A - THE SELECTION OF MALE-STERILE LINES IN ALFALFA.

B - THE WITCHES' BROOM VIRUS DISEASE OF ALFALFA IN BRITISH COLUMBIA.

by

FREDERICK DOUGLAS PETTEM

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ABSTRACT

In the hope of finding male-sterile alfalfa strains for use in the production of hybrid alfalfa, a microscopic study of the pollen produced by approximately 1000 lines of alfalfa grown at the University of British Columbia was conducted. This study revealed 4 lines that consistently showed an absence of viable pollen under different environments. 2 of the male-sterile lines produced no seed on selfing and 2 produced very small quantities of seed. In plants grown from open-pollinated seed of the 4 male-sterile lines, the F_1 segregation for male-sterile to male-fertile were as follows: 1:7.9, 1:7.3, 1:6.8, and 1:8.3, for an incomplete count. These ratios suggest that the male-sterility is controlled by cytoplasmic factors in addition to recessive genes. However, the mode of inheritance will only be factorially interpreted by F_2 and backcross data. It appears that the sterility is caused by a breakdown in meiosis, as the pollen sacs are full of an amorphous material suggestive of arrested development of the pollen grains. The male-sterility should eventually prove of great economic value in the production of hybrid alfalfa seed. Male-sterility has not previously been isolated in alfalfa, although it is common in the plant kingdom.

Over the past 10 years the Witches' Broom virus disease of alfalfa has developed into serious proportions in the Interior of B.C. - this was first pointed out by quadrat results obtained in 1942-44. The disease is shown to be distributed throughout the low rainfall areas of the province where it is widespread although sporadic.

The disease is shown to be the same disease that occurs in Washington and in Australia. Witches' Broom of Alfalfa causes severe dwarfing of the affected plants and a decimation of alfalfa stands. Drastic shortening of internodes and reduction in size of leaves is accompanied by proliferation of crowns and nodes. Up to 3000 thin spindly shoots are commonly produced by a single diseased crown. Typically, leaves are marginally chlorotic; inflorescences are reduced to 3-4 florets per raceme; and, very little, if any, seed is produced. Crowns and roots are symptomless until late stages of the disease are reached, when they show severe rotting. Affected plants gradually die in a period of 3 months to 2 years. However, 2 plants were shown to apparently recover from the disease when brought into the U.B.C. greenhouse from the Nicola Valley.

A histological comparison of the healthy and diseased plants showed gummosis of the xylem vessels, a breakdown and degeneration of the chloropasts of the affected leaves, and a mechanical breakdown of the palisade layer in the outer edge of diseased leaves. Storage of starch in the crowns of plants was found to be depleted according to the stage of the disease, with no storage starch present in severely diseased crowns. However, sucrose was found to be present in storage in diseased crowns but not in healthy crowns.

The disease was found to be easily transmitted by crown grafts. Out of 142 attempted grafts, union of scion and stock was achieved in 31 cases with positive transmission in 27 cases. Seed transmission and inoculations by expressed crown juice have given negative results to date.

Quadrats were plotted in 1950 in the interior of B.C. to further study the disease. Twinning experiments were set up to study natural resistance of the members of the genus Medicago. Results from both of these studies will not be ready for publication for at least 2 more years.

A nursery plot was established at U.B.C. and a replicate plot at Kamloops, B.C. One year after planting the Kamloops plot, several of the alfalfa plants were found producing symptoms of the disease, and to be badly diseased in 15 months time. None of the plants at U.B.C. showed any signs of the disease.

From a potted plant yield trial conducted at U.B.C. the diseased plants were shown to have a statistically significant reduction in yields as compared to healthy plants.

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INTRODUCTION

An alfalfa breeding program has been carried on at the University of British Columbia since 1918 and has constituted a major activity of the Department of Agronomy. Work had progressed to such a point that in 1949 a new variety of alfalfa designated as Rhizoma (*Medicago falcata* x *M. sativa*) was registered in Canada.

An alfalfa breeding program should be continuing. Although rated by Morrison (64) as the "ideal" hay crop, an alfalfa suitable for all agronomic purposes has yet to be developed. New diseases and problems that can be controlled only through breeding methods are continually presenting themselves. There is also in alfalfa much vigour attributable to hybridity (heterosis) yet to be tapped.

One aspect in breeding alfalfa which seems worthy of special attention at this time is the possibility of utilizing heterosis or hybrid vigour. Marked heterosis is exhibited by certain plants resulting from cross-fertilization. Now, a necessary factor in utilizing the heterosis in a practical way by agronomists involves certain pollination controls. In alfalfa, which possesses perfect flowers, containing both male and female parts, this necessitates the rendering of the

male parts of the female parent plant nonfunctional. However, hand-emasculation of the male parts of the very small flowers, a very slow, tedious and expensive process, is not practicable for large scale operations. One way in which this obstacle can be overcome is through the use of male-sterile, female-fertile plants. In the hope that such plants could be found and utilized, an investigation was initiated to determine their presence in our B.C. alfalfa stocks. The first section of this dissertation then is a report upon our search for, and study of, male-sterile lines. (Since two growing seasons only have been available for this study, and, since only one seed set has been obtained as a result, a report on the inheritance of the putative male-sterility in certain lines cannot yet be made.)

While similar investigations are being taken by other interested institutions (in Nebraska, Saskatchewan, Sweden, among others), alfalfa is such an important crop in British Columbia that an attempt to produce hybrid alfalfa would be worthwhile here. Although production in British Columbia is not extensive, alfalfa is nevertheless this province's most important forage crop. This crop is grown in all parts of the province and is the principal hay species. While total acreage figures, although available are inaccurate, an estimate of its importance to the provincial economy can be determined by the following facts. Alfalfa is the prime source of winter hay for the range livestock industry which has an annual production value of $19,000,000. One and one-

half tons of hay are required for each animal unit on the range each winter. In addition, seed production is an important industry in the province, principally in the Peace River area and, to a minor extent, in parts of the Okanagon and Upper Fraser Valleys. There was an estimated seed acreage in 1950 in B.C. of 8,985 acres with a production of 363,000 lbs. clean seed. 700,000 lbs. of seed was produced in 1948 and 220,000 lbs. in 1949. (2)

A second section of this dissertation concerns a disease, first positively identified in B.C. by Brink (17) in 1942, which is now becoming rather destructive to alfalfa in parts of the province. The disease, Witches' Broom of Alfalfa, is attributed by Menzies (63) to a virus pathogen. Very few additional facts, however, respecting the disease are known. Its seriousness, geographical distribution, vectors, or rate of spread are unknown. It is not known, furthermore, whether any natural resistance occurs in alfalfas (Medicago spp.). To obtain much information in respect to these features will require several years of research. Some hope that this disease might be controlled by plant breeding methods is offered by the recent production (49) of an alfalfa resistant to another virus disease("Alfalfa Dwarf") in California. 14 years of research, with ample support, were required to produce this variety. Accordingly, our studies into Alfalfa Witches' Broom disease in B.C. must be of a preliminary nature.

A - THE SELECTION OF MALE-STERILE LINES IN ALFALFA.

INTRODUCTION

Striking advances were obtained in corn breeding by the development of hybrid strains. Inbred lines, developed at considerable cost, were later combined in suitable outcrosses to produce hybrids superior in yield, disease and insect resistance, and uniformity. Advances were so striking that in the U.S.A. alone 90% of the corn acreage is now of hybrid sorts. Acreage has been retired for soybeans and other crops, and yet national corn yields have increased 25%. Uniformity is so marked in hybrid corn that mechanical harvesting techniques are now eminently successful.

Alfalfa breeders, it is not surprising, therefore viewing the progress in hybrid corn breeding have wondered if similar successes might not be realized in alfalfa breeding.

(1) Like corn, alfalfa is heterozygous and open-pollinated, and marked heterosis is demonstrated in hybrids.

(2) In addition, alfalfa, a perennial, can readily be propagated vegetatively, and as such the maintenance and retention of a given genotype is easily accomplished.

(3) Unlike corn, which is monecious, alfalfa flowers are small, hermaphroditic, and gathered in tight racemes.

(4) Unlike corn, alfalfa is insect pollinated.

(5) Unlike corn, in alfalfa only a few seeds are produced per pollination.

(6) Alfalfa is polyploidy in nature which complicates

inheritance, and makes transfer of characters difficult. In addition, there is probably cytoplasmic influences.

The fact that only a few seeds are produced from a single pollination rules out the production of hybrid alfalfa by physical means. As in other plants of similar nature where the advantage of a hybrid program is being investigated (flax, tomatoes, onions, etc.), considerable interest has been drawn to the possibility of avoiding the morphological difficulties inherent in the flower structure by the selection of male-sterile, but female-fertile, lines which are capable of setting seed but not of producing pollen. Such plants, if of agronomic value, would serve as female parents for seed produced by natural crossing in an isolation block.

The obvious necessity of simple inheritance of male-sterility becomes apparent if we want to transmit this character from one line to another. Hope for the existance of such lines has been indicated by Tysdal and Kiesselbach (91), who in 1944 stated "male-sterility, as such, has not been identified in alfalfa, but its possible occurrence and use should not be ignored", and by Armstrong and White (3) in 1935, Tome (89) in 1947 in Argentina, and Clarke and Fryer (27) among other workers. In our work, attention was directed to both male and female sterile lines but the emphasis was directed to male-sterility.

Many difficulties are inherent in alfalfa in working out the inheritance of male-sterility, or of any character. The common alfalfa is shown by Fryer (44a), Ledingham (57),

Nilan (65), Julen (54), Nilsson and Andersson (66) to be a polyploid and as such the inheritance is rarely simple. As Brink, Jones and Albrecht (16) pointed out in discussing Bacterial Wilt disease of alfalfa, a factorial interpretation of the inheritance of disease resistance is at present impossible. Duplicate genes are often involved in inheritance which will obviously add to the difficulties. Atwood (5) wrote that alfalfa is highly heterozygous and as such is using hybrid vigor naturally to a considerable extent. Inbreeding is a very costly procedure in alfalfa, requiring a great amount of tedious and slow hand labour, and the plants soon become weak and sterile, and as such outcrossing may result in very little, if any, seed set. For this reason, and the foregoing ones, a program cannot be translated from corn to alfalfa. However, as Tysdal and Kiesselbach (91) pointed out in 1944 in a crop such as alfalfa, which is perennial and easily propagated by clones, a given genotype can be maintained indefinitely, thus eliminating the need of self-fertilization for its continuance.

In the past few years, prompted to a large extent by Tysdal and associates work (91)(93), attention has become directed to a general assessment of combining ability. This is evidenced by the general theme of such papers as Tome's (89) discussion of alfalfa breeding in Argentina, Bolton's (10), and Hayes' (48).

Due to the foregoing reasons, and especially due to the difficulties inherent in polyploidy, breeders on this

continent have largely given up the idea of the production of hybrid alfalfa. The lack of lines showing complete male-sterility has been responsible as well for the trend away from hybrid seed production.

However, studies at the University of B.C. are being conducted with the realization that the application of the work may be some time away. It is felt that there is a possible use of hybrid vigor even though there are many difficulties. In some respects alfalfa lends itself to a "hybrid" program: it propagates readily by vegetative means. It is felt that in a few years a large proportion of alfalfa seed producing fields will be propagated by cuttings, and as such the interplanting of rows of male-sterile plants with male-fertile plants with a selective harvest could become a method of production of hybrid seed.

REVIEW OF LITERATURE

A. Some general remarks on sterility and incompatibility in higher plants.

(a) Defining sterility and incompatibility.

Sterility is not easily defined as it may take several forms. East (38) in writing of sterility seems to include any phenomenon that results in reduced or no seed set, and considers male-sterility to be a special case of "self-sterility". Armstrong and White (3) write of pollen sterility in alfalfa as "a very definite factor in seed-production - both in determining the pod-setting and in the number of seeds per pod". (Incompatibility?). Crane and

Lawrence (33) draw a distinction between incompatibility and sterility, confining the terms 'self-sterile' and'cross-sterile' to sterility which arises from defective pollen and ovules and other aberrations. They would restrict the term 'incompatibility' to the failure of the pollen to grow down the style and effect fertilization due to some physiological hindrance. They point out that although self-incompatibility is frequently referred to as self-sterility and cross-incompatibility as cross-sterility the terms are misnomers as in incompatibility the pollen and ovules are functional at least on certain plants. Beadle (7) divides sterility into two types: 'impotence' - due to morphological or physiological derangement of the sexual organs resulting in an inability to produce viable germ cells; and, 'incompatibility' wherein normal gametes are formed, but fertilization may be impossible in certain matings while in other matings are normally productive of offspring. Riley (78) in 1932 admits that the terminology employed by various investigators in this field has not been consistent. He writes that the term 'self-sterility', a much older term than 'self-incompatibility' or 'self-parasterility', is applied to the inability of an hermaphrodite with functional gametes to self-fertilize when there are no mechanical barriers to prevent self-pollination. He thinks that there should be no confusion between the use of 'self-sterility' and true 'sterility' as the prefix 'self' qualifies the expression and differentiates between the two.

For the purposes of this essay, I will restrict the term 'sterility' to denote those cases where pollen (or

female gametophyte) is incapable of fertilizing (or being fertilized) in any case; and the term "incompatibility" to denote those cases where fertilization is prevented by genetical differences between normal functional pollen and normal functional ovules and styles.

(b) Causes of sterility

Sterility in plants, as has previously been noted, may stem from a variety of causes and it is not surprising that many classifications have been proposed to aid in its description.

Sterility is classified by Crane and Lawrence (33) into a) "Generational sterility, due to the failure of any of the processes concerned with the normal alternation of generations, namely, development of pollen, embryo-sac, embryo, and endosperm, and the relations of these to one another and their parents, regardless of the cross made, and b) morphological sterility due to suppression or abortion of the sex organs". Dohbzansky (36), on the other hand, classes sterility into genic and chromosomal sterility, within species and within species hybrids. He defines "genic sterility" as sterility due to changes in the individual genes of the organism, and not due to dissimilarities in the gross structure of the chromosomes; and, "chromosomal sterility" due to structural dissimilarities of the chromosome bivalents. Genic sterility in his sense rarely involves any interference with the chromosomal mechanism as shown by the fact that meiotic chromosome pairing and subsequent divisions may occur before any disturbances leading to sterility are noted. Some genic

sterility may, however, involve failure of pairing of homologous chromosomes (ref. "synaptic" gene mutation in corn). Chromosomal sterility, in Dohbzansky's (ibid) usage, has at its base, translocations, inversions, deletions, and other gross morphological changes in the chromosomes. Another method of classifying sterility, according to Dobzhansky (ibid), (attributable to Federly and Renner and to Muntzing), divides sterility into gametic (haplontic) and zygotic (diplontic). The former refers to sterility of pollen or female gametophyte (or for all practical purposes, the unfertilized egg) and the latter to failure of the zygote to grow after fertilization has been accomplished.

Whatever the classification of the causes of sterility, many phenomena may be responsible for the sterility of an organism. Dealing first with the male parts of a flower, breakdowns resulting in sterility may occur at any stage of the life history. Anthers may be replaced by petals thus rendering the plant male-sterile (very common in horticultural flowers, such as the double geranium, or the chrysanthemum). Failure of the anthers to develop whatsoever may occur (73)(88)(90); (It is probable that many plants recorded as gynodioecious, as for example many Labiate, are in effect species in which a certain population of individuals show male-sterility of this type). Anthers may develop partially and then abort entirely (82)(83). Anthers may develop but produce no pollen through failures of reduction-division (4)(7)(9)(40)(42)(43)(44)(45)(72)(73); or failures in meiotic activity due to sticky genes (42, 43), asynaptic genes (73), chromo-

somal rearrangements (43). Pollen grains may be affected before the thickening of the wall, or at the beginning of thickening, or after thickening but before the first division. Partial or complete sterility may be caused by translocations, inversions, deletions, duplications etc. The production of sex cells carrying abnormal gene complements in individuals heterozygous for various chromosomal aberrations leads to a certain degree of sterility. Non-disjunction of chromosomes may lead to partial sterility.

Another irregularity in meiosis may arise from autopolyploidy: mechanical difficulties may be set up to pairing of chromosomes due to excess of "choice" of partners.

Although anthers may develop and pollen be produced, the anthers may fail to dehisce and thus the plant may remain male-sterile (4, 9, 81). Pollen may be produced but due to some innate characteristic it will not germinate (59). (Dwyer (37) in 1932 in an extensive test in Australia found that only 80% of normal pollen germinated). Pollen may not germinate on stigmas if proper moisture conditions are not met (62). Pollen may be fully viable but not be able to germinate due to the stigmatic membrane being too well developed (3) or unruptured (60). The stigmatic membrane may be ruptured but the pollen-tube may fail to grow down the style (38, 39); or, if grown down the style fail to reach the ovary (38, 39, 3, 15, 31, 50, 60 etc); or bypass the ovary (31), or fail to fertilize any ovules (31) or fail to fertilize the basal ovules of the ovary (31, 13) resulting in partial sterility. The ovules themselves may be non-functional and even if the

pollen-tube reaches the ovule, fertilization cannot be effected.

Many of the irregularities of meiosis affecting the male-gametophyte may also affect, but not necessarily, the female gametophyte development.

Ovules may remain infertile even though the pollen-tubes are present and the generative nucleus of the pollen tube and the egg cell of the ovule are in conjugation (36). Even if fertilization is effected the ovules may abort. Collapse of fertilized ovules, or somatoplastic sterility, may occur following abnormal growth of the somatic tissues adjacent to the embryo sac (12, 13, 14).

All of the foregoing may occur in the ordinary diploid 2N plants. In triploids, pentaploids or other unbalanced polyploids the picture is somewhat more complicated. Sterility may be due to unbalance in chromosome complements as an odd number cannot divide evenly.

(c) Incompatibility.

There has been a great amount of literature published in recent years on incompatibility in plants, much of which has been discussed under the heading of sterility. The following review highlights some of the notable literature.

Jones (50) credits Kolreuter with reporting the first case of incompatibility in 1764 in Verbascum. Since that time many instances have been recorded. Jones (ibid) in 1928 estimated that about 100 different species scattered among 50 plant families show incompatibility. Crane and Lawrence (34) in 1934 state that sexual incompatibility is wide-spread

throughout the plant kingdom; a view substantiated by many authors.

The best known, and most satisfactory explanation of incompatibility was presented by East (39) with his oppositional-factor hypothesis. By this postulation, incompatibility is determined by genes usually designated by the letter S and commonly forming a multiple allelomorphic series: S_1, S_2, ...S_{15}, any 2 of which may be carried by a given plant. Pollen cannot function, or shows very little growth in the style of a plant carrying the same incompatibility factors as the pollen; and normal pollen tube growth occurs in stylar tissue carrying a different genetic factor for self-incompatibility. There was also discovered by East (ibid) a self-fertility factor, Sf, functional with any of the above series and dominant in crosses.

Riley (78) in 1932, working with the genus Capsella at Princeton University found that the inheritance of self-sterility in C. grandiflora could not be explained by the theory of oppositional factors. This species is completely self-sterile, and had remained so through 18 generations. The F_1 from a cross between 3 other self-fertile species and the self-sterile C. grandiflora were all fertile; the F_2 split into 3 self-fertiles to one self-sterile. 1/3 of the self-fertiles breed true to self-fertility; while the other 2/3 split into a 3:1 ratio of fertiles to steriles. All the self-sterile segregates of any F_2 family are cross-sterile among themselves. Riley (ibid) in 1932 attributed self-

sterility to a gene simply recessive to self-fertility. In later papers (79, 80) in 1934 and 1936, he explained this self-sterility on the basis of the sporophytic nature of the parent plants and on the interaction of two pairs of genes, a dominant gene T which is epistatic to S^c and their recessive homologues.

The incompatibility reaction can be modified by several factors. Crane and Lawrence (34) state that the incompatibility reaction is greatly affected within a species by temperature. Higher temperature increases the growth rate of compatible tubes whereas it decreases the growth rate of incompatible tubes. This shows that the incompatibility reaction is due to a positive chemical reaction between the pollen and the style, and not merely due to a lack of some growth substance. Self-pollinations of flowers late in the season of growth and also of flowers in the bud stage in certain incompatible forms of Nicotiania has resulted in some seed set. Such seeds give rise to plants either of the same genetic constitution as the parent or homozygous plants. The former fail reciprocally with the parent. The latter are effective when used as females, but fail when used as males in pollinations with the parent. This phenomenon is known as "pseudo-fertility" or"end-season" fertility.

Crane and Lawrence (ibid) further point out that the incompatibility reaction can be modified by autopolyploidy. In tetraploids of pear, Petunia, and Oenothera a breakdown of incompatibility has been found to be due to the competitive

interaction of two different S alleles in the same pollen grain. S_1S_2 pollen is compatible, while S_1S_1 and S_2S_2 pollen is incompatible in a $S_1S_1S_2S_2$ style. However this may break down if one allele is dominant over the other.

Brink and Cooper have published at least 4 papers (12, 13, 14, 31) dealing with self-incompatibility and somatoplastic sterility in alfalfa and other plants. They report that self-incompatibility in alfalfa is only partial and varies considerably from plant to plant. They are of the opinion that ovule abortion or somatoplastic sterility may be unrelated to self-incompatibility and may vary independently of it. Following self-fertilization they found that one of three things may happen: pollen tubes frequently fail to reach the basal ovules, many ovules remain infertile even though pollen tubes are present, and the abortion of fertile ovules are of common occurrence. Crossing was found to raise the proportion of ovules becoming fertile from 15% to 65%, and to reduce the abortion of fertile ovules from 34% to 7%. The higher ovule fertility results from more extensive growth of the pollen tubes within the cavity of the ovary and an increased tendency for pollen tubes which reach the ovules to enter the micropyles and accomplish fertilization. There appears to be no correlation between the percentage of the ovules becoming fertile after self-fertilization and the frequency of fertile ovules collapsing in the same individual.

Cooper (30) described the normal embryology of alfalfa. An ovary of alfalfa contains 10-12 ovules arranged alternately

along the ventral suture in serial order. The necessary condition for the development of the seed is that fertilization of the egg within the ovule occurs. A large disparity in the number of seed which develop after self and cross-fertilization is caused by the difference in the proportion of ovules that become fertile following the 2 types of matings. Cooper and Albrecht (32) studied, histologically, alfalfa pistils following self- and cross-pollination and found a declining gradient in fertility of ovules from the apex to the base of the ovary. In selfed series about 1/3 of the ovules in the top position at the apex of the ovary became fertile with the frequency declining to zero towards the base. In the crossed series the fertility gradient is somewhat equal to this, although the proportion of ovules becoming fertile in each position is a little higher than after selfing - 85% of the ovules in position 1 down to approximately 33% at the base.

Brink and Cooper (ibid) also described somatoplastic sterility in which a high percentage of fertile ovules fail to develop into mature seeds: the ovules collapse frequently during the early stages of post-fertilization particularly after self-pollination. This collapse follows abnormal growth of the somatic tissue adjacent to the embryo sac. Shortly after fertilization active cell division is initiated in the integuments as well as in the endosperm mother cell and the zygote. The critical factor for survival in this stage is the manner in which translocated food is shared between the

endosperm and the integument and this partition depends on the rate of growth or cell division inside and outside the embryo sac. The endosperm and embryo of hybridized ovules grows very quickly and can compete with the integuments for food supply; but the selfed ovules, growing slower, fail in competition and the balance shifts in favor of the integuments. Growth may continue for several hours, using breakdown of the endosperm cells as nutrients, before collapse. The chalazal portion of the endosperm is ruptured, the cells of the embryo become starved, and is followed by a collapse, with the resultant abortion of the remaining endosperm and embryo.

