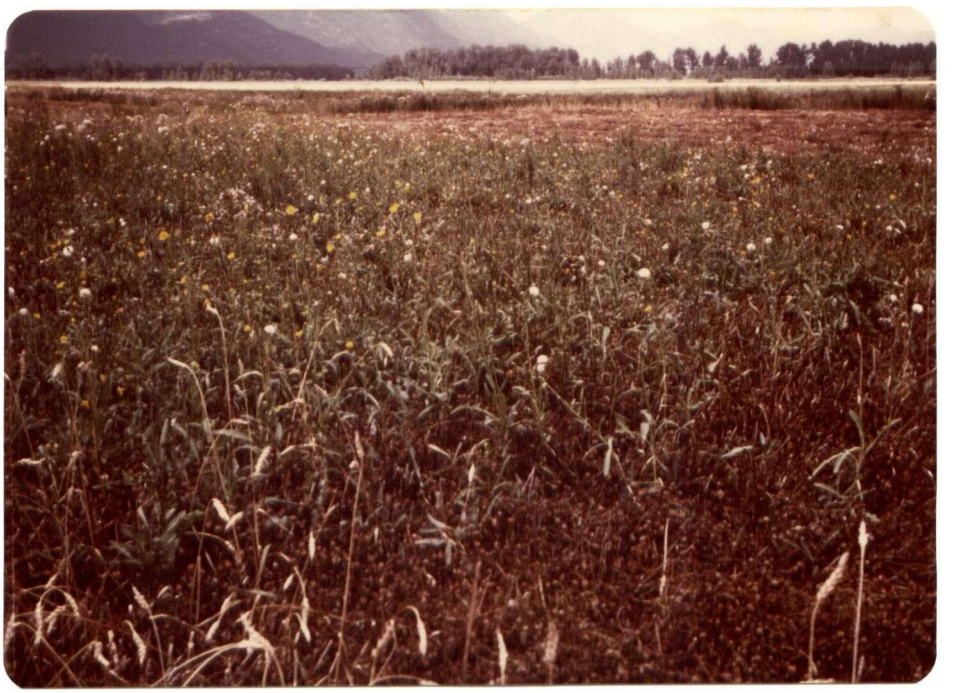

Figure 7.4(d) ....and of course tall weeds such as perennial sow thistle.

Figure 7.5(a) Volunteer grain from previous years harvest.

Figure 7.5(b) ....long stubble remaining after the previous year's grain harvest...such obstructions could lead to poor bee activity.

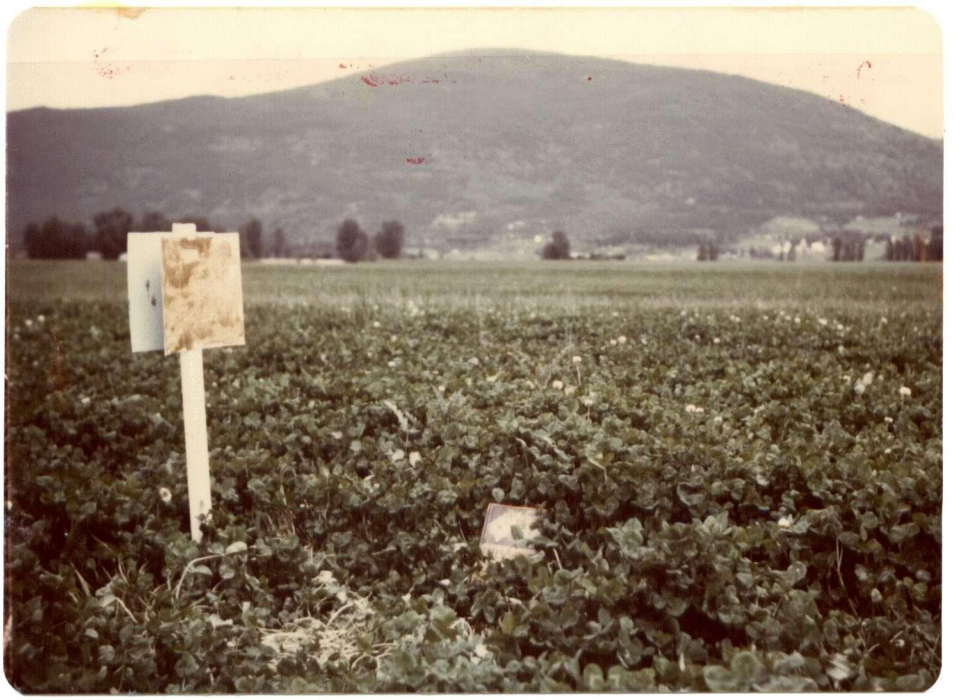

Figure 7.6 ....the sticky traps consisted of 9 inch by 11 inch yellow railroad board.

Figure 7.7(a) ....white clover in certain areas of different soil texture would flower prematurely.