B. Male-sterility in the higher plants.

(a) Some general remarks.

A literature search of recent work on male-sterility in plants brings to light 3 main modes of inheritance of this character. Male-sterility may be governed by a) one or a few recessive Mendelian factors, or b) by dominant factors, and c) by cytoplasmic factors in addition to Mendelian factors. In some cases, the phenomenon is reported but the mode of inheritance is unknown at present. Cytoplasmic male-sterility was reported, apparently erroneously, by Rick (75) in 1948, and by Lewis (61) in 1941, in light of present day findings, to be much less common in cultivated plants than genic male-sterility.

Lewis (ibid) writing in 1941 on male-sterility in natural populations of hermaphrodite plants, stated that male sterility due to a recessive gene is common as a mutant in inbred plants.

Single examples are reported by Lewis (ibid) in Antirrhinum, Capsella spp., Lathyrus odoratus, Lycopersicum esculentum, Oryza sativa, and Rubus idaeus. More than 15 types of male-sterility were known in Zea mays at that time, all being due to the segregation of a single recessive gene. He reported two other cases of male-sterility in maize due to a single dominant gene, but which since have been shown to be in reality due to an interaction of cytoplasmic factors and Mendelian factors. In 1931, male-sterility had not been reported in wild populations of these plants, or in species naturally polymorphic for male-sterility. In all the gyno-dioecious species fully investigated, according to Lewis (ibid) male-sterility is inherited through the cytoplasm.

(b) Male-sterility governed by one or a few recessive Mendelian factors. (If male-sterility is inherited as a simple recessive, inbreeding would favor the production of the phenomenon.)

Bohn and Whitaker (9) in 1949 writing on the male-sterility in andromonecious muskmellon report that staminate flowers from male-sterile plants are normal in size and shape of all parts except the anthers. These are small, fail to dehisce and contain empty microspore walls. In the male-sterile plants meiosis is apparently normal and follows the usual steps up to the formation of the tetrads; at this stage the development ceases and the contents of the young pollen grains disintegrate. The units of the tetrad remain cemented together. They conclude that in muskmellon male-sterility

is diplontic and is governed by a single recessive gene.

Crane (33) in 1915 is credited with the first report of male-sterility in the tomato. He found that male-sterility behaved as a recessive character and believed that it depended on a mutant gene.

Rick (74) in 1948, reported on a search conducted for male-sterile tomato mutants, potentially useful in producing F_1 hybrid seed and in cross-breeding. Among 150 unfruitful plants, 12 were found to be genetically male-sterile. Breeding results indicate that the male-sterility of each mutant is determined by a single recessive gene. One gene ms_5 was recovered 4 times and 8 other non-allelic genes ms_6 to ms_{13} were demonstrated. Only one mutant produced any pollen that was functional, and by using this pollen in self-fertilization pure breeding male-sterile populations for this gene was obtained.

Rick in a previous paper (73) in 1945, reported on a survey of cytogenetic causes of unfruitfulness in the tomato. Gametic sterility is largely responsible for the unfruitfulness of 66 plants discovered in approximately 55,000 field plants of 3 tomato varieties. Failure of normal gamete formation was resolved to a cytogenetic basis in every case investigated. Rick (ibid) found in the 14 diploids discovered in this population that 3 plants were aberrant in gross morphology. Sterility of these plants is probably conditioned either by a pleiotropic effect of the gene determining the deformity, or by the deficiency of a chromosomal segment

which includes a gene affecting the morphological character and another affecting sterility. Rick (ibid) found in 3 other plants that the male-sterility is determined by a single recessive gene. 5 plants showed complete pollen and ovule sterility resulting in one case from asynaptic meiosis.

Roever (81) in 1948, writing on a promising type of male-sterility for use in hybrid tomato seed production, reports a mutant in which natural selfing was prevented because the anthers failed to dehisce. The mutant can be selfed by hand but does not self in the field. He concludes that the character appears to behave as a simple recessive.

Lesley and Lesley (59) working at Riverside, California, reported in 1939 on male-sterility in the tomato. They found that male-sterility is completely recessive and depends on at least 2 recessive genes, ms_1 and ms_2, both of which are necessary for male-sterility. Male-sterile plants are homozygous for both of these recessive characters and produced non-viable pollen. Segregation of sterile and viable pollen was accomplished by means of germination tests in van Tieghem cells using 15% cane sugar media plus ground up stigmas.

Scott and Riner (82) in 1946, report on the inheritance of male-sterility in winter squash in which the male flowers produce no pollen. No differences were observed in the size or shape of the male-sterile versus the normal flower, but there is a marked difference in the appearance of the androecium of the male-sterile and normal flowers. In the male-sterile flowers the androecium aborts in the bud stage

before the staminate flowers open so that no pollen is produced. The factor for male-sterility is inherited as a simple recessive. In the backcross progenies a 1 : 1 segregation for male-sterile: male-fertile flowers was observed. In the F_2 progenies a 3:1 segregation occurred. Male-sterile plants were fully female-fertile, producing abundant seed and fruit when foreign pollen is added.

Beadle (7) reported in 1932 on a study of 15 genes for pollen sterility in maize. In all cases development of megaspores and female gametophytes was normal. Cytologically, the male-sterility was characterized by degeneration of the microsporocytes or of the microspore cells. The time of degeneration varied with the different male-steriles ranging from the 'synizetic' stages of meiosis almost to pollen maturity. The ms genes were recessive and non-allelomorphic.

In a previous paper in 1921, Eryster (41) states that recessive Mendelian genes are the cause of male-sterility in maize.

Emerson, Beadle and Fraser (40) in 1935 reported 20 genes had been described to date for male-sterility in maize. All of these gene-steriles are or were independent recessives, each capable of bringing about either complete or a high degree of sterility in the male inflorescence. Meiosis occurs normally and the pollen grains are formed but before anthesis these grains abort.

Burnham (20) reporting on cytogenetic studies of a case of pollen abortion in maize in 1941 found a gene for pollen abortion (pa) in an established inbred line. Plants hetero-

zygous for this gene had semi-sterile pollen but normal ears. The gene was lethal, or nearly so, to pollen carrying it; but had no lethal effect on the ovules. As a result pa is transmitted mainly, if not entirely, through the ovules, and is located on the first linkage group.

Shifriss (83) writing in 1945 on male-sterilities in cucurbits, found 3 lines among 200 one-generation inbreds of Cucurbita pepo L. which segregated for male-sterile plants. F_1, F_2 and backcross populations showed that the male-sterility is inherited as a recessive character whose expression is due to a single gene. This morphological male-sterility is expressed in complete abortion of the androecium at the bud stage before the staminate flowers open.

Gregory (45) reported in 1905 on the abortive development of the pollen in certain sweet peas whose anthers were contabescent. These peas were produced by self-fertilization by Bateson. Sterility was found to be correlated with a somatic character, the sterile plants possessing a green leaf axil, while fertile plants had red axils. A cytological investigation revealed that the whole nucleus showed irregularities. Reduction-division followed the normal course, and young pollen grains were formed. In the contabescent anthers, growth ceases at this point, the pollen grains remain small and enclosed within the walls of the pollen mother-cells. Development of spindle fibres in the cytoplasm was weak or absent. Gregory (ibid) believed the male-sterility character was inherited as a simple recessive.

Punnett (70)(71), working with Gregory's (ibid) plants, showed that genetically the female gametogenesis of the male-sterile sweet pea plants was normal, and that sterility of the pollen was complete. He also demonstrated that the gene for sterile anthers was inherited as a simple recessive.

In 1937, Faberge (42) at the John Innes Horticultural Institution reported the results of a detailed cytological and genetical study of male-sterility in Lathyrus odoratus. The recessive gene for male-sterility was linked with light axil color. On a cytological basis he found that the following occurred:

(1) Only occassionally a metaphase plate of normal aspect was formed; generally the chromosomes in meiosis remained scattered in cells.

(2) Very few second divisions of meiosis were seen. Of those that occurred, many had cell walls extending only 2/3 of the distance across the cell. He also found that often more than 4 chambers were partitioned off with some chambers being left without any nuclear material whatsoever.

(3) Meiosis may be arrested by a change in the colloidal state of the cytoplasm as early as diakenèsis or as late as second anaphase. The cytoplasm became granular very rapidly arresting meiotic activity.

Shull (84) in 1927 reported on finding pollen-sterility in crosses between species of shepherd's purse. The male-sterility in the interspecific Capsella hybrid was found to be simply recessive to male-fertility.

Suneson (88) reported in 1940 on a 4 year test that he

performed on a male-sterile mutant of barley. Results of F_1 and F_2 showed that the mutant was male-sterile; and that the male-sterility was inherited as a simple recessive. Anthers remained shrunken, rudimentary while pistillate parts were normal.

(c) Male-sterility governed by a single dominant gene.

Rife (76) studying the genetics of cerain common variations in Coleus, found four genes at approximately the same chromosome locus which produce varying combinations of deep versus shallow lobed leaves and male-sterile versus male-fertile flowers; both deep lobes and male-sterility being dominant.

In a later paper, Rife (77) points out that male-sterility (D) and deep lobes (L) are closely linked and both dominant.

(d) Male-sterility governed by cytoplasmic factors in addition to Mendelian factors. To explain the observed data on certain types of male-sterility both cytoplasmic factors and Mendelian factors are required.

Owen (67) states that if a character is to explained by Mendelian, or genic inheritance the male and female parents both influence the characteristics of the offspring, and populations obtained from reciprocal crosses (excepting sex-linked characters) should be identical. If a character, on the other hand, is to be explained by cytoplasmic, or maternal inheritance, the male parent should have no influence on the characteristics of the offspring; and populations obtained from reciprocal crosses should not be identical. If cyto-

plasmic and genic inheritance are both involved, the results are more complicated. The female parent should, in this case, exert more influence than the male parent in the characteristics of the offspring, and populations obtained from reciprocal crosses should not be identical.

Jones (50) in 1950, proposes as new terminology the term "chromogenes" to designate genes in the chromosomes, and the term "plasmagenes" to designate the determiners in the cytoplasm. Pollen abortion is brought about by chromogenes and also plasmagenes, both of which are variable in their control of pollen production. Plasmagenes that condition pollen sterility have no other effect upon the growth and structural details of the plants. Plasmagenes and chromogenes for pollen sterility, when working together in the same plants, apparently have no effect on each other and are independent in their action.

Rhoades in 1931 and 1933 (72)(73) studying male-sterility in Zea mays concluded that pollen sterility is inherited through the maternal cytoplasm. Rhoades (ibid) reported that the egg cytoplasm plays the chief role in the expression of male-sterility. There was no transmission of the character through the pollen of partially sterile plants. Cytologically, microsporogenesis is normal with degeneration of pollen usually occurring before the first vegetative division. There is a pronounced difference between the cytoplasmic elements of microspores in normal races and those of the male-sterile lines.

Josephson and Jenkins (53) reported in 1948 on male-sterility in corn hybrids. Seeking an explanation for seriously low seed set in a number of fields of white hybrid corn, male-sterility was found to be the primary cause of the difficulty. Examination of the tassels during pollen shedding time revealed the presence of male-sterile plants. High sterility resulted only when the hybrids had cytoplasmic contributions from one strain. Sterility was also found to be influenced by certain contributions from the male parent, a minimum of 2 genes apparently being required. The authors point out that the expression of sterility is subject to environmental influence.

Owen (67) working with male-sterility in sugar-beets in 1945 found that several pairs of Mendelian factors may influence pollen development when carried by plants with sterile cytoplasm, but same factors have no effect when carried by plants with normal cytoplasm. Complete male-sterility was characterized by empty white anthers. Owen concluded that in sugar-beets, male-sterility is produced by combined cytoplasmic and genic inheritance.

Artschwager (4) reported in 1947 on the results of a cytological investigation into the cause of pollen abortion in Owen's (ibid) plants. He observed that when male-sterility in sugar-beets is cytoplasmically inherited, completely male-sterile plants bear white, empty anthers. Normal pollen mother cells and normal microspores are produced, but the microspores fail to develop fully and disintegrate by the time the flowers

open. In the semi-male-sterile types, small, non-viable pollen grains are formed but the anthers do not dehisce. Owen (ibid) had observed that sometimes viable pollen is produced by some branches of the inflorescence, and occasionally white anthers and yellow ones are borne within the same flower.

Pollen abortion, especially in hybrids of plants with different chromosome numbers, usually is the result of abnormal meiotic divisions; in sugar-beets, however, Artschwager (ibid) observed a different type of pollen degeneration in which the anther tapetum, through the development of a plasmodium, plays an important role. He found that pollen abortion in anthers of sugar-beets with cytoplasmically inherited male-sterility is associated with either a periplasmodium or a cellular tapetum - both of which may occur in a flower cluster but not within a single flower. The plasmodium's presence somewhat delays pollen abortion; but where the tapetum remains cellular some microspores are destroyed while still in tetrads. He could not offer an explanation of the cause of the development of the periplasmodium which is restricted to sugar-beets exhibiting cytoplasmic male-sterility. Cytology of the young plasmodium suggests hypermetabolic activity to the detriment of the developing microspores.

Jones and Clarke (52) studied the inheritance of male-sterility in the onion with the hope of the production of hybrid seed. The male-sterile onions produced no viable pollen but set seed readily when hybridized with pollen

from male-fertile plants. They found 3 types of inheritance which they explained by assuming that the male-sterile condition results from an interaction between a recessive gene and a non-nuclear or cytoplasmic factor,. All plants with normal cytoplasm (N) produce viable seed. All male-sterile plants possess the sterile type of cytoplasm (S). No light was shed on the nature of the cytoplasmic factor which differs in the 2 types. A gene for male-sterility (ms) also influences pollen development when carried by plants with S cytoplasm but has no effect when carried by plants with N cytoplasm.

Clarke and Pollard (28) found in 1949 that male-sterility in the onion was not complete and that the amount of selfing varies largely from one male-sterile plant to another, but the average of selfing for all lines tested was 4.1%.

Fineman (43) found complete pollen sterility in the potato to be a result of the failure of normal microspore formation during meiosis. This view is supported by numerous meiotic irregularities she observed in sterile-pollen plants. The most common meiotic irregularities she observed were: (1) filure of the chromosomes to pair, (2) lagging of the chromosomes on the spindle and (3) failure to complete the normal reduction-division process. Partial sterility of the fertile plants is conditioned, after what appears to be a normal meiosis, by microspore abortion, which frequently occurs after the spores have reached normal pollen grain size. Fineman (ibid) concluded that female parents transmit more pollen sterility than male parents.

Bateson and Gairdner (6) in 1921 crossed common tall and a procumbent flax and found normal and male-sterile individuals in a 3:1 ratio in the F_2. Sterile plants were obtained only when the procumbent race was used as a female parent. They attributed the results to "anisogeny".

Chittenden and Pellew (26) offered a suggested interpretation of Bateson and Gairdner's (ibid) "anisogeny". They interpreted the sterility as due to the interaction of a specific gene in the cytoplasm of the procumbent race. In another paper (25) Chittenden in 1927 wrote that a single gene apparently determines male-sterility with one type of cytoplasm (procumbent) but not with another (erect).

In 1932, East (38) found that certain S factors from Nicotiania sanderae produced male-sterility in the presence of cytoplasm derived from N. langsdorfii but produced viable pollen in the presence of N. sanderae cytoplasm. Male-sterile plants produced no pollen at all. He found that the ratio of self-fertiles to male-steriles was 266:232.

(e) Male-sterility reported, but mode of inheritance unknown at present.

Welch and Grimball (90) discovered a male-sterile but female-fertile carrot in 1947. Male-sterility consisted of anthers being shrivelled and brown in color before any petals unfolded. Hybrid seed produced on male-sterile plant had F_1 plants that were classified 37 male-sterile and 15 normal.

Frankel (44) working in New Zealand, observed male-

sterility with female-fertility in nature in 8 forms of the genus Hebe, comprising 5 species and 1 species-hybrid. As Hebe only flowers every 2 or 3 years it is unsuited to the study of male-sterility inheritance. However, Frankel (ibid) concludes that the male-sterility is due to genetic causes and not to environmental causes as the phenomenon is regular from year to year and in a new environment after transplanting. A cytological study of the breakdown of the pollen showed degeneration occurred rapidly and with regularity, either in:

(1) Pachytene of prophase - up to this meiosis is normal. Diplotene loops are not formed. "The pachytene threads coagulate individually, approximately to the size of diakenesis bivalents, and collapse into an amorphous mass which is rapidly dissolved". Subsequently the empty cell walls collapse, remaining in this state in the anther cavity. Tapetum cells degenerate simultaneously.

(2) Tetrad degeneration - all meiotic stages up to second telophase are normal. Tetrads are formed but collapse rapidly. No pollen grains seen.

(3) Pollen degeneration -

a) Tetrad cells round off, and about a day later, not having undergone a change either in diameter or in wall structure, the pollen grains shrivel, collapse, and gradually disintegrate. The process is regular and rapid and occurs with concurrent degeneration of tapetal walls of the pollen mother cells.

b) Pollen grains formed, increase in size and cell walls

thickened; immediately followed by setting in of shrivelling leading rapidly to a collapse of pollen grains and tapetum.

c) Pollen grains formed, partially collapse and remain without any further degeneration up to the opening of the flowers. However, the anthers fail to dehisce.

C. Observations relative to possible male-sterility in alfalfa.

Several notable review articles on alfalfa breeding and its inherent difficulties have been published in recent years. Extensive papers by White (97), Atwood (5), and Tysdal and Kiesselbach (92) cover the field very completely. It is not the purpose of this thesis to attempt a summary repeating this work. However, several peculiarities of the alfalfa seed producing mechanism, which may complicate a study of male-sterility, are discussed briefly in this paper.

(a) Self- versus Cross-fertilization in alfalfa.

Alfalfa is normally a cross-fertilized crop, although self-fertilization may also occur except where limited by self-incompatibility. The amount of natural cross-pollination in alfalfa has been studied by several investigators. Tysdal and Kiesselbach (91) report that 89% of the seed produced in Nebraska resulted from cross-fertilization; Knowles (56) in Saskatchewan reported 94.2%, Burkart (18) found 84.5% crossing in Argentina, and Hadfield and Calder (46) reported 44% in New Zealand.

Cross-pollination produces a much higher average number of seeds per flower pollinated than self-fertilization; in fact, Cooper and Brink (31) report a net fertility 6 times

as high per flower pollinated after crossing as after selfing. Tysdal and Kiesselbach (91) state that a consideration of self-fertility relationships in alfalfa is of paramount importance, and plant breeders should select highly self-sterile plants. Not only are more pods set following cross- than self-fertilization but more seeds are formed per pod. This is borne out by many workers: Lesins (60) in Sweden, Armstrong and White (3) in Canada, Hadfield and Calder (46) in New Zealand, Tysdal and associates (91, 92), Carlson (22), Cooper and Brink (31, 32) among others in the U.S.A. Self-fertilization not only results in a marked decrease in seed production, but also to a lesser degree in vegetative vigour (46), whereas cross-fertilization results in a measure of heterosis. Hadfield and Calder (ibid) found that 65 seeds were formed per 100 florets tripped and open-pollinated, and when the florets hand-tripped and the plants covered 43.0 seeds were formed per 100 florets tripped. Piper and associates (69) in a series of experiments in 1912-1914 observed that total seed production and number of seeds per pod was more than doubled on open-pollination over self-pollination. (If 100% crossing were then possible, alfalfa seed production would be increased by 50 - 100%.

(b) Is tripping necessary for alfalfa seed production?

A morphological peculiarity of alfalfa flowers that further complicates the picture of sterility is the necessity of "tripping" of the flower before fertilization can be effected. According to Coffman (29), DeCandolbe (35) in 1832

gave the first explanation of the tripping process in which he stated that the explosion of the flower takes place when a certain stage of its maturity is reached. Hildebrand (again quoting Coffman(ibid)) believed as early as 1866 that fertilization may take place in untripped flowers. Brink and Cooper (15) observed pollen germinating within untripped flowers and pollen tubes entering the styles even in the late bud stage. Carlson (22) found 27% of the flowers producing seed without tripping in Utah in 1930. Hay (47) in 1925 found that 5.9% of untripped flowers set seed. On the other hand, Armstrong and White (3) write that pollen tubes only penetrate the stigma when the stigmatic surface is ruptured. However, they did find that 0.6% of the untripped flowers showed pollen germination. They observed a thin membrane covering the stigmatic surface which is sufficiently thick and impermeable to prevent penetration of pollen tubes and which, if unruptured, will prevent growth of pollen tubes down the style. Stigma scarification, and rupture of this membrane, with the release of the stigmatic content which initiates pollen germination, is normally achieved when the staminal column is released from the keel or "tripped" striking the standard with considerable force. Lesins (60) confirms this theory with observations of the stigmatic membrane, and reports the presence in one case of a broken membrane in an untripped flower.

Hadfield and Calder (46) write that tripping is a prerequisite to seed production. Blinn (8) in Colorado reporting on the results of an experiment on "tripping" wrote

in 1920 that "there was no clear evidence that bees or other insects were essential to alfalfa seed production. Fertilization can and does take place without insects", and that tripping is not necessary for seed production. Brand and Westgate (11) in 1909 stated that pollination is not effective until flowers are tripped. Burkill (19) wrote that alfalfa pollen was shed in the bud stages, to which Coffman (ibid) agrees and adds that tripping is not necessary for seed production.

The question has aroused many papers and is still unanswered. It appears to this writer that the presence or absence of a stigmatic membrane may rest upon a genetic factor or upon a physiological response. The presence or absence of this membrane appears to be the determining factor in whether or not pollen can fertilize the ovules or grow in the styles whether tripped or not tripped.

The tripping of flowers is normally performed by bees, and Peck and Bolton (68) in 1946 showed that the native bee population was often the limiting factor in alfalfa seed production in Northern Saskatchewan and Alberta. This view although contrary to that of Blinn (ibid), is generally agreed upon by the scientists working in this field. An idea of the importance attached to the role of insects in relation to tripping and seed setting in alfalfa, can be gleaned by the report of the Twefth Alfalfa Improvement Conference (1) wherein 9 out of the total of 23 papers read dealt with this phase of seed production.

Dwyer (37) in 1932 reported instantaneous tripping of

all flowers at 104°F under most diverse moisture conditions providing the flowers are in a fresh turgid condition. Armstrong and White (3) state that the controlling factor appears to be duration and intensity of hours of sunshine. Dwyer (ibid) also believed wind to be an important agent of pollen dissemination and insects to play a minor role in pollination. However, this view is certainly not agreed to by the majority of workers.

Kirk (55) reported on the finding of an autogamous alfalfa which is self-tripping. Automatic tripping of autogamous alfalfas occur when "the dynamic force present in the staminal column is sufficiently greater than the static force present in the keel to cause the tripping mechanism to explode simultaneously" to quote Armstrong and White (3). Southworth (85) attempted to raise a high seed producing line by hybridizing M. lupulina (self-tripping) with M. sativa (not self-tripping). He hoped to produce a self-tripping high seed setting alfalfa, and achieved a considerable measure of success in experiments running over 20 years. However, use of highly self-fertile, self-tripping lines is undesirable in developing synthetics, because the seed yields from open progenies from such lines is similar to the seed yields from their selfed progenies (87).

In the past few years the utilization of hybrid vigour to obtain maximum productivity of the concerned plants has assumed importance to many scientists. As Bohn and Whitaker (9) point out, male-sterility enables seedsmen to produce

hybrid seed comparatively easily and cheaply.

Clarke and Fryer (27) writing on seed setting of alfalfa in 1930 found that many plants produced high percentages of sterile (empty) pollen grains. The percent varied among plants but remained constant for particular plants even when the pollen was produced under different conditions. In 1929 counts were obtained from a number of alfalfa plants which were setting practically no seed under field conditions: empty grains ranged from 50 to 90%. However, plants from a normal field contained from 3 to 48% sterile pollen.

Armstrong and White (ibid) add to this in 1935 that plants having a high percent of sterile pollen were deficient in amount of pollen as well; a high proportion of shrunken pollen grains seems "associated with faulty dehiscence of the anthers".

Tysdal and Kiesselbach (ibid)(91) stated in 1944 that male sterility had not been identified in alfalfa, but that its possible use and occurrence should not be ignored.

STUDIES IN FIELD AND LABORATORY.

A - Material and methods (a) Pollen studies

During the summer of 1949 investigations were begun to determine the presence of male-sterile lines of alfalfa at the University of British Columbia farm.

A microscopic examination of the pollen, produced by the 910 clonal lines of Rhizoma alfalfa maintained by the Dep't. of Agronomy, was carried out. Several racemes representative of these lines were clipped while in a fresh turgid state and carried, with their excised ends in fresh water to the labor-

atory. Here 3 fresh florets were selected at random from each sample and tripped on a microscopic slide. The pollen was mounted in mineral oil and cover slips affixed. The slides were examined under the 125x power of a Reichert research binocular-microscope for quantity and quality of pollen grains. Several samples were observed of the lines at either ends of the quality distribution curve.

Following the method outlined by Burton(21) in 1944, the pollen was assessed a numerical value of 1 to 5 both for quantity and quality. A production of very few pollen grains received a numerical rating of 1; large quantities of pollen was assessed a numerical rating of 5. In the case of quality, a numerical rating of 5 represented pollen of uniform large s size and proper shape with an absence of micro-pollen or shrivelled dried-up pollen. (Normal proper shape is rounded, semi-hyaline and finely pitted.) A value of 1 represented pollen that was shrunken, shrivelled or micro-pollen.

44 clonal lines produced no flowers during the summer so could not be categorized. On the basis of the first examination, 44 lines were rated 5 for quality of pollen; and 5 lines were tentatively designated as "sterile" as all pollen that was produced was either shrivelled or amorphic. New slides were made up for these 49 lines, and the pollen was recategorized. This time, 20 of the 44 lines rated 5, were again rated 5 both for quantity of pollen and quality of pollen. One of the so-called sterile lines, showed a production of pollen, so was given a higher classification. The

remaining 4 "sterile" lines showed on all observations, in the cases of any pollen production at all, only shrunken, shrivelled pollen. Table I shows the categorization at this date.

2 clonal cuttings of each of the 20 lines designated as "high pollen producing lines" (See Tables I and III), and 5 cuttings of each of the 4 lines designated "sterile" (See Tables I and II) were made, and planted in the greenhouse November 4 and October 31, 1949 respectively.

The cuttings grew well in the greenhouse and through the use of artificial light were "forced" into flower in April, 1950. Pollen from the different lines was again examined under 125x with results comparable to the previous summer's observations. (See Table I). None of the lines designated as "male-sterile" produced viable pollen.

The plants were transplanted from the greenhouse to the Alfalfa Nursery plots in May 1950. Pollen was examined on the 18th of September, 1950, under 125x, with results as shown in Table I.

A pollen examination of the following varieties of alfalfa, contained in the Alfalfa Nursery plots at U.B.C., was also undertaken in 1949 and 1950 for the presence of male-sterility. In 1949 Viking, Ranger, Grimm, Ladak, Ferax, Buffalo, Don (creeping M. falcata), and M. lupulina pollen was examined along with pollen from 4 strains of Rhizoma: H71P27, H7, H68, H190, which were not included in the previous pollen examination. In September 1950 an examination

of the pollen from the above plants was conducted plus the following lines: Grimm Summerland S274, Grimm Vidarshov, Grimm Saskatchewan 666, Grimm Saskatchewan 451, erect M. falcata, Nemaston, Kansas Common, Atlantic, Oregon Creeper I and II, M. ruthenica, M. glutinosa, Hunter River lucerne, Booboroogie lucerne, and Australian creeping lucerne. In none of the cases examined was any male-sterility observed.

(b) Self-pollination studies

A counted number of florets of each of the selected lines (Table II) were self-pollinated by the following procedure during the latter part of July and the first part of August, 1950.

Racemes containing fresh untripped florets were selected and all leaves, buds, terminal growth, and opened flowers were cut away with scissors leaving a maximum of 10 untripped florets per raceme. The raceme was washed to remove any adhering foreign pollen on the standards by dipping in clean water. When dry, the florets were artificially tripped by inserting the points of a closed pair of scissors (sterilized between lines with ethyl alcohol followed by a wash) between the standard and the keel, and opening the scissors. This caused the staminal column to be released from the keel and stike the column with some force.

After the florets of a raceme were tripped, a cellulose bag was secured over the raceme to exclude any insects that might effect cross-pollination. (Stevenson and Bolton (87) found that cross-pollination occurred in alfalfa if foreign

pollen was applied one hour or possibly longer after tripping where flowers had not been emasculated. Tysdal and Garl (94) found that if foreign pollen were added to untreated stigmas in addition to its own, the foreign pollen would be the active agent in fertilization 70-98% of the time.)

The cellulose bags were removed in a weeks time to prevent mildewing and to allow any seed formed to ripen. The racemes had been tagged previously so that they could be identified. The developed pods were harvested periodically when nearly ripe and allowed to ripen in the laboratory. When ripe, the seed was threshed by hand and counted. (See Table II).

The seeds were planted in flats in the greenhouse in October 1950, and transplanted in December to other flats (40 per flat), and overwintered in these containers. They will be transplanted to the Alfalfa Nursery in May 1951 for possible use in future genetical studies.

1 plant resulted from the 5 seeds obtained by selfing "sterile" line 144-11A, and 1 plant from the 1 seed obtained from "sterile" line 91-10B. Only the latter plant was in flower by April 25, 1951, and a pollen examination of these flowers was carried out.

(c) Open-pollination studies

All seed pods, when nearly ripe, were collected at intervals of a few days from August 1st to the first part of October 1950, from both the "male-sterile" and high pollen producing lines. The seeds, when ripe, were threshed

and counted with results as shown in Table III. The open-pollinated seed from the "male-sterile" plants was planted in flats in the greenhouse in November 1950, and 100 plants of each line were transplanted to 5" pots in late December.

The plants began to flower on April 11, and microscopic slides were prepared daily of the pollen from the flowers as soon as they unfolded from the bud. By April 25, 71 plants of the 20-DRC line had flowered, 12 plants of 142-10B, 41 plants of 91-10B, and 44 plants of the line 144-11A had come into flower. Microscopic slides of the pollen from these plants was examined under 125x on April 26, with results as shown in Table 6.

B - Observations

Pollen showed a great amount of diversity both in shape and size. Pollen varied from normal, rounded, semi-hyaline, and finely pitted; through elliptical, striated, and with dense cytoplasm; to irregular, angular and of large size. Also observed was very small pollen of normal shape and density (designated 'micro-pollen'), and dark, amorphous, very small granular bodies (designated 'aborted' pollen). On the basis of pollen examination 20 lines were selected as being superior pollen producers, and 4 lines were selected as possessing "male-sterility".

The high pollen producing lines all produced a large quantity of pollen (with a few exceptions) of uniform excellent quality except 3 lines which produced, the second year, some micro-pollen and shrunken pollen. The results

are tabulated in Table I.

The examination of the varieties other than Rhizoma was carried out as a check: most produced very good pollen and none showed any male-sterility. However, we had only 6 plants of each variety in the nursery plot to make observations on, so the examination would hardly be significant.

The lines designated as "male-sterile" were observed in the main production field in August and September 1949 with the following observations recorded. The original 20-DRC line was complete and showed superior vegetative growth. Only 2 plants were living in the original clonal row of 91-10B: both being healthy, vigorous plants. Clonal row 144-11A had only one poor unvigorous plant left in the row. Clonal row 142-10B had only one plant, although vigorous and healthy, remaining. The original observations on the pollen taken in 1949 of all the selected "male-sterile" and " high-pollen" lines are contained in Table I.

In a critical examination of the pollen of the "male-sterile" lines on 18 Sept. 1950 the following observations were made.

20-DRC: No loose pollen. The pollen sacs appear full of a dark amorphous substance.

142-10B: Very little good pollen, much aborted. Pollen sacs full of granular dark substance.

144-11A: Approximately equal amounts of good pollen with misshapen, irregular, shrivelled pollen; however there was not very much of either.

91-10B: Very little pollen was produced, but it seemed normal pollen although very small in size (micro).

On self-fertilization 20-DRC and 142-10B produced no seeds; whereas 144-11A produced .0427 seeds per floret selfed, and 91-10B produced .008 seeds per floret selfed. These amounts of seed setting agree very well with the results as observed on the amount of pollen produced by these lines. (See Table I)

Of the normal or high pollen producing lines 0.654 seeds were set per floret selfed, with a range from 0.009 to 1.877 seeds produced per floret selfed. (Clarke and Fryer (27) found 0.44 seeds set per floret selfed in Saskatchewan in 1930. Cooper and Brink (31) found that an average of 0.80 seeds were set per floret selfed with a range of 0.16 to 1.76. This agrees fairly well with my findiggs.)

Shrivelled, small, and discolored seed constituted 34.84% of the seed produced on self-pollination, and 16.19% of the seed produced on open-pollination. (See Tables II and III)

The amount of seed produced on self-pollination is show in Table II, and on open-pollination of the same lines in Table III.

An examination of the pollen from the 71 F_1 plants of the 20-DRC line that had flowered at time of reporting showed 2 plants that produced no pollen at all, and 7 plants that produced only very shrivelled, misshapen, amorphic, apparently sterile, pollen. The 2 plants producing no pollen at all had empty pollen sacs in abundance, but no signs of pollen, excepting a very fine dust like debris which may be the remains

of early aborted pollen.

An examination of the pollen from the 44 F_1 plants of the 144-11A line showed 4 plants that produced empty pollen sacs without a vestige of free pollen, and 2 plants that produced the shrivelled, amorphic, apparently sterile pollen as observed in the 20-DRC segregates.

The pollen from the 41 F_1 plants of 91-10B showed 1 plant that failed to produce pollen, and 5 plants that produced only misshapen 'sterile' pollen.

Line 142-10B had only 12 F_1 segregates in flower by April 26, 1951, and of these, only 1 plant appeared to produce pollen that could be designated 'sterile'.

Table VI shows the frequency distribution of the pollen of the F_1 segregates of the 4 male-sterile lines as they were observed on April 26, 1951. As previously outlined, a value of 5 indicates pollen of uniform, semi-hyaline, rounded shape. 1 indicates pollen that is misshapen and probably sterile or incapable of germination. A value of 0 indicates an absence of pollen. The intermediate grades indicates pollen of intermediate quality. That is, a value of 3 would indicate the presence of rectangular, striated pollen. A value of 4 would designate the presence of normal pollen and also the abnormal pollen of 3. A value of 2 indicates the presence of presence of aborted pollen along with both the normal and abnormal.

TABLE I - RESULTS OF POLLEN EXAMINATIONS.

LINE	OBS. AUGUST 1949		OBS. 18-24 APRIL 1950		OBS. 18, SEPT. 1950.	
	Quan.	Qual.	Quantity	Quality	Quantity	Quality
20-DRC	0	0	-	0	0	0
91-10B	2-	0	1	1	1+	1
142-10B	0	0	0	0	0	0
144-11A	2-	1-	1	1	3	2
106-DRC	5-	5	5	5+	5	4+
37-10B	5	5+	4+	4+	3	4+
68-10B	4	5-	4+	3	4+	4
83-10B	5	5-	5	4	5	5-
102-10B	5-	5	5	4	5	3 #
103-10B	4+	5-	5	4+	5	3 #
128-10B	5-	5-	5	4+	5	5
144-10B	5+	5-	5	5	5	4+
15-11A	5-	5-	5	5	4+	3 #
30-11A	5-	5-	5	4+	5	5
34-11A	5-	5-	5	5+	5	3- #
35-11A	5-	5-	5	5+	5	4-
59-11A	5-	5-	3	4	5+	4+
71-11A	5-	5-	5	4+	4+	4+
73-11A	5-	5-	5	5+	5	4-
81-11A	4+	5-	4+	4+	5	4+
94-11A	5-	5-	5	5	5	4+
95-11A	5	5-	4	5-	5	5
90-11B	5+	5-	5-	4+	5-	5
115-11B	5+	5-	4+	4	5	5

Note: # indicates presence of micro-pollen or aborted pollen.

TABLE II - SEEDS PRODUCED UPON SELF-POLLINATION.

Line	Full seeds	Small brown seeds No.	Small brown seeds % total	Total seeds	No. florets selfed	Seeds/floret
Male-sterile lines						
20-DRC	0	0	-	0	217	0
142-10B	0	0	-	0	142	0
144-11A	5	0	-	5	117	0.0427
91-10B	1	0	-	1	118	0.0085
High pollen producing lines						
106-DRC	118	20	14.49	138	106	1.302
37-10B	0	1	100.	1	106	0.009
68-10B	8	10	55.56	18	105	0.171
83-10B	172	27	13.57	199	106	1.877
102-10B	10	41	80.39	51	106	0.481
103-10B	24	14	36.84	38	107	0.355
128-10B	44	15	25.42	59	99	0.596
144-10B	125	12	8.76	137	100	1.370
15-11A	27	31	53.44	58	108	0.537
30-11A	25	11	30.56	36	103	0.350
34-11A	85	8	8.60	93	103	0.903
35-11A	60	33	35.48	93	110	0.845
59-11A	106	20	15.87	126	105	1.200
71-11A	5	1	16.67	6	103	0.058
73-11A	25	13	34.21	38	106	0.358
81-11A	12	0	-	12	100	0.120
94-11A	39	71	64.55	110	109	1.009
95-11A	70	16	18.60	86	103	0.835
90-11B	5	12	70.59	17	48	0.354
115-11B	33	5	13.16	38	107	0.355
		Average	34.84%		Average	0.654 seeds per floret selfed.

TABLE III - SEEDS PRODUCED UPON OPEN-POLLINATION

Line	Full seeds	Small brown seeds		Total seeds	No. of plants	Seeds/Plant
		No.	% total			
Male-sterile lines						
20-DRC	491	89	15.34	580	5	116
142-10B	258	139	35.01	397	5	79.4
144-11A	528	111	17.37	639	5	127.8
91-10B	909	60	6.19	969	5	193.8
High pollen producing lines.						
106-DRC	295	44	12.98	339	2	169.5
37-10B	697	54	7.19	751	2	375.5
68-10B	74	20	21.28	94	2	47.0
83-10B	1314	376	22.25	1690	2	845.0
102-10B	654	58	8.15	712	2	356.0
103-10B	773	31	3.86	804	2	402.0
128-10B	41	54	56.84	95	2	47.5
144-10B	354	78	18.06	432	2	216.0
15-11A	400	54	11.89	454	2	227.5
30-11A	86	46	34.85	132	2	66.0
34-11A	622	36	5.47	658	2	329.0
35-11A	569	108	15.95	677	2	338.5
59-11A	470	69	12.80	539	2	269.5
71-11A	106	6	5.36	112	2	56.0
73-11A	1165	118	9.20	1283	2	641.5
81-11A	565	148	20.76	713	2	356.5
94-11A	368	53	12.59	421	2	210.5
95-11A	309	52	14.40	361	2	180.5
90-11B	550	61	9.99	611	2	305.5
		Average	16.19%		Average	287.8 seeds per plant.

TABLE IV

CORRELATION OF SEED SET UNDER OPEN & SELF-POLLINATION.

Line	Seed set			
	Open-pollinated		Self-pollinated	
	# seeds	Rank	# seeds.	Rank
68-10B	47	1	0.171	4
128-10B	47.5	2	0.596	12
71-11A	56	3	0.058	2
30-11A	66	4	0.350	5
106-DRC	169.5	5	1.302	18
95-11A	180.5	6	0.835	13
94-11A	210.5	7	1.009	16
144-10B	216	8	1.370	19
15-11A	227.5	9	0.537	11
59-11A	269.5	10	1.200	17
90-11B	305.5	11	0.354	6
115-11B	316	12	0.355	7.5
34-11A	329	13	0.903	15
35-11A	338.5	14	0.845	14
102-10B	356	15	0.461	10
81-11A	356.5	16	0.120	3
37-10B	375.5	17	0.009	1
103-10B	402	18	0.355	7.5
73- 11A	641.5	19	0.358	9
83-10B	845	20	1.877	20

Using ranking correlation

$\Sigma x = 210$ $\quad\quad$ $\Sigma y = 210$

$\Sigma x^2 = 2581.0$ $\quad\quad$ $\Sigma y^2 = 2581.5$

$(\Sigma x)^2 = 44{,}100$ $\quad\quad$ $(\Sigma y)^2 = 44{,}100$

$S.S._x = 376$ $\quad\quad$ $S.S._y = 376.5$

$\Sigma xy = 2223$

$r = +.0478$

$t = .209$

At n-2 (18) degrees of freedom, tabled t equals 2.101 at $p = .05$. Calculated t is smaller than tabled t so no significance can be asserted to the correlation coefficient. That is, there is no correlation.

TABLE V

RANK CORRELATION OF SEED SET UNDER OPEN & SELF-POLLINATION USING WILCOXON'S (98) APPROXIMATE STATISTICAL PROCEDURE.

(Ranks as assigned in Table IV)

Open-poll.	Self-poll.	Rank totals	(Rank totals)^2
1	4	5	25
2	12	14	196
3	2	5	25
4	5	9	81
5	18	23	529
6	13	19	361
7	16	23	529
8	19	27	729
9	11	20	400
10	17	27	729
11	6	17	289
12	7.5	19.5	380
13	15	28	784
14	14	28	784
15	10	25	625
16	3	19	361
17	1	18	324
18	7.5	25.5	650
19	9	28	784
20	20	40	1600
			10, 185

$$r = \frac{x^2r}{p-1} - 1.$$

$$x^2r = \frac{12}{np\,(p+1)} \text{ Sum (rank totals)}^2 - 3n(p+1)$$

$n = 20$ $p = 20$ (rank totals) = 10, 185

$$x^2r = \frac{10,\,185}{70} - 126$$

$$= 145.5 - 126 = 19.5$$

$$r = \frac{19.5}{19} - 1 = 1.03 - 1 = +.03$$

To test whether this differs significantly from zero it may be compared with its standard error which is $1/\sqrt{p-1}$ or $1/\sqrt{19}$ = 1/4.66 = .227. The ratio .03/.227 equals .132, and therefore the correlation cannot be considered significant as its value (+.03) is less than one quarter of its standard error (.227).

TABLE VI - FREQUENCY DISTRIBUTION OF THE POLLEN CLASSES OF THE F_1 SEGREGATES OF THE 4 'MALE-STERILE' ALFALFA LINES ON APRIL 26, 1951.

Line	No. of plants examined	Category for quality of pollen					
		5	4	3	2	1	0
20-DRC	71	2	10	22	28	7	2
144-11A	44	0	2	8	28	2	4
91-10B	41	0	3	12	21	5	1?
142-10B	12	0	0	7	4	1	0
Totals	168	2	15	39	81	15	7

Note
Quality category 1 indicates that no viable appearing pollen is present

Quality category 0 indicates that no pollen is produced.

C - Discussion.

Lines 20-DRC and 142-10B out of over a thousand lines examined show consistent lack of viable pollen and produced no seed on selfing, and as such can be considered male-sterile. 20-DRC being extremely vigorous and a fair seed setter upon open-pollination is worthy of further study. 142-10B, although showing male-sterility, is weakly vegetative and low in seed setting on open-pollination. Due to these reasons 142-10B does not possess the same value agronomically as the 20-DRC line; but even considering these factors it is felt that it is also worthy of further study. It was noted that the F_1 segregates of 20-DRC were much further advanced than those of the latter line right from the seedling stage. This is further evidenced by the fact that 71 plants of the 20-DRC F_1 were in flower by the time 12 142-10B F_1 were at the same stage.

Lines 144-11A and 91-10B appear to possess partial male-sterility. 91-10B, in spite of there being only 2 plants left in the main clonal row, and the fact that it produced seed on selfing, is worthy of further study due to its excellent seed set on open-pollination and its very low percentage of small, brown discolored seed. Although the same objections can be applied to line 144-11A, it is felt that it is especially worthy of further study as 9.1% of the F_1 segregates showed a complete absence of pollen when examined on April, 26, 1951.

Line 37-10B appears to possess a self-incompatibility factor. This line appeared well above the average in seed

set upon open-pollination, while only 0.009 seeds were set per floret selfed on self-pollination.

The production of small, brown, discolored and wrinkled seed was greatly increased by self-pollination (34.84%) over open-pollination (16.19%). (Carlson and Stewart (24) reported in 1931 that shrivelled, dicolored seed ranged from 7.7% to 27% for a 4 year average. They found that an increase in shrivelled dicolored seed was expected late in the season due to insufficient time, before freezing weather, to produce full seed. However, in our case, the self-pollinated seed was collected during the same periods as the open-pollinated seed, so the difference cannot be explained on this basis. Whatever, the reason there is a significant difference. It is possible that partial incompatibility or delayed somatoplastic sterility may be the cause of the difference observed.

The results of the limited F_1 segregation for the 4 'male-sterile' lines as reported in Table VI do not appear to conform to common Mendelian ratios. 20-DRC produces male-steriles in the F_1 in a ratio of 1 to 7.9 tothe male-fertiles. 144-11A produced male-steriles to male-fertiles in a ratio of 1:7.3. In 91-10B the observed ratio was 1:688; and in 142-10B appeared 1:8.3.. If the male-sterility was inherited as simply recessive to male-fertility no male-sterility should appear in the F_1.

However, whatever the mode of inheritance, which will undoubtably be complicated by polyploidy, the F_1 segregation does show that the observed male-sterility is inherited and

and is not wholly attributable to environmental modification. However, before an explanation of the mode of inheritance can be resolved on a genetical basis, a more complete count of the F_1 should be obtained, along with F_2 and backcross segregation data.

To determine the underlying features of the male-sterility a histological (and possibly a cytological) examination of the stamens will have to be performed. However, from the observations made, an estimate of the cause can be made. As the F_1 segregation and observations are by no means complete or final, this estimate is drawn from the observations on the parental 4 "male-sterile" lines.

In the line 20-DRC, no normal pollen was produced, aand the pollen-sacs appeared full of a dark, amorphous granular substance. These granular bodies could probably be units of tetrads cemented together with division arrested, followed by shrivelling and a resultant intensifying of the cytoplasm to give the dark coloration. No differences were observed in the gross morphology of the male-sterile and the male- fertile flowers; nor in the stamens or in the external features of the pollen sacs. It is felt that a histological study of the pollen-sacs would be well worth while.