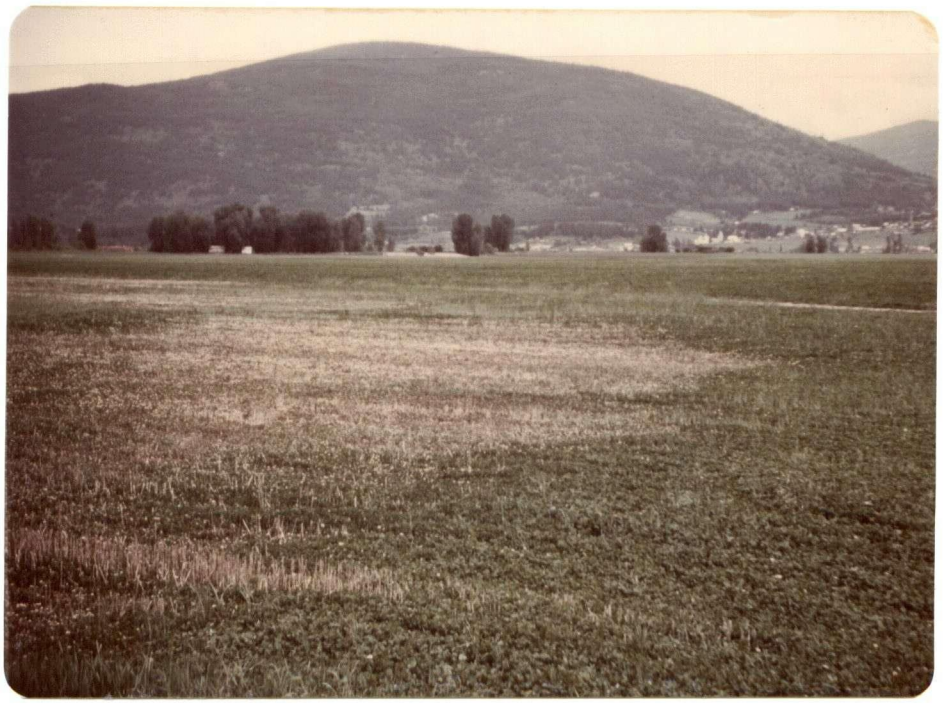

Figure 7.7(b) Flowering in early June

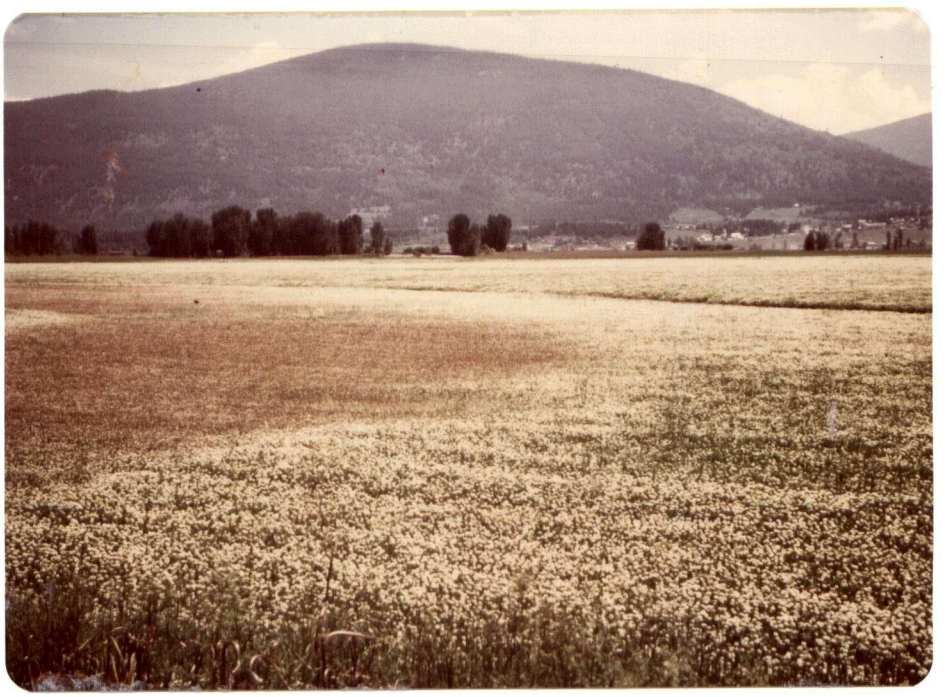

Figure 7.7(c) Matured by late June

Figure 7.8 The average number of inflorescences per hectare was estimated to be 11.5 million.

Figure 7.9 ....beehives are routinely placed in each crop of white clover.

Figure 7.10(a) ....harvesting generally commences when 90% of the heads are "brown".

Figure 7.10(b) ....farmers are experiencing a seed loss, while harvesting, of up to 50%.

Figure 7.11(1) ....it is impossible for the farmer to keep the crop longer than one seed harvest.

Figure 7.11(b) Chisel ploughed

Figure 7.12 .....consequently the trial was abandoned.

Figure 7.13 .....white clover is a short - long day species and different strains respond in their flowering to different combinations of temperature and day-length.

Figure 7.14(a) ...free flowering strains are important for seed production.

Figure 7.14(b) ...the petals are white but are occasionally pink.

Figure 7.15(a) ...Louisiana strains stood out by their consistent presence of the inverted "V" leaf mark.

Figure 7.15(b) Plants with no leaf markings were in the minority.