In line 142-10B the pollen sacs in most instances appeared full of the same dark amorphous substance as in the previously discussed line, but at the same time some normal pollen was produced. No seed was produced on self-pollination. However, the fact that some normal pollen was produced at times, allows the tenetative conclusion that either the male-sterility is

incomplete or that the degeneration of the pollen is effected very late in microsporogenesis and that all pollen-mother cells are not equally affected.

In the line 144-11A approximately equal amounts of good pollen were produced with misshapen, irregular, shrivelled pollen (although very little of either). At the same time a few pollen sacs were observed which appeared devoid of contents. On selfing 4.27% seed was produced by this line. However, the F_1 segregation gives 4 plants out of a total of 44 plants observed to produce no pollen at all (on the basis, of course, of a single examination). It would appear that in this line, that the male-sterility factor, although present, is of low penetrance. The empty pollen sacs could be explained on the basis of the male-sterility factor causing a breakdown very early, or at the onset, of meiotic division; in which case, no remains of the pollen would be observed.

In the fourth line, 91-10B, 0.8% seed was produced on selfing, and although the pollen sacs appeared empty on a pollen examination, a small amount of normal, although minute, pollen was produced. In normal microsporogenesis, after the final division of meiosis, the tetrad resolves itself into pollen grains, of irregular shape, who feed and grow and round out. It appears that in this line, the pollen grains were formed, but did not grow: in which case, it is possible that the male-sterility factor affected the tapetal layer. However, an answer to this can only be obtained by further study.

Seed production of the high pollen producing lines under

self-pollination and open-pollination was found to be not correlated. However, it was observed that the highest seed producing line under self-pollination was also the highest producing line under open-pollination. The comparison of of the seed set, under the different types of pollinations, had certain limitations. A comparison was made between the seed-set per floret selfed selfed and the total open-pollinated seed-set of the plant; whereas, it would have been comparable if an correlation had been attempted between seed-set per floret selfed and seed-set per floret open-pollinated.

It is felt that the isolation of male-sterility, such as accomplished in the past two years work at U.B.C., could have very important effects on the future of alfalffa breeding. Male-sterility could be used as the key to open the door to hybrid vigor, and provide the means of unleashing some of the heterosis that is manifested by alfalfa, in the majority of cases, when it results from hybridization.

In the nursery plot at U.B.C. there are several rows of plants resulting from cross-pollination and beside them several rows of plants resulting from self-pollination. These plants are 1½ years old only, and the cross-pollinated plants, without exception, are so much larger, more spread, and more vigorous than their selfed counterparts that the contrast is startling. When one thinks of what this means, when it can be considered that, through the use of use of male-sterility, whole fields can all be planted to hybrid seed, the potentialities are unlimited.

However, a lot of testing will have to be done before the production of hybrid seed through the employment of male-sterility can become a reality or feasible on an economic scale. The male-sterile lines will have to be checked carefully for agronomic suitability, and combining ability with the desired male-parent lines. Nevertheless, it is felt, and strongly too, that the male-sterile lines have great potentiality in a hybrid vigor program.

SUMMARY

A pollen study of approximately 1000 lines of alfalfa grown at the University of British Columbia, and embracing most of the common alfalfa varieties, revealed 4 lines that exhibited male-sterility, and 20 lines that produced very high quality pollen. These lines were clonally propagated in the fall of 1949, overwintered in the greenhouse, and transplanted to the Nursery plots at U.B.C. in May 1950. A counted number of florets were selfed of each line; and open-pollinated seed was collected. No correlation between self- and open-pollinated seed set was observed. 2 of the male-sterile lines produced no seed on selfing, and 2 produced very small quantities.

The F_1 segregation for male-sterile: male fertiles were as follows: 1:7.9, 1:7.3, 1:6.8, 1:8.3 in the plants grown from open-pollinated seed of the 4 male-sterile lines.

Possible modes of inheritance and causes of the observed male-sterility is discussed.

Literature on male-sterility and incompatibility is reviewed. No references were found citing male-sterility in alfalfa.

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B - THE WITCHES' BROOM VIRUS DISEASE OF ALFALFA IN B.C.

INTRODUCTION

Although Witches' Broom of Alfalfa was first reported in B.C. only recently (1932 - Foster (17)), the disease has been recognized in Australia for 40 to 55 years. However recent its dicovery in this province, the disease is developing into serious proportions in parts of the Interior. The ravages of the disease had assumed enough importance by 1948 that the Department of Agronomy at U.B.C. felt the need for some investigation into the nature and extent of the disease. Since its discovery in North America, Witches' Broom of Alfalfa has as yet received comparatively little attention due probably to its apparent sporadic occurrence. In Australia, on the other hand, the disease has been sufficiently serious to command continuing financial support of fairly extensive investigations.

Investigations performed in Australia and in the United States has shown the disease to be caused by a virus and to be difficult to transmit mechanically. The pathogen is probably disseminated by insects. Symptomology is well described by a number of workers: Edwards (12), Menzies (34), and Smith (42). However, very little basic information has been acquired so far on the true nature and fundamentals of the disease.

Out of the studies initiated at U.B.C. in 1949, data regarding spread, distribution, seriousness are to be acquired along with information regarding any resistance inherent in

members of the genus Medicago. The final goal is the production of strains or varieties capable of resisting the disease. It is hoped that information on methods of transmission, insect vectors of the disease, and the host range of the virus will be obtained by plant pathologists and entomologists.

A program, such as outlined, will require many years to consummate. Therefore, my part of the investigation will consist of setting up foundation experiments, and the compilation of data of other workers relevant to the investigation.

LITERATURE REVIEW

A. - History and distribution.

Witches' Broom of Alfalfa was first recognized as a disease in Australia. McCleery (36) reported that in 1924 the disease was prevalent throughout the drier parts of New South Wales. Noble and associates (39) reported in 1947 that, of the 417,000 acres under lucerne (alfalfa) in New South Wales, 235,000 acres occur in areas where the Witches' Broom disease is known to be very prevalent. In these areas, the disease is considered to be the main factor limiting the profitable life of lucerne stands. Economic stands could only be maintained 4 to 5 years before ploughing up and resowing was necessitated. In areas where lucerne was grown for seed, the diseased plants represent a total loss, as seed is not produced normally.

Edwards (12) reported in 1936 on investigations into the disease which began in 1931 (with preliminary work dating

back to 1929). At that time he reported the disease to be very widespread throughout the inland areas of New South Wales where, under an average annual rainfall of 17 to 21 inches, lucerne is grown for grazing purposes. Fields that were 4 to 5 years old showed 20 to 25% infection while stands more than 7 to 8 years old commonly showed 70% infection. However, he reported that the Witches' Broom disease is not known to occur in crops less than 15 to 18 months old. Edwards (ibid) also reported the disease to be present, although less severe than in New South Wales, in Queensland, Victoria, and South Australia.

The first North American record of Witches' Broom of Alfalfa was made by Haskell (20) in 1925 with observations of the disease as affecting some plants in Salt Lake County of Idaho. In the same year, Richards (41) reported the appearance of the disease in Utah. In 1932, Foster (17) reported the disease as occurring in 2 widely divergent parts of B.C., but with the cause unknown. Interest in the disease in North America was crystallized by the work of Menzies (34) in Washington published in 1946. At that time the disease distribution was limited to the area between the Cascade and Rocky mountains excepting an eastern extension of the disease into Alberta, and a single report from Vancouver Island.

The Canadian Plant Disease Survey Annual Reports for the years 1922 to 1949 contains 27 mentions of the Witches' Broom of Alfalfa, and a chronological listing of the citations points out clearly the trend of the disease. Some references

to a (possibly related) witches' broom condition in some other legumes is also cited.

1922-1931: No mention.

1932: Foster (17) reported 2 year old alfalfa plants affected with a witches' broom were sent to the laboratory from Smithers and from Saanichton. He reported the disease as being found on clover at the same places.

1933: No mention.

1934: Anonymous. At Lytton and McGillivray's Flats there were certain patches in the fields, which contained several plants affected with Witches' Broom. The disease was also noted in other fields in Cariboo county and a single diseased plant was recorded from Summerland.

1935: Anonymous. 15 - 20% of the plants in the irrigated section, Cariboo county, B.C. were infected, being worst in older fields.

1936: No mention.

1937: Jones (37) reported 'Dwarf' to be prevalent in the Cariboo districts and other parts of the interior of B.C.

1938: Jones (38) reported a few plants of the Ladak variety were affected at the Agassiz Station, B.C. It was widely distributed in the interior of the province, principally in the irrigated areas.

1939: No mention.

1940: Cormack (3) reported about 1% of the plants in a plot at Edmonton, Alberta, were moderately to severely affected and many others were beginning to show the symptoms.

1941: Cormack (4) reported a few plants to be moderately affected in 3 fields at Cherhill, Alta. 5% of the plants were dead or severely infected in the plot at Edmonton.

1942: Brink (2) reported the disease to be present in one or two fields in the Nicola Valley of British Columbia, where alfalfa stands last for only 3 or 4 years as a result of the disease. Brink (ibid) reported the disease to be identical with that described in Washington.

1942: Heald and Menzies (22) report the disease to be present in 4 counties in the state of Washington, and to be serious in the Methow valley area of Okanagon county. They stated that the disease was identical with that described by Edwards in Australia (12).

1942: Cormack (5) reported the damage from Witches' Broom to be increased in the plots under observation at Edmonton, Alta.

1943: Woolliams (48) reported the disease as affecting at least 5% of the plants in a field at Armstrong, B.C.

1943: Cormack (6) reported the disease had advanced somewhat in the fields at Cherhill, Alta. and also in the plot at Edmonton, Alta.

1944: Cormack (7) reported that the field under observation at Edmonton was so thinned out by the disease that it was ploughed up. In the same year, he reported occasional plants of alfalfa were severely infected in a field at Bremner, Alta. and in an old plot at Lacombe, Alta.

1945: Cormack (8) reported slight damage in the University plots at Edmonton, Alta.

1946: Cormack (9) reported the disease for the first time from Saskatchewan, where it was affecting 2 fields in the White Fox district and one field in the Loon Lake district.

1946: Wright (50) reported that a relatively high proportion (10 - 15%) of diseased plants occurred in 5-6 year old alfalfa stands in the North Okanagon.

1947: Wright (51) reported that 20% of the plants in an eight year old alfalfa field in the Cariboo district were affected, and damage was estimated at 15%. A much lower percentage of infection was observed in recently seeded stands.

1947: Gilpatrick (18) reported a few plants were infected in four of the stands examined in central and northern Alberta. In the same year, he reported a few plants of Alta-swede red clover were severely affected at Edmonton, Alta.

1948: Woolliams (49) reported the disease as being quite prevalent around Lytton and Lillooet in B.C., and the odd plant was affected in a field at Shuswap, B.C.

1948: Mead (33) found a trace of the disease in an old field east of Tisdale, Sask.

1948: Thomson and Lebeau reported (43) a few plants were found affected in 2 stands in central Alberta.

1948: Munro (35) reported a clover plant showing symptoms resembling Witches' Broom of Alfalfa in the Cariboo.

1949: Lebeau (31) reported infection was 5-tr 3-sl/240 fields in central Alta. and Peace River district.

1949: Anonymous. A few affected plants were seen in an old field at Hudson Bay Junction, Sask.

1949: Wright (51) observed Witches' Broom for the second year in a 1/10 acre plot of alsike clover at the Expt. Sta. Prince George, B.C.; about 15% of the plants were affected. It also affected 25% of the plants of Birdsfoot Trefoil (Lotus corniculatus) in 2 25 foot rows at the station. "Witches' broom was also found to be affecting at least 10% of the wild lupin plants (Lupinus sp. indet.), which grow on uncultivated land about Prince George. Only 3-4 plants of red clover growing wild were found affected at Quesnell in the Cariboo district".

1949: Payette (40) reported that in some plots of Ladino white clover at the station, Ste. Anne de la Pocatiere, P.Q., plants failed to flower, but instead numerous little leaves developed giving the appearance of witches' broom. The same trouble was observed in all stands of Ladino clover over one year old inspected in L'Islet county.

1949: MacLeod (37) found 5 red clover plants showing symptoms resembling witches' broom in a field in York Co., N.B.

B - Nature of the losses due to Witches' Broom of Alfalfa.

Witches' Broom of Alfalfa causes a definite decrease in yield of forage as a result of the dwarfing, but the chief source of loss is the reduction of stands by the early death of the plant. Infected plants succumb very readily to winter killing, undoubtably due to their weakened condition brought about by the greatly increased demands on stored food reserves in the production of "myriads" of proliferations.

Edwards (ibid) reported on a yield experiment in

Australia conducted over a period of 3 years (1932-34) to determine the effect of the Witches' Broom disease on the amount of fodder produced by diseased plants under field conditions. He found that during the period the experiment was in progress the diseased plants have given a mean yield of 37.4% less green weight of fodder than the unaffected plants.

Seed production is almost totally inhibited in diseased plants. The majority of the diseased plants fail to flower, but occasionally blooms may be produced which are considerably smaller and paler in colour than the normal inflorescence according to Smith (42). The occasional flowers that are produced are usually in groups of 2 or 3 instead of the multi-flowered racemes of the normal inflorescence. Both Menzies (ibid) and Edwards (ibid) report very little seed is produced from the diseased flowers in either North America or Australia respectively. Edwards (ibid) adds that in areas where lucerne is grown for seed production the affected plants represent a total loss.

C - Other alfalfa viruses and virus diseases.

Kenneth Smith (42) lists 4 viruses as affecting alfalfa, namely:

1) Medicago virus 1 Weimer - causing Common Alfalfa Mosaic

2) Medicago virus 2 Pierce, Zaumeyer and Wade - causing Alfalfa Mosaic,

3) Medicago virus 3 Weimer - causing Alfalfa Dwarf Disease,

4) Medicago virus 4 Edwards - causing Witches' Broom of Alfalfa.

1. Common Alfalfa Mosaic.

Previous to 1931, records of field observations only were made of this disease: the existance of a transmissable virosis of the mosaic type affecting alfalfa had not been proven experimentally. Weimer (45) transmitted it successfully in 1931 with the aphid Illinoia pisi. It might be noted however that in 1922, Dickson (10) working at MacDonald College, transmitted a mosaic disease of clover to Medicago sativa using the same vector.

Weimer (46) reporting on further studies in California found no severe losses from Alfalfa Mosaic, and stated that the disease damage was limited to a very slight dwarfing of most of the seriously infected plants.

The first evidence of the disease in a leaf is the appearance of one or more small, more or less circular, greenish-yellow spots. These areas frequently consist of a yellowish band of tissue ½ to 1 mm. in width, surrounding an island of apparently normal color ½ to 2 mm. in diameter. There may be one or more concentric rings of green surrounding narrow bands of chlorotic tissue. The chlorosis spreads until the rings are more or less obscured and the leaves sometimes completely chlorotic. In some severe cases the leaves are reduced to 1/3 their normal size, are crinkled and more or less deformed. Although there may be some dwarfing of diseased stems in severe cases, normally there is no reduction in size. Necrotic lesions have not been observed on the stems, and the disease does not cause premature defoliation.

Weimer (46) attempted several mechanical methods of transmitting the common mosaic disease but with negative results. He did achieve, as noted earlier, successful transmission with the pea aphid (Illinoia pisi). The period of incubation was found to be 7 to 14 days from time of inoculation to the appearance of primary symptoms.

Common alfalfa mosaic, frequently recorded from California, is probably existant in other states. The disease is favored by moderately cool damp periods in fall and spring. Interestingly enough, in the last report of the Alfalfa Improvement Conference (1) the disease is not recorded.

2. Alfalfa mosaic.

Weimer (46) considered the virus causing this disease to be a strain of Medicago virus 1, as the disease that it causes somewhat ressembles Common Mosaic. Inasmuch the causal agent differs in several important properties from the former virus, this pathogen has been considered to be a separate entity and classified accordingly by Smith (ibid). The virus is sap-transmissable, whereas the former was not. The vector, Illinoia pisi, is common to both viruses. Whereas the former virus caused a mosaic only of alfalfa, this virus has a wide host range: Hyacinth bean, adzuki bean, mung bean, rice bean, common and Turkestan alfalfa, white sweet clover, crimson clover, red clover, garden peas, spring vetch and soybeans are all infected.

Its affect on alfalfa is more severe than the Medicago virus 1, in that the affected plants are decidedly dwarfed

and the leaves are distinctly mottled and crinkled.

Mosaic has been reported by MacLeod (37a) in 2 fields of Grimm alfalfa at Fredericton, N.B., and in a field of alfalfa near Oromocto, N.B. Hurst (26a) reported in 1945 that mosaic affected an occasional plant in Prince Edward Island fields. However, in the United States, previous to 1949, the disease was recorded only from Wisconsin.

In 1949, McWhorter (38) reported a necrotic strain of alfalfa mosaic virus which he isolated from alfalfa and peas in eastern Washington and Oregon, and which was usually accompanied by yellow bean mosaic. The host range was typical of alfalfa mosaic, but the thermal inactivation point is 10° to 15° lower than those previously recorded for that virus (62-64°C for 10 minutes).

3. Alfalfa Dwarf Disease.

When and where this disease originated is unknown. However, in 1919 - 1921 farmers in California south of the Tehachapi mountains could maintain satisfactory stands of alfalfa for 8-10 years, whereas in 1931 stands were seldom worth maintaining over 3 years. There has been no evidence of the disease outside of southern California. Dwarf disease is mainly responsible for the short-lived stands of alfalfa in this area. The symptoms were first described by Weimer (44) in 1931, and a short time later the same investigator (47) showed the virus nature of the disease.

The earliest symptoms of the disease cannot be detected above ground, and Alfalfa Dwarf is very well established in the root before it is evident in the top. The first sign in

the tops is a shortening of the stems and a slight reduction in the size of leaves. Blossoming is often retarded or inhibited. Progressive reduction in size of stems and leaves follows each cutting and is accompanied by a reduction in the number of buds developed each time, which results in a gradual reduction in the number of stems. No chlorosis or other color change is evident until the last few stems die. In the final stage of the disease only one stem, or at the most a few stems, are produced and are only a few inches high. Not infrequently, the leaves of the affected plants are darker green than healthy plants. In roots, the earliest stage of the disease, that can be detected, is a slight yellowing of the wood just beneath the bark. This yellowing, a result of gum formation in the vessels, spreads until the entire active part of the xylem is involved.

In 1941, Hewitt and Houston (24), struck by the geographical association of Pierce's Disease of Grapevines and Alfalfa Dwarf in California, started investigations into the two diseases, the results of which were published in 1946 (25). They noted that both diseases were spread by an insect vector and that they appeared in adjacent areas. Both diseases appear more frequently and more severely in wet portions of fields or along ditches or ponds where there is a luxuriant growth of grasses heavily infested by leafhoppers. Through a series of experiments they proved that the same 4 species of leafhoppers that transmitted the Pierce's disease of grapevines also transmitted the Alfalfa Dwarf disease; and that these insects

could effect an intertransmissability of the two diseases. Root pieces from diseased grapevines were inserted into the roots of healthy alfalfa plants and transmitted the virus in 12 out of 35 trials; but root pieces from diseased alfalfa, similarily inserted in the stems of rooted grape cuttings, failed to transmit the disease due to failure of union of the scions and stocks.

In 1949, Houston (26) reported the release for commercial production of a dwarf resistant strain of alfalfa developed through selection in California common alfalfa. Observations in fields of California common alfalfa showed that, after practically 100% of the plants were either dead or badly diseased, about 1 plant in each 2000 square feet was still making a normal top growth, even though root symptoms showed that the plant had been diseased for a period of one to two years.

A number of such plants were selected, transplanted and selfed to produce progeny. The progeny were artificially inoculated using viruliferous vectors and observed over a period of 2 years. A portion of the progeny appeared quite tolerant to the virus and maintained normal growth 2 to 3 times that of California common check plants. These results indicated the possibility of selecting a Dwarf resistant strain from California common, without changing in any way the other desirable qualities of this variety.

With the foregoing determined, 800 plants showing natural field resistance were selected and planted in an isolation

block. Here they were allowed to interpollinate naturally and set seed the following year. Seed was harvested separately from each of the remaining plants (425 plants, the rest had died in transplanting, showed virus effects, or failed to set seed).

This seed was planted in 3 row blocks replicated 3 times in each of 3 counties of Southern California incommercial fields where dwarf had been very prevalent the past years. The final results from these plats in the spring of 1949 showed that the progeny from 60 to 70 of the original selections were quite tolerant to the dwarf virus and held a normal growth producing a good stand.

Simultaneously, to further test the Dwarf resistant qualities of the original selections, cuttings from 320 of thebetter plants were rooted in the greenhouse, artificially inoculated by a vector, and transplanted to an isolated block in a field along with a number of common plants inoculated at the smae time. At the end of the second deason of growth, all the latter were dead, whereas about 65 of the former showed no effect of the virus with the exception of root symptoms.

Based on these results, and those of the field plots, the more resistant selections were saved to cross-pollinate and set seed, which was released to the growers as California Common '49 last year.

D - Other alfalfa diseases resembling Witches' Broom.

There are two other diseases of alfalfa that superficially resemble Witches' Broom, and may be confused with this disease. They are the bacterial wilt disease and the dwarf disease, and the distinguishing features are:

(1) Bacterial wilt disease. In common with Witches' Broom, the Bacterial Wilt disease causes a marked dwarfing of the plant, owing to shortening of the internodes and reduction in leaf size. The great proliferation characteristic of Witches' Broom is not normally a symptom of Bacterial Wilt, but it is not the distinguishing criterion. In Bacterial Wilt the characteristic yellowish or brownish-yellow discolouration of the vascular cylinder of the tap root serves as a positive distinction since the roots of Witches' Broom are normal in colour in the primary stages of disease.

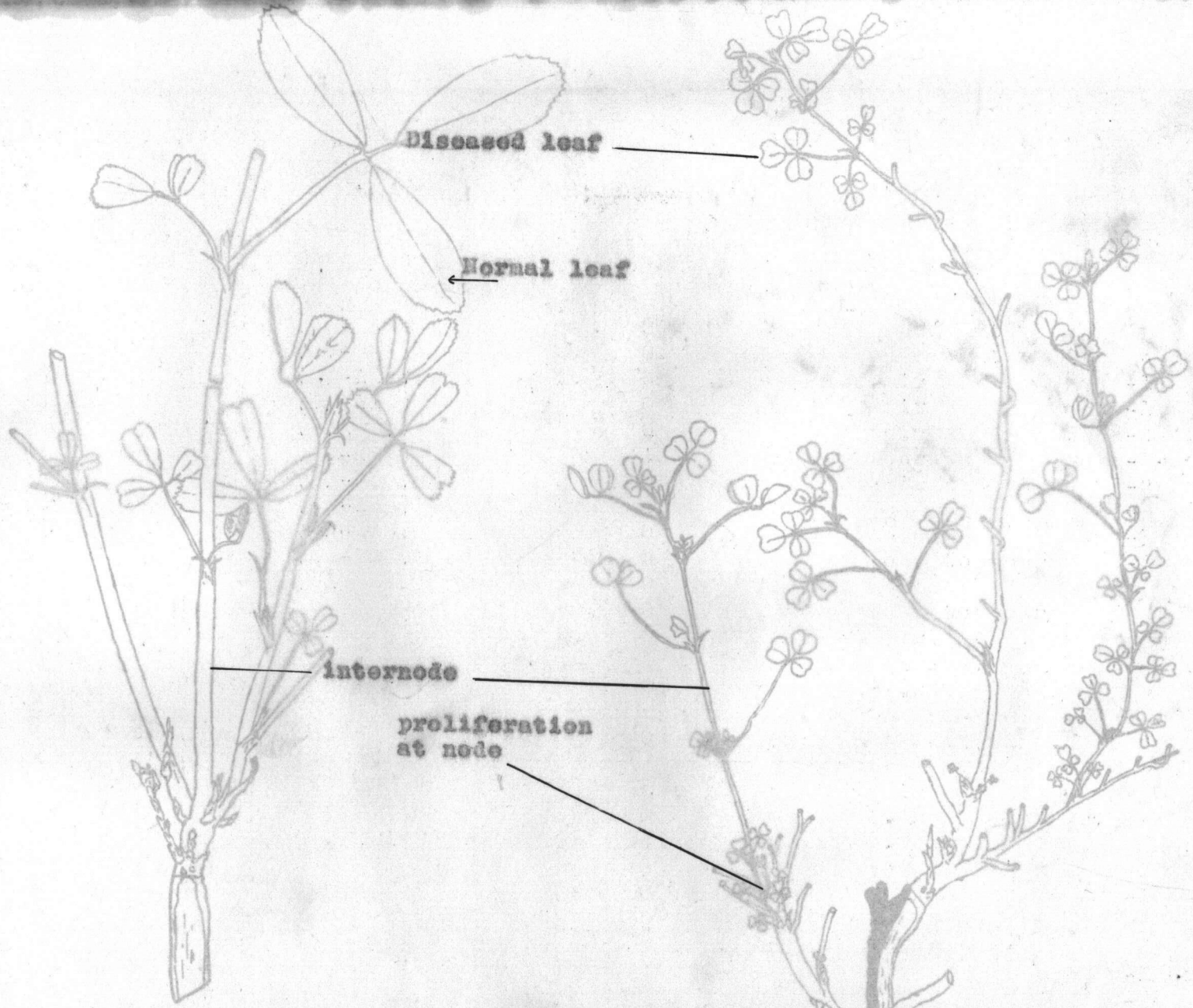
(2) Alfalfa Dwarf disease. In common with Witches' Broom this disease causes a dwarfing of the alfalfa plant both in reduction in total size and in the length of internodes and size of leaves. However, in Dwarf disease the roots are discolored, leaves retain their normal colour or may be intensified, and there is a progressive reduction in the number of shoots produced. In Witches' Broom the roots remain normal for some time, leaves are definitely chlorotic, and there is a progressive increase in the number of shoots produced. Also of note is the fact that in the former disease the roots are very badly infected before any symptoms appear above ground.

WITCHES' BROOM OF ALFALFA SYMPTOMOLOGY.

Diseased plants can be observed and identified from afar in the field by their greatly reduced size (hypoplasea) and their pale yellowish coloration, and the patchy appearance of the field due to the reduction in the number of plants. On several occasions in B.C. and in Washington, the disease has been spotted while driving along the highway on the basis of these symptoms.

The most characteristic symptom of Witches' Broom of Alfalfa is the marked dwarfing of the plant. However, as the appearance of infected plants is slowly modified over a period of months or years, a wide range of symptoms can be observed.

In the advanced stage of infection, there is typically a dense proliferation of shoots produced from the crown accompanied by a severe dwarfing of thw whole plant (Photo 1). In very advanced stages of the disease, however, the number of shoots may be greatly reduced due to the severe rotting of the crown and upper portions of the root, with the consequent death of the buds in that portion. Internodes are greatly shortened. (Internodes on healthy plants are normally $1\frac{1}{2}$ to $2\frac{1}{2}$ inches long, while in diseased shoots when severely dwarfed are but a fraction of an inch long. (Plate 1, Photo 2). In fact, leaves were observed on occasion so greatly reduced in size, that it was only after examination with a 10x hand-lens that the three leaflets could be differentiated. The normal alfalfa leaflet is rather narrow, oblong or elliptic-oblanceolate with spinose denticulations towards the apex. Diseased



HEALTHY ALFALFA BRANCH OR 'SHOOT' (x1)

WITCHES' BROOM DISEASED ALFALFA SHOOT. (x1)

leaflets are smaller, rounder, sometimes lack apical denticulations and are frequently wrinkled or puckered. (Plate 1).

The first symptom of the disease to be seen is a slight marginal chlorosis of the leaves giving the plants a yellowish cast or tinge. Primary symptoms of infection usually are seen when new growth starts after cutting, and the symptoms seem to be general all over the plant. At this time, there may be little or no dwarfing of the plants, but the diseased plants can be recognized easily by the greatly increased number of stems, the yellowish cast, and a tendency for the growth to be very erect. (Photos 3, 4, 5)

After the next cutting, the new growth will usually be decidedly dwarfed and the leaflets much reduced in size. Proliferation and stunting is progressive with each successive cutting. Both Edwards (ibid) and Smith (ibid) report a colour change in infected plants when proliferation has occurred: the foliage is often very dark in colour and may have a purplish tinge. Menzies (ibid), on the other hand, does not mention this phenomenon as occurring in Washington. Nevertheless, it has been observed, in a few cases at U.B.C., that the foliage becomes very dark, hardly purplish, before the leaves become chlorotic, and before crown proliferation is pronounced. This darkening of the foliage has occurred in conjunction with a rosetting or clustering of the nodal foliage about the nodes.

Several hundred spindly stems are common on infected plants and Menzies (ibid) reports that severely infected crowns have been found with as many as 3000 very fine, densely

Witches' Broom diseased alfalfa plant, showing very erect growth.

Severely dwarfed Witches' Broom diseased plant on left; normal alfalfa plant on right.

matted stems. This figure is in agreement with some of the plants observed, but, in most of the plants observed, the rotting of the crown had occurred to such an extent, by this period, in the progress of the disease that the number of shoots is much reduced from this number (3000).

Immediately preceding death of the plant the foliage wilts severely and becomes prostrate. At this time an examination of cross-sections of these crowns has shown almost complete disintegration of cellular structure; also blocking of the vascular system has cut off the normal water and nutrient supply to the foliage, a feature which results in wilt and eventual death. Menzies (ibid) made no observations on the rotting of the crown, although it was observed to precede the death of the plant in all cases at U.B.C. Edwards (ibid) stated that in the later stages of the disease, the crown and upper root tissues are often severely rotted and the plants gradually die. He made isolations from the decaying tissues which yielded an unidentified sterile white fungus, Rhizoctonia batatícola, and various types of unidentified Fusaria. The most apparent fungus observed at U.B.C. was an unidentified Basidiomycetes.

Edwards (ibid) observed partial and complete recovery of diseased plants when transplanted to the greenhouse and maintained under conditions favourable for growth. In many instances, the recovery was only temporary with renewal of symptoms at a later date, but in some cases he reported complete recovery. Menzies (ibid) did not observe this phenomenon in Washington. However, two plants in the greenhouse at U.B.C., that exhibited

Alfalfa plant showing apparent
recovery from Witches' Broom disease.

Witches' Broom diseased alfalfa plant
showing proliferation and dwarfing.
Nicola Valley, B.C.

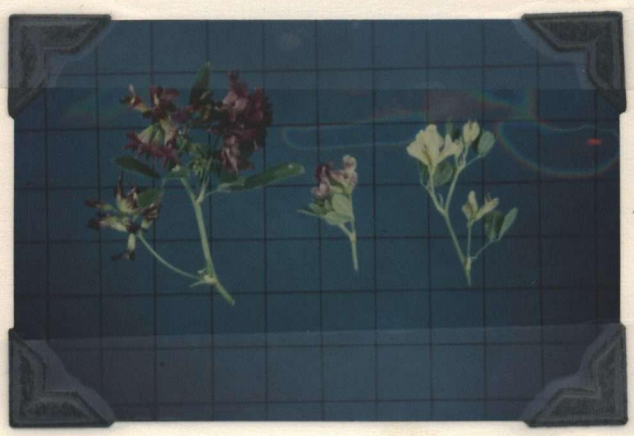
positive disease symptoms in the fall of 1950 (they were clones of diseased plants obtained in June 1950 from the Nicola Valley) had shown apparent complete recovery from the diseased condition in April, 1951. Photograph 6 shows one of these recovered plants. Several diseased plants were noted on April 11, 1951, to be producing normal shoots mixed among the diseased stems, while the fall before, only diseased shoots were produced. These normal shoots bore flowers by April, while very few of the diseased stems bore flowers or even buds at this date.

It was commonly observed that newly diseased plants produced normal growth at the periphery, with reduced or dwarfed foliage in the centre of the plant.

Not only was crown proliferation observed, but also an abnormally large number of fine, elongated shoots have been observed arising from the nodes of the stems, and then more shoots arising from the nodes of these elongated shoots, with the resultant production of a thick, abnormal, bushy type of growth.

The majority of diseased plants failed to flower, a rather striking symptom when seen during the normal flowering time in the field. The few flowers formed on diseased plants appear smaller in size, and paler in colour. However, the latter point is hard to determine, as normal alfalfa flowers (M. media) show a tremendous colour diversity, ranging from pale blue-white, light greens, several shades of purple, to the odd yellowish flower. Nevertheless, in the few cases where healthy periphereal shoots were producing flowers at the

Normal alfalfa shoot on left, and Witches' Broom diseased shoot on the right. Note chlorosis, shortened internodes, reduced leaflet size and rounder shape, and small pale inflorescence.



Normal alfalfa inflorescence on left; the two on the right are Witches' Broom diseased alfalfa inflorescences. Note reduced number of florets and paler color.

same time as the central diseased shoots were producing flowers, the central flowers appeared paler in colour. The flowers borne on diseased shoots were borne singly, or in groups of 2 to 6 with an average of 2.7 (average of 20 clusters examined at random); this contrasts sharply with the multi-flowered raceme bearing approximately 15 flowers, produced by healthy plants.

As is to be expected in light of the reduced number of flowers, very little seed is produced. The small amount of seed that was collected seemed normal (perhaps a little small) and germinated well.

INVESTIGATIONAL WORK

I - TRANSMISSION EXPERIMENTS

A. Grafting studies

(a) Literature review.

Edwards (11, 12, 13), Heald and Wellman (23), and Menzies (34) carried out extensive graft transmission tests in investigating Witches' Broom of Alfalfa. Edwards (ibid) achieved the first success in obtaining positive transmission of the disease by grafting.

Edwards (ibid) used, in a majority of his cases, scions from diseased plants introduced by means of a side or veneer graft into the upper portion of the tap root of healthy plants just below the crown. He also used healthy scions and diseased stocks in an attempted cleft or wedge graft into the crown and into the upper portion of the tap root. He encountered

considerable difficulty in establishing a successful graft whenever a diseased plant was used as a stock. The grafts were performed in a greenhouse, bound with raffia and then coated with wax. Two months after the grafts were attempted the plants were transplanted to the field; at this time he had achieved 57 unions out of 100 attempted using diseased scions and healthy stocks; and 11 unions out of 58 attempted using healthy scions and diseased stocks. Out of the latter 11 unions, only 2 persisted after transplanting to the field and positive transmission of the disease occurred in both instances. Out of the former 57 unions, only 28 scions persisted for 2 weeks after transfer to the fields, and a month later there were only 15 of these scions still surviving. Out of the instances where the scions had died out prior to transplanting, 4 showed positive disease transmission when examined $2\frac{1}{2}$ months after transplanting. At this time 13 of 15 plants showing scion survival also showed positive disease transmission; and 9 of the plants were diseased whose scions only persisted for 2 weeks after transferring to the field. In all, Edwards (ibid) achieved transmission in 28 plants out of 158 attempted grafts, or out of 68 achieved grafts.

Heald and Wellman (ibid) reported that inoculations by grafting from diseased to healthy plants had reproduced the symptoms in a limited number of cases.

Menzies (ibid) achieved very little success in root-grafting tests, so he used instead a modification of the shoot-grafting technique. This modification consisted of keeping the delicate grafts, firmly bound with fine thread,

in a glassed-in humidity chamber for 10 days after grafting. He reports 75% successful grafts using this method, with disease transference occurring in all cases where the diseased scions became united with the healthy stocks. In a scion removal experiment, he found that virus transfer commenced between the 10th and 12th day following grafting and was completed by 25 days. Using shoot grafts, disease symptoms were apparent on the average in 61 days; while with root-approach grafts, symptoms took on the average 118 days to be manifested. It thus appears in the former case, that the actual latent period of the virus is from 40 to 50 days using shoot-grafts.

Menzies (ibid) also attempted a study of the host range of Witches' Broom of Alfalfa by means of cross-grafting. He obtained 87 apparently successful grafts on 12 species of legumes. Serious difficulties arose from the fact that several of the legumes were annuals, and did not remain alive long enough for the symptoms, if any, to be recognized. However, he was successful in transferring the disease to Black Medic (Medicago lupulina L.), andto California Bur-Clover (Medicago hispida Gaert.).

(b) Experiments.

1. Several diseased plants were dug in the Nicola valley of British Columbia, brought to the University and planted in a greenhouse deep bench in June 1950. 142 cleft crown grafts, using healthy 8 months old Rhizoma plants as stocks and Witches' Broom scions from the above plants, were attempted from June 13 to 16, 1950. Grafts were bound with raffia, thickly coated with paraffin, and planted in a

deep greenhouse bench. The healthy foliage was cut back at this time, and several times during the summer and fall the healthy foliage was cut back to allow the diseased scions any advantage that this might give. Although in several cases an apparent disease transfer was suspected, the nature of a crown graft rendered it impossible to observe whether the disease symptoms appearing were from disease transfer to the healthy stocks, or if the diseased foliage appearing was scional only in origin. The plants were dug up for observation on January 10, 1951, at which time there were 124 plants remaining alive. Whether the 18 plants that died, did so as a result of the disease or from cultural methods was not determined. (All grafted plants were transplanted to pots in November 1950.) The results obtained are given below:

Plants healthy on January 10, 1951.

Union of scion and stock, but scion dead	7
No union, scion never established or disappeared	79
No union, both scion and stock living separately	4

Plants diseased on January 10, 1951.

Union, but scion dead	5
Union, scion living in firm union with stock	14
Union, scion living in very feeble, if any, union	2
No union, no evidence of establishment of scion	3
Plants doubtful or suspicious on Jan. 10, 1951.	10
	124

The 10 plants belonging to the latter category were replanted in the greenhouse. On the 5th of February, 1951, 3 plants showed positive symptoms of the disease, and an additional one was suspicious in showing a large number of small, clustered leaves; and when observed on the 16th of February this plant was showing proliferation and marginal chlorosis -

typical disease symptoms. Final examination of these plants was carried out April 16 1951, when besides the 4 diseased plants, 2 appeared suspicious. ~~One plant (35)~~ Plant No. 35 showed a clustering effect of the leaves around the stem and a much darkened color; and the ~~other plant (29)~~ plant No. 29 showed the clustering effect and had a chlorotic tinge to the whole plant.

Adding in the above plants to the above totals results in figures given below.

Plants healthy	
Union of scion and stock, but scion dead	7
No union, scion never established or disappeared	83
No union, both scion and stock living separately	5
Plants diseased	
Union, but scion dead	6
Union, scion living in firm union with stock	14
Union, scion living in feeble union with stock	4
No union, no evidence of establishment of scion	3
Plants doubtful of suspicious	2
	124

Out of 142 attempted crown grafts, 124 plants were living after 7 months. successful union of scion and stock was achieved in 31 cases, and disease transference was observed in 27 cases. All 18 cases wherein the scion and stock were in union and both living at time of digging, showed a disease transference. (XX). 7 cases were observed, where the scion and stock had united, but the scion was dead by January 10. How long the union lasted is not known, but judging from the results of Menzies experiment in removing scions every five

days, the union must have lasted less than 2 weeks before the scion died. In 3 cases, scion or remains of any part of the scion could not be found although there was a positive disease transference. In these 3 cases it is probablethat a union did exist for a period long enough for a virus transfer before a rotting of the scionoccurred. Evidence that a union must exist is shown by the five cases where both scions and stocks were living in very close proximity to one another, but with no disease transmission. A rough measure of the upper limit of time required for the appearance of symptoms - February 16th or 8 months. If the 2 doubtful or suspicious plants subsequently develop disease symptoms, the period of incubation will of course be again lengthened.

2. In May 1950, 12 stem cleft grafts, using diseased scions and healthy plants were attempted in the greenhouse in co-operation with M. S. Wright of the Dominion Plant Pathology Laboratory. The grafts were bound with raffia and kept moistened by a string-wick extending from a jar of water, However, union was not observed, and the scions died in about 2 weeks time. Menzies' modification of using a humidity chamber was not attempted.

3. At the same time, another method of transferring the disease was attempted. This consisted of cutting longitudinal sections (approximately 2 inches long) out of the healthy and diseased shoots growing in adjoining pots, and firmly binding the freshly cut areas together with raffia. However, both the diseased and healthy shoots, above the cut, died in all cases in about 10 days time. No disease transference was observed

in any case during the summer, fall and winter in the greenhouse.

B. Mechanical Inoculation Studies

(a) Literature

Edwards (ibid), employing as inoculum the freshly expressed and undiluted sap of diseased foliage, attempted several methods of mechanically inoculating healthy plants. The methods, that he employed, are outlined briefly as follows.

1. Individual leaves were brushed lightly with a small cheesecloth bag saturated with inoculum and containing a mixture of sand and crushed infected leaf tissue.

2. Stems were pricked with a fine hypodermic needle and a drop of inoculum allowed to flow into the wound.

3. Stems scratched longitudinally, with a fine hypodermic needle in several places, and inoculum allowed to flow into the wound.

4. Multiple pin inoculations (using 2o-3o entomological pins bound together) into terminal leaves of vigorously growing shoots, leaves and stems.

5. Small cotton woolplugs soaked in inoculum were inserted in the tap root just below the crown in contact with the vascular tissue.

The inoculated plants were observed for periods ranging from $2\frac{1}{2}$ to $3\frac{1}{2}$ years, with no symptoms developing in any case.

Menzies (ibid) inoculated 250 plants using macerated diseased tissue on a swab as inoculum and dusting carborundum powder on leaves to be inoculated. However, Menzies failed to secure disease transmission in any case.

(b) Experiments

1. Crowns of Witches' Broom diseased alfalfa was finely ground with meat grinder, and strained through 8 thicknesses of cheesecloth. The resultant excised juice was inoculated into crowns of 30 healthy 15-month old Rhizoma seedlings on February 16th, 1951, using a "London" Luer Fisher and Burpe 5 cc. size hypodermic syringe fitted with a B-D 16 needle. At the same time, the macerated crown material from which the juice was extracted was wrapped around the broken roots of 12 healthy 15 month old Rhizoma seedlings. All plants were potted in the greenhouse, together with 23 controls and observed from time to time.

Final observations on the plants were made on April 28th, 1951. 2 plants of the inoculated series showed very deep coloration and rather intense proliferation. However, this same condition was observed on one of the control plants. One plant of the macerated series showed the intensification of color and proliferation habit, and produced no flowers or flower buds; whereas, the other plants in the series were flowering vigorously. These plants will be transplanted to the field in May for further observations.

2. On February 14th, 1951, 8 healthy 6 month-old Grimm seedlings were planted in a 14-inch size earthen-ware pot containing shredded foliage of diseased plants well mixed with the soil. The seedlings were planted so that the roots and crowns were in close contact to the diseased material. Final observations made April 28th, 1951 showed all plants to be

normal.

C - Seed Transmission of Witches' Broom of Alfalfa.

Edwards (ibid) conducted a seed transmission experiment with a limited number of seed from infected plants. Several of the young plants showed, by the first fall, marked foliage abnormalities. The leaves, considerably reduced, were clustered along the stems producing a rosette-like effect, which was suggestive of infection. However, these plants flowered normally after transplanting to the field, and no definite evidence was obtained to show that any of them were infected with Witches' Broom. Final observations, taken 2 years later, showed several suspicious plants, but no plants showed positive symptoms of the disease.

Menzies (ibid) grew 488 plants from seed collected from infected plants, but failed to secure any evidence of Witches' Broom infection in a year's time.

Experiment

Seed was collected from diseased plants at Savona and at the Basque ranch, B.C. in August 1950. This seed, threshed by hand, was scarified, germinated in pitre plates, and transplanted to the greenhouse to flats in October, 1950. Germination was normal. 162 plants were transferred from flats to pots in late December, and the remaining plants were transplanted to other flats, 40 to a flat. Larger than normal numbers of small, weak seedlings were observed at time of transplanting; however, as no seed was collected from healthy plants in the same districts (Savona, Basque Ranch) from which the seed from

diseased plants was gathered, a count of these plants was not made. Observations made on April 28th, 1951, showed that although no plants showed Witches' Broom symptoms, several plants were very small and the odd plant chloratic and exhibited rosetting of nodal foliage.

D. Plant multiplication through cuttings

One of the difficult, time consuming, and expensive procedures in the study of this virus disease will be the securing of diseased plants in sufficient numbers for use in the various required replicated field tests. The diseased plants will have to be laboriously dug out of normally tough sod in fields in the Interior, and then carried without delay to the coast. Here the plants will be divided and planted as quickly as possible. However, very high mortality has been observed in all such transfers in the past.

Accordingly, on the 2nd of October, 1950, 3 sets of 25 stem cuttings of diseased alfalfa were treated with the following procedure. The basal ends of sets of cuttings were dipped for 30 seconds into 4000 ppm solutions of indole butyric acid (IBA) alpha-napthalene acetic acid (NAA), with the 3rd set, as control, dipped into distilled water. The cuttings were planted in clean sand in glazed pots, kept watered, and left untilDecember 4th when they were dug up, observed for root formation, and planted in flats. 7 of the cuttings treated with IBA none of the cuttings treated with NAA, and 8 of the cuttings treated with distilled water were alive and showed root formation. The cuttings treated with IBA, although less

numerous than the controls, showed considerably greater root formation both in size of roots and extent of rooting area. 2 of the cuttings subsequently died after transplanting, but the remaining 13 have grown into fairly large plants by the time of reporting (April 28th, 1951), all showing advanced symptoms of the disease.

The experiment did point out, besides being able to get the proliferation effect in the absence of a crown, thatit is possible to root cuttings of Witches' Broom diseased alfalfa and thus increase the number of diseased plants for use in field experiments. If a diseased plant produces 500 stems, this means that the one plant can produce, using my results of 26% rooting, 130 diseased plants; and this could be done about once a month. It isfelt, however, that with improvement in techniques much higher percentage of rooting of cuttings could be achieved, and that this method should prove to be very usefulto the agronomist and plant pathologist in multiplication of diseased stocks. It has the advantage to the plant breeder inthat a great number of plants, all with the same genotype, and at the same stage of the disease, can be secured readily for use in resistance and breeding studies.

E. Insect transmission studies

Edwards (ibid) attempted severalexperiments in an attempt to transmit the Witches' Broom of Alfalfa using insects as agents of inoculation (or vectors). A brief resume of his work is given below.

1. 2 to 4 Jassids, field collected, were allowed to feed

on a diseased plant for 7 to 9 days and then transferred to healthy plants for 5 - 10 days. Twenty eight 5 - 6 month old plants were treated this way. 12 - 24 thrips, also field collected, were allowed a 5 - 7 day acquisition feed on a diseased plant and then transferred to a healthy plant for 5 - 7 days. 16 healthy plants were treated in this manner. No transmission was observed in any case.

2. Two 24 plant plots were established for preliminary work with possible insect vectors. Plots were enclosed with wooden frames covered with fine white madopolam. Mixed populations of insects collected on diseased plants in the field were enclosed in each frame. After 3 months, the experiment was modified by replacing the central 4 plants by a large diseased plant to produce a continuous source of inoculum. At the same time, massed populations of field collected insects were liberated inside the cages. 80% of these insects belonged to the Thysanoptera and the Hemiptera. The remaining 20% consisted of species belonging to the Diptera, Coleoptera, and Lepidoptera.

In addition, in the following year 6 more plots were added to the experiment, 2 of which were kept sprayed and served as controls. The plots were cut as required, and massed populations of insects collected in highly diseased fields were added periodically. 9 such additions were made to the latter 4 transmission cages, and 11 to the former 2. 15 months after establishment of the first part of the experiment, one plant was found in the first 2 cages, that showed definite symptoms of Witches' Broom disease. However none of the other

plants in any of the plots, although observed carefully for a period of 3 years, developed any evidence of infection.

Menzies (ibid) made mass collections of suspected species of insects, caged them on infected alfalfa plants for a short feeding period, after which they were segregated into species groups and transferred to healthy plants. In the course of several hundred tests with sucking insects one case of transmission occurred where the leafhopper Platymoideus acutus Say. was used. In this case, an acquisition feed of 7 days on diseased plants was allowed, then insects transferred to healthy plants for 12 days. Witches' Broom symptoms were first considered definite in approximately 2 months after inoculation.

In another experiment, Menzies (ibid) used large cages containing one infected plant and 4 to 6 healthy plants. Field collections of Platymoideus acutus were added to some of the test cages from time to time for a couple of months, while other cages were retained as checks. First symptoms appeared on 2 plants in 5 months after inoculation, and by 8½ months, 18 of the 29 surviving test plants were definitely affected with Witches' Broom, whereas none of the check plants became infected.

However, Menzies (ibid) could not find this leafhopper Platymoideus acutus in 2 sweeps made in June and August of 1944 in the Methew valley of Washington at the time the disease was spreading rapidly. He concluded from this, that Platymoideus acutus, although a vector, is probably not the principal vector of the disease.

It was felt that a search for a vector, involving by necessity, trained entomologists and plant pathologists, is somewhat outside the province of the Department of Agronomy. Some work in breeding of Witches' Broom resistant varieties of alfalfa may, regrettably, have to be attempted without valuable research being conducted regarding vectors.

II - YIELD TRIAL

(a) Literature

Edwards (ibid) selected and enclosed an 1/7 acre alfalfa field containing from 30% to 35% of Witches' Broom diseased plants. The whole plot was cut to establish uniform conditions. 25 diseased and 25 healthy plants, selected at random in the field, were cut whenever the unaffected plants reached the flowering stage. The foliage was weighed immediately after cutting and the weights recorded to the nearest ½ ounce. Averaged over a period of three years, the diseased plants gave a mean yield of 37.4% less green weight fodder than the unaffected plants. (This is hardly a true figure, as it does not take into account the yield reduction which would result from the higher mortality of the diseased plants thus lessening the stand.)

Menzies (ibid) reported a serious reduction in yields but did not report on any tests to determine the amount of reduction in yields due to the disease.

(b) Experiment.

Ten comparable sized clonal cuttings of each of Witches' Broom diseased plants secured from the Interior, and Grimm

TABLE VII - YIELD TEST

WEIGHT IN GRAMS OF AIR-DRY HEALTHY AND WITCHES' BROOM DISEASED ALFALFA FODDER.

A - WITCHES' BROOM DISEASED PLANTS

Pl. No.	1st cutting	2nd cutting	3rd cutting
1	10.68	1.91	3.31
2	5.51	0.89	2.13
3	4.53	0¾88	2.44
4		0.07	0.57
5	7.66	3.41	4.78
6	3.81	0.68	0.89
7	3.12	0.92	2.78
8	4.18	2.16	1.72
9	3.38	0.30	1.13
10	3.31	0.17	1.01
Totals	46.18	11.39	20.76
Averages	4.62 gms.	1.14 gms.	2.08 gms.

B - HEALTHY PLANTS

Pl. No.	1st cutting	2nd cutting	3rd cutting
11	6.42	3.78	4.77
12	8.55	2.04	3.35
13	4.83	1.29	2.95
14	6.36	3.63	4.20
15	3.42	1.02	1.59
16	6.52	2.80	4.65
17	5.49	1.85	2.88
18	3.97	1.72	2.06
19	4.70	2.92	3.42
20	4.79	2.35	2.68
Totals	55.05 g.	23.40 g.	32.55 g.
Averages	5.51 g.	2.34 g.	3.26 g.

Table VIII - Correlation of air-dry weights of fodder of healthy and diseased alfalfa on first cutting.

Witches' Broom plants		Healthy plants	
Σx	= 46.18	Σy	= 55.05
$\bar{x}$	= 4.62 g.	$\bar{y}$	= 5.51 g.
n	= 10	n	= 10
$(\Sigma x)^2$	= 2132.5924	$(\Sigma y)^2$	= 3030.5025
Σx^2	= 287.7224	Σy^2	= 323.2593
S.S.$_x$	= 74.4632	S.S.$_y$	= 20.2090
S.D.$_x$	= ± 2.3874	S.D.$_y$	= ± 1.4916
S.E.$_x$	= ± .9089	S.E.$_y$	= ± .4717

S.E. of diff. between the 2 means = ± 1.024 g.

Diff. between the 2 means = 0.89 g

$$t = \frac{.89}{1.024} = .86914$$

Tabled t at p = .05 is 2.101 at 18 D.F.
Tabled t greater than calc. t - so no significant difference between the fodder produced by Witches' Broom and Healthy Alfalfa plants at beginning of the experiment.

Table IX - Correlation of air-dry weights of fodder of healthy and diseased alfalfa plants on second cutting.

Witches' Broom plamts		Healthy plants	
Σx	= 11.39	Σy	= 23.40
$\bar{x}$	= 1.14	$\bar{y}$	= 2.34
n	= 10	n	= 10
S.D.$_x$	= 1.052	S.D.$_y$	= .9333
S.E.$_x$	= .3327	S.E.$_y$	= .2952

S.E. of diff. between the 2 means = .445
Diff. between the 2 means = 1.20 g

$$t \quad \frac{1.20}{.445} = 2.69$$

Tabled t = 2.101 at 18 D.F.

Calc. t is greater than tabled t - therefore there is a significant difference in yield between the fodder produced by Witches' Broom and Healthy alfalfa plants on the second cutting.

Table X - CORRELATION OF AIR-DRY WEIGHTS OF FODDER OF HEALTHY AND DISEASED ALFALFA PLANTS ON THIRD CUTTING.

Witches' Broom Plants		Healthy plants	
Σx	= 20.76	Σy	= 32.55
$\bar{x}$	= 2.08	$\bar{y}$	= 3.26
n	= 10	n	= 10
$S.D._x$	= 1.304	$S.D._y$	= 1.052
$S.E._x$	= .4124	$S.E._y$	= .3327

S.E. difference between the 2 means = .5298

Diff. between means = 1.18 gms.

t = 2.227

Tabled t = is 2.101 at 18 D.F. and p = .05

Calc. t is greater than tabled t.

Therefore there is a significant difference between the air-dry weights of healthy and diseased plants' fodder at the third cutting.

alfalfa obtained from the nursery plot at U.B.C. were propagated in 10 pairs of 8-inch glazed pots in the greenhouse on Sept. 10, 1950. Plants were watered, and periodically randomized to avoid position effect. When the healthy plants had reached 1/12 bloom, February 16, 1951, all plants were harvested. Tops were dried for 1 hour in an oven at 100°C, and allowed to return to air-dry-weight before weighing on a rough balance. They were harvested a second time on March 16, and a third time on April 16, 1951. The results of these weighings are given in Table VII.

It was found that on the first cutting there was no significant difference between the yield of the Witches' Broom diseased plants and the healthy plants. (Table VIII). This shows that the cuttings were comparable in size at the beginning of the experiment.

The yield of the Witches' Broom diseased plants and the healthy alfalfa plants upon the 2nd and the 3rd cutting are statistically analyzed in Tables IX and X. It was found that on the 2nd cutting, the fodder produced by the diseased plants was but 48.8% of that produced by healthy plants. On the 3rd cutting, the fodder produced by the diseased plants was 63.4% of that produced by the healthy plants. Both of these decreases in yield were found to be statistically significant.

III - FIELD STUDIES ON THE WITCHES' BROOM DISEASE OF ALFALFA.

A. Quadrat studies

1. Introduction

A true picture of the decline in yields due to a disease can only be given by an experiment conducted under normal plant environment. A potted-plant yield trial, as previously reported, has many limitations. However, it does afford the scientist with a very good comparison of the healthy and diseased plants and as such the prodedure contains much merit. Nevertheless, in a greenhouse experiment diseased and healthy plants are maintained under ideal growth conditions. The effects of overgrazing, winter injury, and drought on diseasd weakened plants are not observable.

The only factual method of obtaining a reliable and accurate report on the decline in yield (all factors taken into account) is through the use of quadrats. In quadrats (a measured area of field), every plant is recorded and its hitory followed. It is possible, through quadrat study, to observe when a plant becomes sick, and when it dies and disappears. Management factors can be taken into account. In short, quadrat reports are one of the most valuable tools of the agronomist in studying the progress of a disease, or of any other factor affecting range or pasture.

We had available quadrat sheets covering quadrats laid in the Interior of B.C. in the spring of 1942 and observed in 1944 by Dr. V.C.Brink. The results of this quadrat data are reproduced in this thesis. Feeling the inadequacy of this data (over 2 years elapsed between laying and reading the quadrats) additional quadrats were plotted in the fall of 1950.

2. Study of quadrat data from 1942-1944 (V.C.Brink).

Dr. V.C.Brink (unpublished) noticed in 1941 that a great many fields of alfalfa were being ploughed up and being replanted to grasses in the rich Nicola valley of B.C. due to the ravages of a new disease which produced a serious dwarfing of alfalfa plants and a decimation of the stands. In order to study the disease, the following year Dr. Brink plotted 7 square meter quadrats, largely in the Clark field, Nicola Stock Farms, Nicola B.C. These quadrats were plotted in June, 1942 and final observations were taken in September 1944.

Alfalfa plants healthy - 1942	140	
Plants healthy - 1944		33
Plants diseased - "		27
Plants dead or disappeared 1944		80
Alfalfa plants diseased 1942	93	
Plants dead or disappeared 1944		93
	233	233

The significant feature of the above data is that there was no vestige in 1944 of any of the 93 Witches' Broom diseased plants charted in 1942: all had died or disappeared.

Of the 140 healthy plants plotted in 1942, only 60 were remaining by 1944, and of these 60 plants 27 showed advanced symptoms of the Witches' Broom disease. Other factors may have contributed to the disappearance of the healthy plants besides Witches' Broom, but it is strongly suggested that many of them may have succumbed to this disease. Other factors which may have contributed to their loss were management factors, too dry in summer, and overgrazing in the winter.

3. Quadrat studies initiated in 1950 in the Interior.

To further study the progress of the Witches' Broom of Alfalfa disease, Dr. V.C.Brink laid 6 quadrats in alfalfa fields in the interior of B.C. at, or near, the following geographic points: one near Spence's Bridge, three in the vicinity of Savona, one near Wallachin, and one on the Basque Ranch near Ashcroft. The first quadrat was plotted July 11, 1950; and the remaining five were laid September 18, 1950. The quadrats were laid without particular choice in the field to position: the meter-quadrat frames were carried into the field and thrown at random - and the plant life recorded where it fell. Foot long, one-inch square metal pins were driven out of sight into the ground at the four corners of the quadrat frame so that future records could be made of the same area.

The quadrat data sheets for the 6 quadrats plotted, enclosed as Appendix I of this essay, are briefly summarized as follows.

1.	July 11, 1950.	Spence's Bridge, B.C.	19 healthy alfalfa 0 Witches' Broom
2.	Sept. 18, 1950.	Savona? B.C.	8 healthy 3 diseased
3.	"	"	11 healthy 10 diseased
4.	"	"	7 healthy 4 diseased
5.	"	Wallachin, B.C.	7 healthy 5 diseased
6.	"	Basque Ranch, Ashcroft	19 healthy 9 diseased.

B - DISTRIBUTION OF ALFALFA WITCHES' BROOM IN B.C.

During the summer of 1949 and 1950, alfalfa fields were examined at many points throughout the province of British Columbia (and parts of the state of Washington) for the presence of the Alfalfa Witches' Broom disease. A knowledge of the disease distribution would point out several salient features of the disease. It would show us the ecological habitat and the climatic conditions under which the diseased condition thrives. (In Australia, Edwards (12) points out that the disease is restricted to areas that have an annual rainfall of 17 to 21 inches.) If continuing, a disease distribution would also give us pertinent information on direction of spread which may, or may not, be correlated with the possible vector spread.

Fields were examined during the two years in the districts as indicated in the following tables XI & XII and on the accompanying distribution map. All fields (except where indicated by #) were examined by Dr. V.C.Brink (accompanied in some cases by the author). Alfalfa fields in 35 districts were found to contain at least 5% of Witches' Broom diseased plants. The disease was not observed in fields in 31 other districts, but the conclusion is not to be drawn that the disease is not present in these districts, but just not observed.

A survey of the North Thompson valley is planned for later on in the investigation.

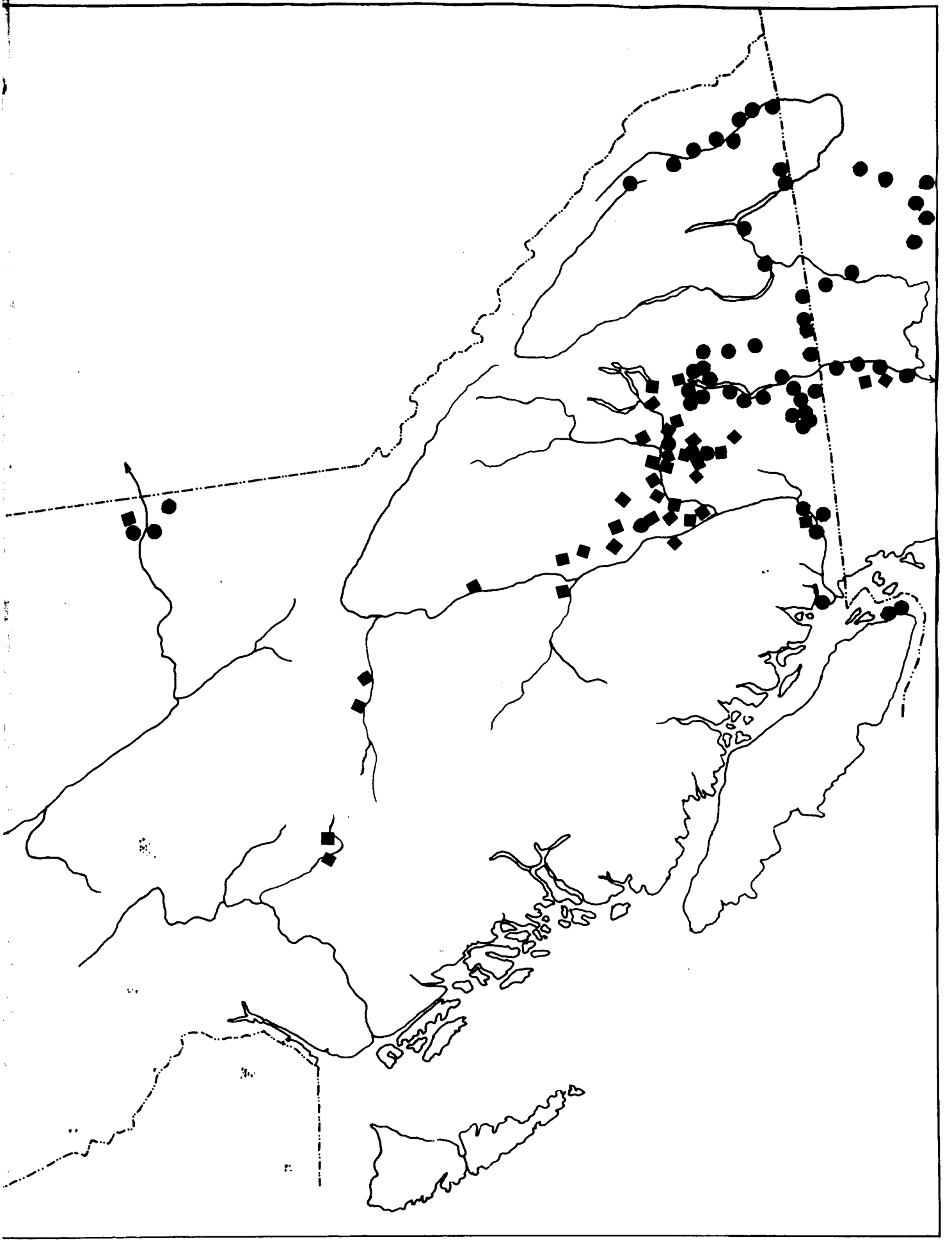
THE DISTRIBUTION OF WITCHES' BROOM OF ALFALFA

IN BRITISH COLUMBIA

On April 30, 1951.

■ - Alfalfa fields containing at least 5% Alfalfa Witches' Broom plants.

● - Alfalfa fields that did not contain Alfalfa Witches' Broom plants.



alfalfa
Table XI- Districts in which/fields were examined that did not contain Alfalfa Witches' Broom Plants.

Saanich Peninsula
U.B.C.
Nicoman Island
Chilliwack
Rosedale
Princeton
Hedly
Keremeos
Okanagon Falls
Summerland
West Bank
Kelowna
Vernon
Falkland
Lumby
Bridesville
Rosk Creek
Grand Forks
Castlegar
Balfour
Creston
Moyie
Cranbrook
Newgate
Canal flats
Edgewater
Shumway Lake
Kamloops
Pavillion
Stump Lake
Lytton (south of)

Table XII - Districts in which alfalfa fields were examined that contained at least 5% Alfalfa Witches' Broom Plants.

Peace River #
Lake Cathlyn (near Smithers)
Smithers
Fort Fraser
Vanderhoof
Quesnel
Williams Lake
Riske Creek
Spring House
Alkali Lake
Canoe Creek
Clinton
Pavillion
Hat Creek
Lillooet
Lytton
McGillvray
Ashcroft
Wallishin
Savona
Tranquille
Kamloops
Vinsula
Nicola
Nutsford
Shumway Lake
Merritt
Dot
Aspen Grove
Monte Creek
Falkland
Armstrong #
Salmon Arm
Methew Valley (Washington)
(Agassiz?)#

# indicates fields in which Withhes' Broom of Alfalfa has been reported by other investigators that those of the Department of Agronomy, U.B.C.

C - Notes on Alfalfa grown at the Dominion Range Station, Kamloops, B.C. on September 17, 1951.

At the same time that the Nursery Alfalfa Plot was planted at the University of British Columbia, a replicated plot was planted at the Dominion Range Station, Kamloops, B.C. Alfalfa plants, grown from seed at the U.B.C. greenhouse, were transplanted to the field at Kamloops on June 1, 1949.

Seed for the Rhizoma strains was harvested in the fall of 1948 from open-pollinated clonal rows in the main production field at U.B.C. Seed of Viking, Ladak, Grimm, Ferax, Buffalo and Ranger was received from the Dominion Department of Agriculture, Ottawa. Grimm Vidarshov seed was obtained from Sweden; Grimm Summerland S274 from Dom. Exp't. Sta., Summerland, B.C., and Grimm Sask. 666 and 451 were both obtained from the Dom. Forage Lab., Saskatoon, Sask.

The Kamloops nursery was furrow irrigated both in the summer of 1949 and 1950. Observations in the fall of 1949 showed all plants to be vigorous and healthy. However, in the spring of 1950 some of the plants appeared very poorly and during the summer began to develop disease symptoms. On Sept. 17, 1950 several plants were observed to be showing various stages of the Witches' Broom disease: this is shown in the accompanying table. Observations of the replicated plants in the Alfalfa Nursery at U.B.C. failed to disclose any plants showing any symptoms of the Witches' Broom disease. The nursery at U.B.C. had received comparable cultural treatment to the Kamloops plot, with the exception that sprinkler irrigation was used at U.B.C. and furrow irrigation at Kamloops.

TABLE XIII - OBSERVATIONS ON ALFALFA GROWN AT THE DOM. RANGE STA., KAMLOOPS, B.C. 17/9/50

Row	No. plants	Variety	No. of	No. of plants showing symptoms of Witshes' Br6om of Alfalfa disease	Vigor	Seed set (graded 1 - 5)
1	6	Rhizoma	(30-11A)	0	Excellent	0
2	6	"	(24-DRC)	0	Excellent	0 - 1
3	6	"	(29-11B)	0	Fair	0-1 dry
4	6	"	(45-11A)	0	Fair	0-1
5	6	"	(H71P27)	0	Fair	0-2 dry
6	5	"	(4-11A)	4	Poor	0-1-2 dry
7	6	"	(39-DRC)	0	Fair except #6	0-2 dry
8	6	"	(66-10A)	0	Good	0-3
9	6	"	(51-DRB)	0	Good	0-3
10	6	"	(28-11B)	0	Good to poor	0-1
11	6	"	(H156P18)	0	Good	3
12	6	Ranger		0	Good to poor	5
13	4 (6 orig)	Buffalo		0	Fair(2 pl. missing)	5 on one 3 on others
14	6	Ferax		0	Fair (3 weak)	5 onone 2-3 others.
15	4 (6 orig)	Grimm		0	Good (2 miss.)	3
16	6	Ladak		6 (primary)	Fair	5+
17	6	Viking		0	V. good (2 poor)	4+
18	6	Grimm Sask.	451	4	V. good	5+ rest pl.
19	6	" "	"	3	Good	0-1 dry
20	6	" "	666	0	Fair	0-1
21	6	" "	"	0	Very good	2-3 dry
22	6	Summerland	S274	5 (advanced)	Good	0 dry
23	6	"	"	0	Good	0-1 dry
24	6	Grimm Vidarshov		0	Good	0-2 dry
25	6	"	"	0	Good butone	5+

IV. Histological studies of Witches' Broom diseased alfalfa.

a) Literature review.

Neither Edwards (12) or Menzies (26) reported on any attempt at studying the tissues of Witches' Broom affected alfalfa. However there exists several fine review articles on the hisological aspects of plant virus diseases in general.

The anatomical aspects of plant virus disease problems are reviewed very completely by Miss Esau (14, 15, 16). She reports that gum deposition and tylose development in the tracheary elements of the xylem are found to be frequently occurring phenomenon. Gummosis may occur in the xylem of various plants under normal conditions, but is frequently more often observed in the tissues of plants affected by various pathogens and physiological disturbances. She reports that most workers think that the gum is derived from the decomposition of starch, and to a lesser extent, other carbohydrates; and that the products of decomposition migrate from the living cells into the tracheary elements. Gummosis is a very frequent phenomenon of virus infection, but is not specific to this type of disease. Jones and McCulloch (27) report the presence of gum in the vascular system of alfalfa affected with the Bacterial Wilt disease.

Wynd (53) reviews the metabolic phenomena associated with virus infection in plants. He found that the two major physiological effects of virus infection in plants are:
(1) Respiratory activities are markedly increased very early during the course of infection which corresponds to the period of arrival of virus particles from the point of infection. The

plant recovers from this initial shock, although the quantity of virus protein continues to increase.

(2) The permeability of cytoplasm or its membranes is greatly altered in respect to soluble substances. This accounts for the accumulation of carbohydrates in the leaves, and the accumulation of nutrient ions in the roots at the expense of the tops.

Wynd (ibid) in discussing the cause of diminished chlorophyll in chlorotic leaves, points out that part of the diminution of the total chlorophyll is due, not only to actual decrease per chloroplastid, but also to the lessened number of chloroplastids. He believes that the mottling of leaves may be caused by the virus, not directly, but through products of a deranged metabolism. However, other investigators, according to Wynd (ibid) believe that the increased acidity which accompanies virus diseases is a factor in the destruction of chlorophyll. Although chlorophyll is decreased, carotene content of leaves (mosaic) is enhanced; in fact, the light green areas showed an increase of 96% over normal colored leaves.

The histology of normal alfalfa is very fully described by Hayward (21); and the crown and root tissues are described by Jones (28). Graber and associates (19) describe the normal food reserves of alfalfa plants. Roots of high reserve plants have a greater proportion of parenchyma in relation to the fibrous tissue; and the parenchyma cells are packed with reserve foods, principally starch.

b) Experimental studies

1. Comparison of the cellular structure of healthy alfalfa with alfalfa affected with the Witches' Broom disease.

Materials and Methods

(1) Diseased and healthy rootlets, stems, flowers and buds were obtained from Clinton - Ashcroft in June 1950. Fixed with Randolph's modification of Navashin's solution. Imbedded in paraffin, transverse sections were cut with the rotary microtone at 15 to 20 mu. Stained with safranine and methylene blue. Mounted in Canada balsam.

(2) Normal Grimm crown and root sectioned with sliding microtone at 40-50 microns (Nov. 17/50). Killed with B.C. Fix. Stained safranine and methylene blue. (Found better staining when mordant for methylene blue, ammonium molybdate, not used) Mounted in Canada balsam.

(3) After transmission experiment was completed in Jan. 1951, several of the crowns of plants that had become diseased were sectioned using sliding microtone cutting at 40-50 mu. B.C. Fix. Safranine and methylene blue. Mounted in Canada balsam.

(4) Crowns of diseased plants, transplanted from the Cariboo the previous summer to the greenhouse at U.B.C. were sectioned transversely on sliding microtone. As for (3)

(5) Leaves from diseased and healthy plants in Feb. 1951 (grwwn in greenhouse), dipped in 90% alcohol to remove air, Fixed with Rand. mod. Navashin. Imbedded in parawax, transverse sections at 10-14 mu with rotary microtone. Sections secured to slides with Haupts adhesive, stained with safranine and meth. blue. Mounted in Canada balsam.

Observations

CrossSections of roots, stems, nodes, buds, were compared for differences between healthy and diseased tissues. The main, if not only, difference observed was in size. The diseased plant tissues cells were very much smaller than those of healthy plants (pygmismic).

In the healthy material, it was noted that in the young, sqarish in cross-section, stem, the vascular bundles are well separated; whereas in older stems the vascular bundles more or less form a continuous ring. In the diseased plants, the stem has a tendency to remain square in cross-section and without a continuous vascular bundle ring being formed.

The normal crown (see photo) has a relatively simple cellular structure, consisting of a radiating network of vascular tissue interspaced with undifferentiated storage and supporting cells; the whole structure being surrounded by a corky epidermis.

The diseased crown does not appear greatly altered. Xylem elements were frequently found filled with gum (photo) which stained either yellowish or red. Secondary organisms, one of which was a member of the Basidiomycetes class appear in great numbers in the outer rotted tissue. Interspersed throughout the crown tissue of diseases plants were found, infrequently, small areas, appearing somewhat gummotic, that were surrounded by a dfferentiated healthy cambial area.

In the leaf tissues, several points of contrast were observed. Quite frequently, in the outer portion of the diseased leaf, a breakdown of the palisade layer was noted.

The palisade cells lost their typically elongate shape, became rounded and resembled spongy mesophyll cells. It was also noted that these cells, if they contained any chloroplasts at all, possessed but a very few. The macrosymptoms of the leaves of diseased plants, have often shown a puckering and wrinkling of the marginally chlorotic edges of leaves. The breakdown of the strength-giving palisade layer would allow this puckering condition to occur.

Also noted in the leaf-comparison, was the greatly increased number of calcium oxalate crystals surrounding the vascular bundles of diseased leaves over those of the healthy leaves.

2. Comparison of food reserves of healthy and diseased plants.

Material and Methods.

The diseased alfalfa material was obtained from plants showing varying stages of the disease and which originated in the Nicola valley. the previous summer. The healthy plant material was obtained from 6 months old Grimm seedlings, and from 2 year old Rhizoma plants grown in the greenhouse.

A series of diseased plants showing a graduation from slightly diseased to very badly diseased conditions were examined for starch reserves, along with representative healthy plants. Cross-sections of crown and root were made freehand with a razor blade and stained with I_2KI. Leaves from plants of the same series, were boiled in 70% ethyl alcohol until all color was removed from them, and then they were tested for starch using I_2KI.

At the suggestion of the author, Mr. George Setterfield of the Department of Botany carried out a somewhat more extensive microchemical tests on root crowns and stems of Witches' Broom diseased plants (2 stages - mildly diseased and severely diseased) and healthy alfalfa plants. This was performed as a microchemistry problem for Botany 534. He carried out tests for 14 of the organic materials and food storage forms commonly found in plants.

Observations

The crown series showed a definite correlation to the amount of starch stored with the stage of the disease. Healthy crowns contained a large quantity of storage starch, with a graduation down to no starch being present in the crowns in the poorest plant in the series. Although the poorest plant still showed considerable starch in the leaves, a progressive decline, as in the crowns, was observed.

Setterfield's results with the crown tissues and their starch storage support my findings. However, he found that there was more starch present in the diseased stems than in the healthy stems.

He found a very significant correlation between the stage of the disease and the storage of sucrose in the crowns. Healthy plant crowns showed negative tests for sucrose in all cases. Mildly diseased plants stored sucrose in moderate quantities in the crown; while severely diseased plants, that showed no starch storage in the crowns, showed a large quantity of sucrose stored in the crown tissue. Sucrose was not stored in the stems in either healthy or diseased plants.

DISCUSSION

Considerable additional knowledge on the distribution in British Columbia of the Witches' Broom of Alfalfa disease has been gained already by the studies completed. The centre of distribution in this province appears to be in the vicinity of Kamloops and the Nicola Valley, and extends northward (and radially) in the low rainfall regions between the Cascade and Rocky mountains. An unpublished oral report of the disease occurring in Alaska on alfalfa would extend the distribution quite far north. The presence of the disease is also reported from parts of Alberta and Saskatchewan, where again, it is appearing in the low rainfall regions. With the exception of a report in 1937 (not since observed) of the disease as occurring at Agassiz in the heavy rainfall area, all noted occurrences of the disease have been restricted to the low rainfall areas. This same distribution pattern is observed in Australia, where the disease only affects plants in the 17-21 inch annual rainfall areas.

Leafhoppers, notorious virus vectors, need a dry climate to reach a maximum population; and it is possible, that this is correlated with the observed distribution. The phenomenon of masking of disease symptoms, and the recovery of diseased plants, observed in the greenhouse studies at U.B.C., and also reported from Australia, may explain the observed distribution picture. Under good management and sufficient rainfall, it is possible, that the disease symptoms would be masked.

Besides the observation of recovery of the diseased plants in some cases, the study, so far, has brought out other salient points in disease symptomology. Witches' Broom disease has been shown to affect young alfalfa plants - one year after 6 months old seedlings were planted in the Kamloops nursery plot, primary symptoms were appearing in some cases. A continuing observation of these plants showed that the onset of the disease is very rapid, as by the end of 4 months very advanced symptoms of the disease ~~was~~ were noted. Observations on the disease transference by grafting at U.B.C. have shown that plants recorded as suspicious had inside of 2 weeks time developed the marked primary symptoms of proliferation and marginal chlorosis. This was followed by severe dwarfing in another months time, with necrotic symptoms appearing in yet another month.

Histologically, there appears to be very little mechanical differnce between diseased and healthy tissues, with the exception of size, and the further exceptions, detailed later, of leaf palisade abnormalities. All tissues and cells, of the diseased plants were observed to be much smaller than in thenormal plants. Gummosis was found frequently in the xylem elements of the crown of diseased plants especially in the older portions. Food storage of starch in healthy crowns was found to be diminished with the progress of the disease; and was replaced by food storage in the form of sucrose which is not stored in healthy alfalfa crowns. It is possible, and indeed probable, that the virus

may upset, or modify, the enzymatic balance of the crown so that this food storage change is brought about. It was also noted that all diseased crowns were severely rotted, and the rot was accompanied by (caused by?) an undetermined Basidiomycetous fungus. The presence of this fungus was not observed in any sections of the normal healthy crowns. This raises an interesting conjecture: Does the production and storage of sucrose, by implementing the growth of the fungus (sucrose is a favored fungous medium or nutrient), help to hasten the death of the plant by the rotting of the crown by the fungus?

In a comparison of cross-sections of healthy and diseased leaves(exhibiting marginal chlorosis) very marked differences were observed. In the outer portion of marginally chlorotic leaves a mechanical breakdown of the normal palisade layer was observed.to occur. The palisade cells rounded up and lacked orientation, and appeared devoid of chloroplasts (which would give the chlorotic coloration to this area of the leaf). The breakdown of the palisade layer would lower the structural strength of the leaves in their outer portions - this is manifested by the common symptom of diseased leaves showing a marginal puckering.

The chlorotic appearance of the infected plants produced one of the more pronounced field symptoms of the disease. The chlorotic appearance, accompanied by dwarfing of the plant combined to produce symptoms that were visible at considerable distances. In fact, many fields were observed

from the highway to contain diseased plants - observations confirmed by actual visits to the fields.

Over 135 fields were examined in the Interior of the province during the two summers, and 50 to 60% of these fields showed at least 5% infected plants. Disease incidence was generally correlated with management practices of the fields, but not always. Several diseased plants were observed in the alfalfa hay field at the Trnaquille farm, and this field was anything but mismanaged. The results of a quadrat study initiated last fall (1950) should point out many features in this respect.

A startling fact emerged from a study of quadrat data collected by Dr. Brink in 1942-44. When plotted in 1942, the quadrats contained a total of 140 healthy plants and 93 diseased plants. In 1944, no vestige remained of any of the 93 diseased plants; and only 33 out of the 140 healthy plants remained in that state, 27 of them were diseased, and 80 had disappeared altogether. To gain additional information on the fate of alfalfa plants in the field, 6 more quadrats were plotted in the Interior, largely in the Savona district. These will be observed at intervals for the next few years and more quadrats will be charted.

To augment the quadrat reports, a yield trial was carried out ~~at~~ in the greenhouse at U.B.C. during the past winter. Cuttings of comparable size were propagated in pots, and the 10 healthy and 10 diseased plants were harvested whenever the healthy plants started to flower. It was shown that there was a statistically significant differnce in yield

of the healthy plants over the diseased plants. On the 1st cutting, the healthy and diseased plants were shown to belong to the same population as far as weight of fodder was concerned. However, the 2nd cutting showed a reduction of 51.2% and the 3rd cutting a reduction of 36.6% in production of fodder by the diseased plants when compared to the healthy plants.

Quadrat reports may possibly cast light on the host range of the virus, and also on resistance in native stocks of alfalfa or closely related genera. In the hope of compiling data on the host range and on resistance, a twinning experiment has been designed. During the summer of 1950, 40 varieties and closely related species of alfalfa were planted in a randomized block in the nursery at U.B.C. To these will be added, this spring, plants received from other sources this past winter. Cuttings from diseased plants will be planted in close proximity to the healthy plants and observations made to determine if disease transference occurs. Included in the varieties under test are plants received from Dr. J.D.Menzies in Washington that are supposed to contain inherent disease resitance.

Disease transference from diseased plants of alfalfa to healthy alfalfa plants by means of graft experiments carried out in the summer of 1950. was observed at U.B.C. Out of 142 attempted crown grafts, using diseased scions and healthy Rhizoma stocks, 124 plants were living at the end of 7 months. Successful union of scion and stock was achieved in 31 cases, and disease tranference was observed

in 27 cases. All cases wherein the scion and stock were in union and both living showed a disease transference.

A grafting experiment, such as was performed, outside of showing a very good symptom sequence for disease recognition purposes, is of more interest to the plant pathologist than to the agronomist. A crown graft places strains on a plant that it seldom (if ever) receives in nature. The plant breeder requires techniques for testing large numbers of plants without seriously modifying the tested plants. In dealing with bacterial and fungous diseases, and, indeed, even with the easily transmitted mosaic type virus diseases, several procedures are available to the breeder.

However, in the difficultly transmitted virus diseases, transmission and testing is not such a simple matter. When the insect vector of the virus disease is known and available, resistance tests then become a relatively simple performance. The only known vector of Witches' Broom of Alfalfa, the leafhopper, Platymoideus acutus, has successfully resisted attempts at being reared, and indeed, is not considered to be the chief vector of the disease. Anyway, an easily employed vector, although it would be advantageous, is not essential to the plant breeder in determining field resistance.

Field resistance may not imply true immunity, but merely the fact that the plants can grow successfully in the presence of the disease. The possession of field resistance can readily be determined by interplanting diseased and healthy plants, as in the twinning experiment previously

discussed, preferably in an area where the disease occurs naturally and where the natural agents of inoculation reside. With this in mind, experiments of this type are planned to be initiated this year at points in the Interior where the disease is endemic.

SUMMARY

The distribution of the Witches' Broom disease of Alfalfa in British Columbia is shown to be confined to the areas of low rainfall. Severity of the disease is discussed on the basis of field and quadrat observations.

Quadrat and yield data are included. Diseased plants are shown to have a statistically significant reduction in yield compared to healthy plants under same conditions.

Symptomology of the disease is enlarged. Recovery of diseased plants is noted. A histological study of the disease shows gummosis in the vessels of the crowns, and mechanical breakdown of palisade cells of the leaves. Storage starch is shown to be depleted in the crowns, and instead food is stored in form of sucrose.

Successful transmission of the disease was achieved by crown grafts. Seed transmission, and inoculations with expressed sap gave negative results at present.

Experiments designed for the future study of the disease are discussed.

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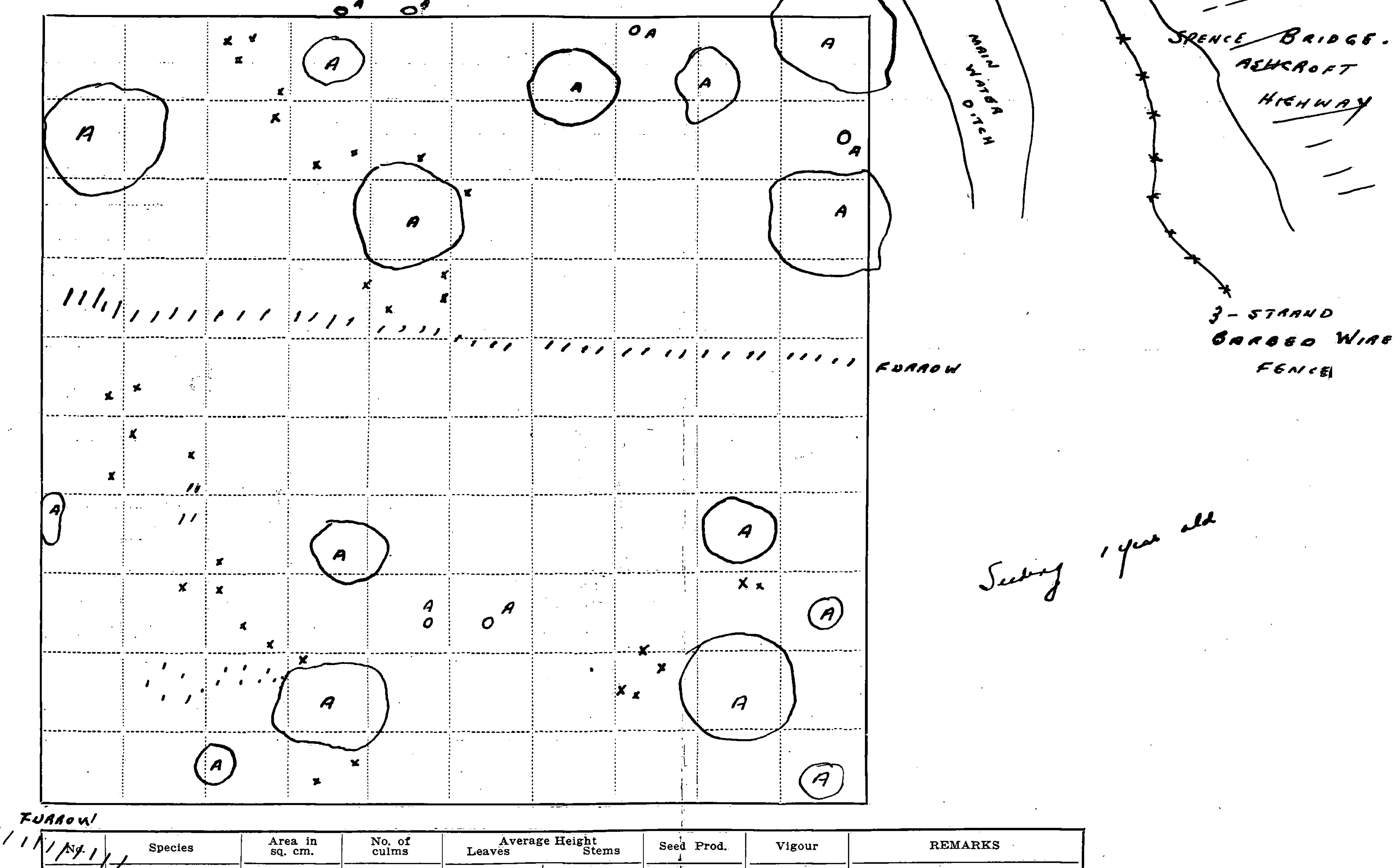
Quadrat No. 1-50   Date Charted Jly. 11/50



	No.	Species	Area in sq. cm.	No. of culms	Average Height Leaves	Stems	Seed Prod.	Vigour	REMARKS
Ⓐ	19	ALFALFA			18" cut	back to	4" - late	Seed	
/	13	DOWNY BROME							
x		SMOOTH BROME							
		SISYMORIUM ALTISSIMUM							

QUADRAT No. 1-50

Date Charted JULY 11, 1950 **Location** SPENCE'S BRIDGE

FIRST ALFALFA N.E. OF SPENCE FLAT (BLACK LOCUST GROVE) ON BENCH ABOVE S.W. SIDE OF MAIN BLACKTOP HIGHWAY; FIRST CORNER OF FIELD; CAIRN OF ROCKS MARKER IN ROADSIDE "DITCH". HAY CORRAL JUST IN SIGHT.

Character of Site. (a) Exposure and slope S.W.

(b) Altitude 800' (c) Soil LIGHT BROWN

Plant Type. (a) Formation CULTIVATED - ALFALFA - BROME (b) Association

(c) Principal species

SUMMARY OF QUADRAT DATA

(a) Grasses and Sedges.

									Totals
Symbol									
No. of Specimens									
Average height — leaves									
Average height — culms									
No. of culms									
Seed production									
Area occupied — sq. cm.									
Area occupied — %									

(b) Non-Grasses.

									Totals
Symbol									
No. of specimens									
Average height — veg. growth									
Average height — flower stalks									
No. flower stalks									
Seed production									
Area occupied — sq. cm.									
Area occupied — %									

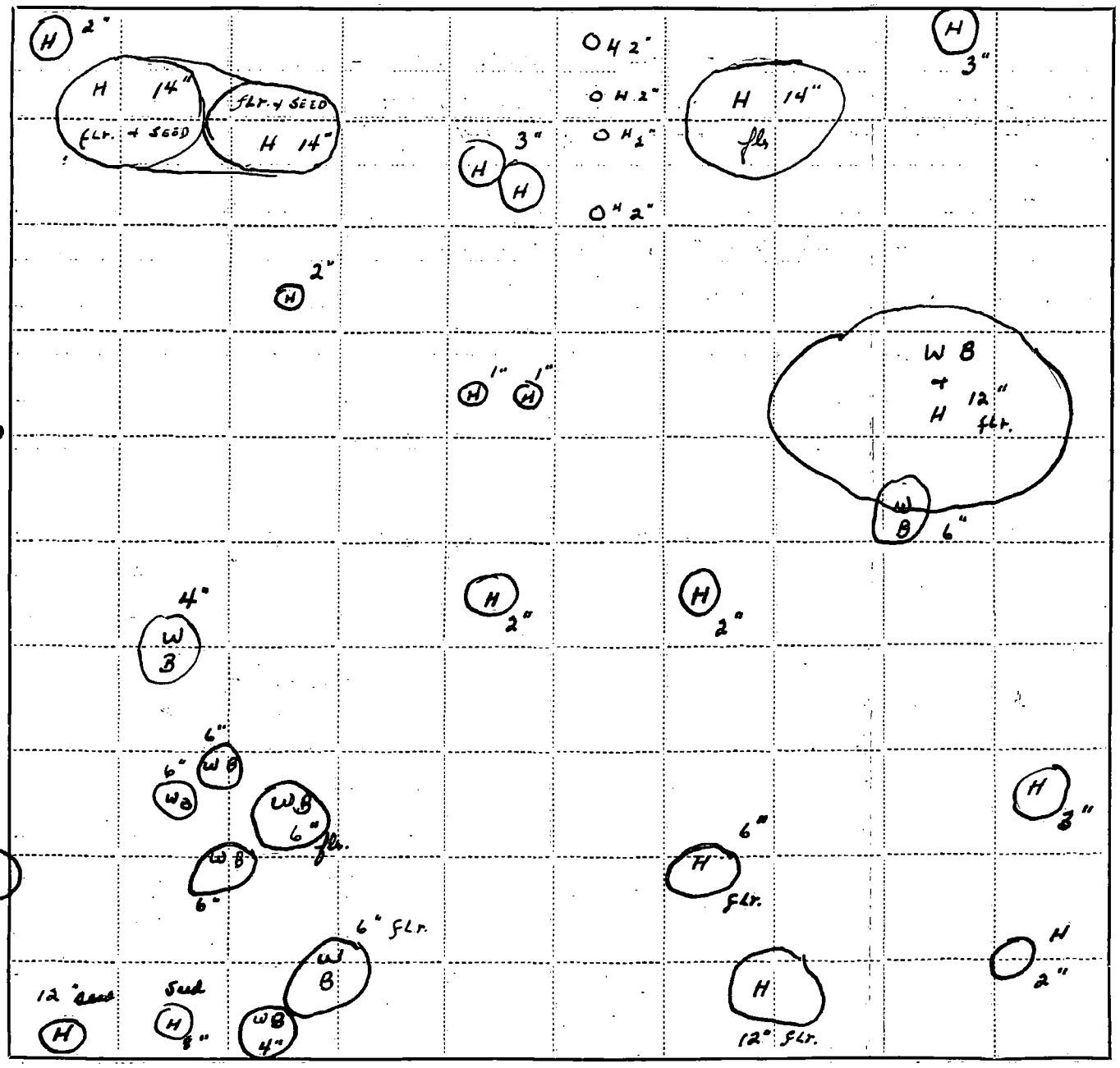
Relative Forage Value of Quadrat % **Carry-over**

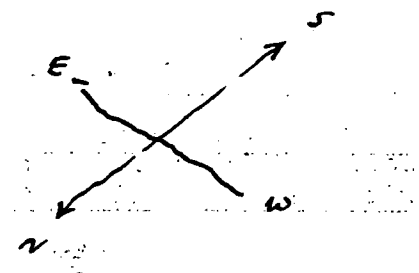
Distance of Quadrat from nearest watering place

Other Factors affecting grazing

Remarks

Quadrat No. 6-50 Date Charted Sept. 15/50





Second cut or third cut after;
Recovery peculiarly erratic both as to height and stage of development!

No.	Species	Area in sq. cm.	No. of culms	Average Height Leaves	Average Height Stems	Seed Prod.	Vigour	REMARKS
19	HEALTHY ALFALFAS							
9	W.B. ALFALFAS							

QUADRAT No. 6 - 50

Date Charted Sept. 18, 1950 Location Ashcroft Manor - Basque Ranch

near main highway at the foot of the Oregon Jack Creek Road; line up outhouse and conspicuous red soil outcrop; 8 paces from deep open furrow at edge of field and 12 paces from a brier patch with Clematis ligresticifolia.

Character of Site. (a) Exposure and slope N

(b) Altitude 1600' (c) Soil light brown, silt, alkali

Plant Type. (a) Formation Alfalfa - downy brome. (b) Association

(c) Principal species

SUMMARY OF QUADRAT DATA

(a) Grasses and Sedges.

									Totals
Symbol									
No. of Specimens									
Average height — leaves									
Average height — culms									
No. of culms									
Seed production									
Area occupied — sq. cm.									
Area occupied — %									

(b) Non-Grasses.

									Totals
Symbol									
No. of specimens									
Average height — veg. growth									
Average height — flower stalks									
No. flower stalks									
Seed production									
Area occupied — sq. cm.									
Area occupied — %									

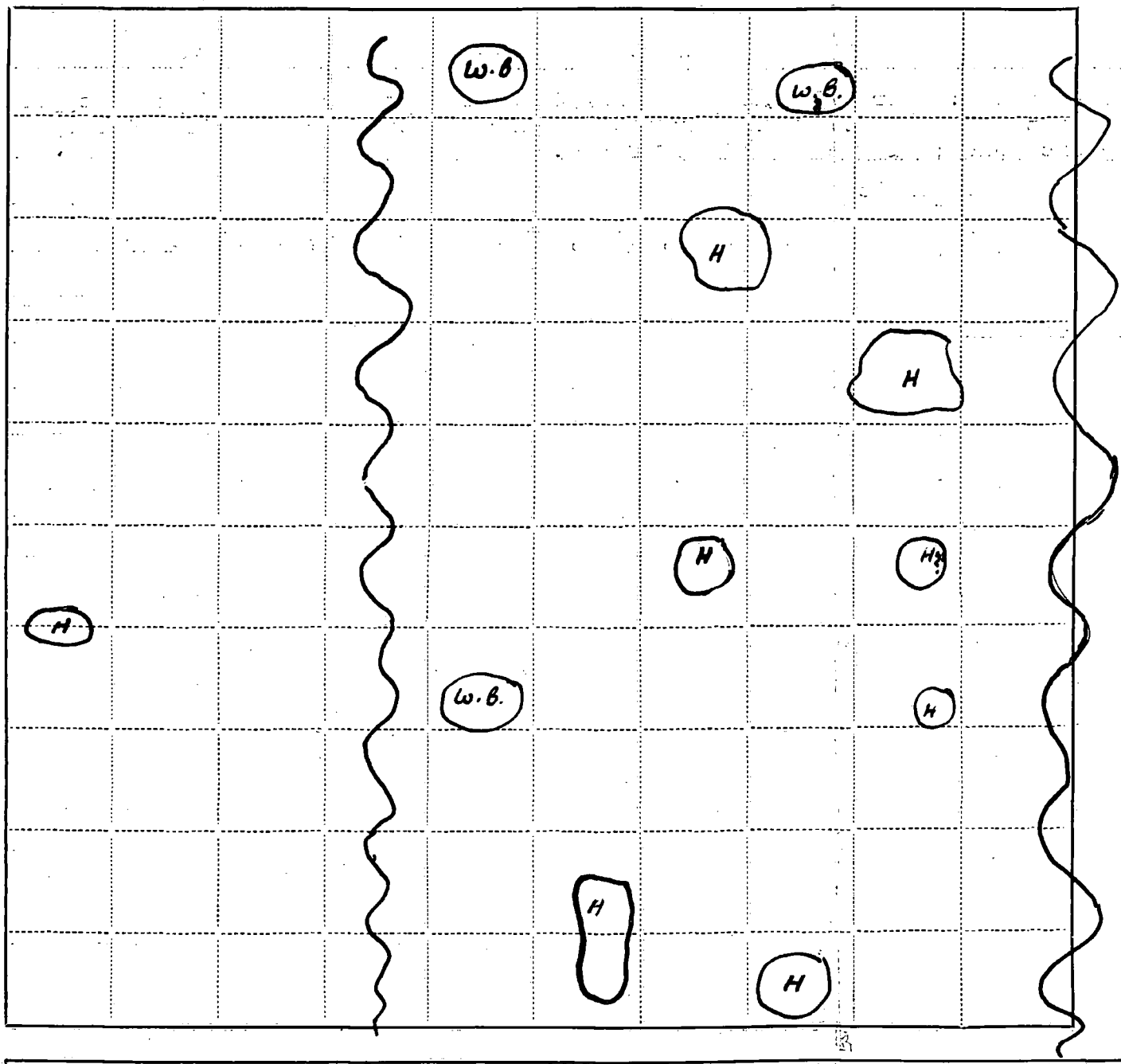
Relative Forage Value of Quadrat % **Carry-over**

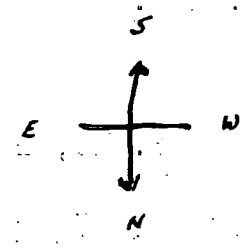
Distance of Quadrat from nearest watering place

Other Factors affecting grazing

Remarks

Quadrat No. 2-50 Date Charted SEPT. 18, 1950





IRRIGATION FURROWS

QUADRAT VERY DRY

DODDER NEAR BY - A MODERATE INFESTATION

No.	Species	Area in sq. cm.	No. of culms	Average Height Leaves	Average Height Stems	Seed. Prod.	Vigour	REMARKS
8	HEALTHY ALFALFAS			" "				RECENTLY MOWN
3	WITCHES' BROOM -			4"				

Mileage No. 139.2

V.C.Brink

QUADRAT No. 2 - 50

Date Charted Sept. 18, 1950 Location SAVONA

S of main highway - Kamloops - Savona; near telephone pole 11/34 KA 112 with cross support on opposite side (S) side of highway; quadrat in field S. of highway between irrigation furrows which line up with 2 above mentioned telephone poles; 21 paces south of fence.

Character of Site. (a) Exposure and slope N, gentle

(b) Altitude 2200' (c) Soil light brown, till, fine sandy loam, small stones

Plant Type. (a) Formation (b) Association

(c) Principal species Alfalfa - salsola

SUMMARY OF QUADRAT DATA

(a) Grasses and Sedges.										Totals
Symbol										
No. of Specimens										
Average height — leaves										
Average height — culms										
No. of culms										
Seed production										
Area occupied — sq. cm.										
Area occupied — %										

(b) Non-Grasses.										Totals
Symbol										
No. of specimens										
Average height — veg. growth										
Average height — flower stalks										
No. flower stalks										
Seed production										
Area occupied — sq. cm.										
Area occupied — %										

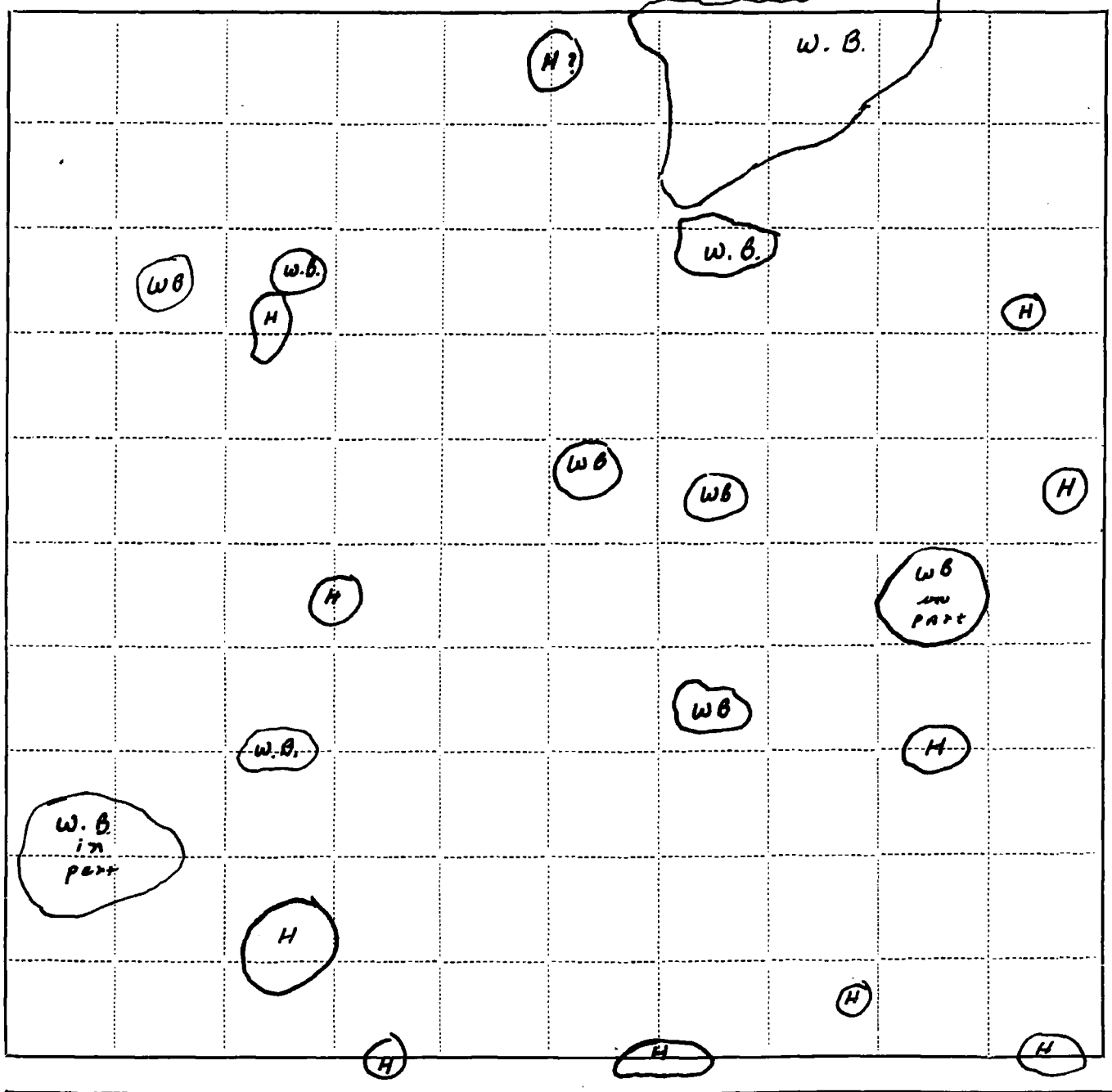
Relative Forage Value of Quadrat % **Carry-over**

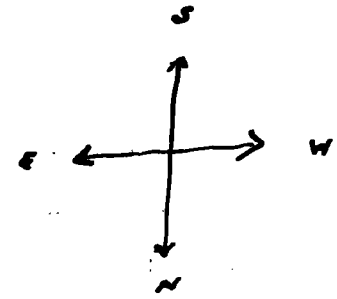
Distance of Quadrat from nearest watering place

Other Factors affecting grazing

Remarks

Quadrat No. 3-50 Date Charted SEPT. 18/50





No.	Species	Area in sq. cm.	No. of culms	Average Height Leaves	Stems	Seed Prod.	Vigour	REMARKS
10	ALFALFAS	WITH	WITCHES'	BROOM 5"				
11	"	HEALTHY		10"				

V.C.Brink

QUADRAT No. 3 - 50

Date Charted Sept. 18, 1950 **Location** Savona

Near (W of) quadrat 2 - opposite pole 11/27 between fence posts 3 & 4 west of one carrying a 'No Hunting' sign; quadrat located 21 paces S of fence

Character of Site. (a) Exposure and slope As for Q-2-50

(b) Altitude do (c) Soil do

Plant Type. (a) Formation do (b) Association

(c) Principal species do

SUMMARY OF QUADRAT DATA

(a) Grasses and Sedges.

									Totals
Symbol									
No. of Specimens									
Average height: leaves									
Average height: culms									
No. of culms									
Seed production									
Area occupied: sq. cm.									
Area occupied: %									

(b) Non-Grasses.

									Totals
Symbol									
No. of specimens									
Average height: veg. growth									
Average height: flower stalks									
No. flower stalks									
Seed production									
Area occupied: sq. cm.									
Area occupied: %									

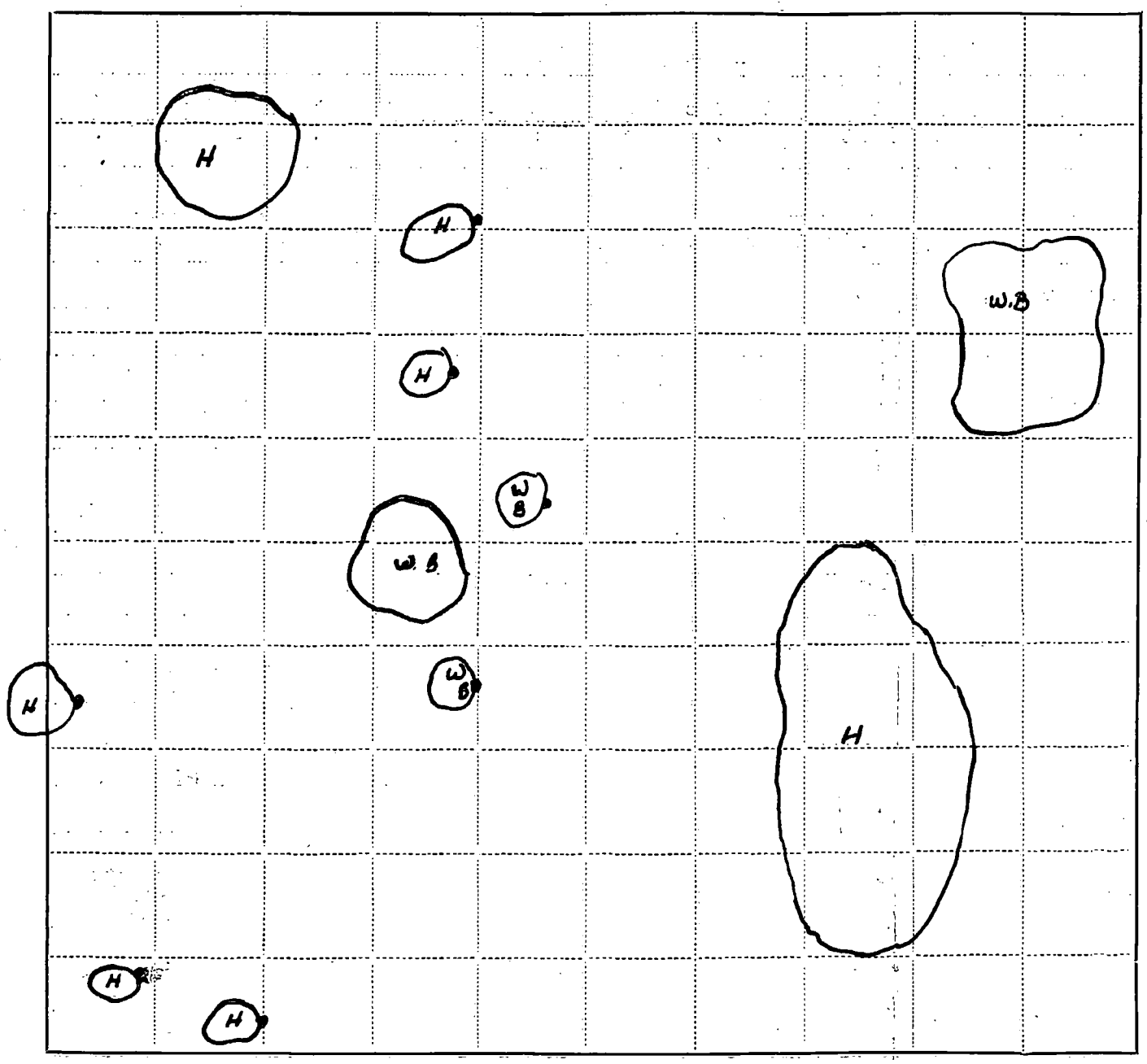
Relative Forage Value of Quadrat % **Carry-over**

Distance of Quadrat from nearest watering place

Other Factors affecting grazing

Remarks

Quadrat No. 4 - 50 Date Charted Sept. 18/50



A new seeding with many old plants remaining in the field, these marked ○ were plants of the new seeding less than one year old; some showed witches' broom!

No.	Species	Area in sq. cm.	No. of culms	Average Height Leaves	Average Height Stems	Seed Prod.	Vigour	REMARKS
4	WITCHES BROOM ALFALFAS			7"				
7	HEALTHY ALFALFAS			12"	Recently mown			

mileage No. 142.4 V.CBrink

QUADRAT No. 4 - 50

Date Charted Sept. 18, 1950 Location SAVONA

West of Q 2-50 and Q 3-50about 2 miles along highway in field on south side of highway between telephone poles 9/7 KA 59 and 9/6; quadrat located about 2 paces inside (S) fence. West of gate.

Character of Site. (a) Exposure and slope

(b) Altitude (c) Soil light brown, sandy

Plant Type. (a) Formation Alfalfa-downy brome, cultivated (b) Association

(c) Principal species

SUMMARY OF QUADRAT DATA

(a) Grasses and Sedges.

									Totals
Symbol									
No. of Specimens									
Average height — leaves									
Average height — culms									
No. of culms									
Seed production									
Area occupied — sq. cm.									
Area occupied — %									

(b) Non-Grasses.

									Totals
Symbol									
No. of specimens									
Average height — veg. growth									
Average height — flower stalks									
No. flower stalks									
Seed production									
Area occupied — sq. cm.									
Area occupied — %									

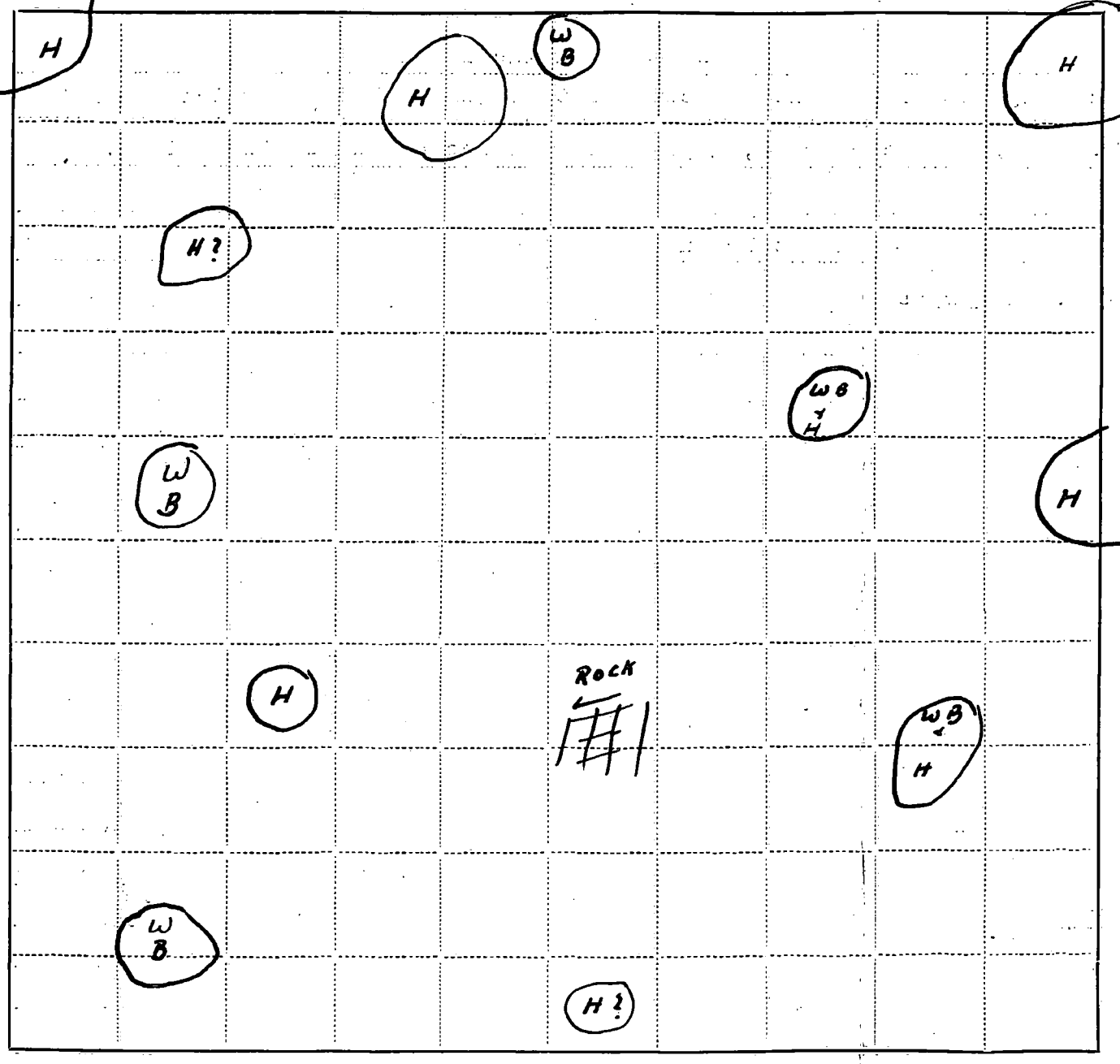
Relative Forage Value of Quadrat % **Carry-over**

Distance of Quadrat from nearest watering place

Other Factors affecting grazing

Remarks

Quadrat No. 5-50 Date Charted SEPT. 18/50



field amazingly productive in spite of this stand. large lay stacks plants tall & vigorous. grassing is with Kentucky Bluegrass.

No.	Species	Area in sq. cm.	No. of culms	Average Height Leaves	Average Height Stems	Seed. Prod.	Vigour	REMARKS
3+2	DOUBTFUL	W. B. ALFALFAS		17"				
5+2	"	HEALTHY	ALFALFAS	22"	BLOOM			

Mileage No. 165.65

QUADRAT No. 5 - 50

Date Charted Sept. 18, 1950 Location Wallachin

Near "Hoodoo Cliffs" Wallachin; Hay stacker and lone deciduous tree make a line on which quadrat is located in the field, S of highway to Cache Creek, 9 paces from fence and irrigation ditch. (Haywards)

Character of Site. (a) Exposure and slope S

(b) Altitude 1600' (c) Soil light brown

Plant Type. (a) Formation Alfalfa - K. bluegrass (b) Association

(c) Principal species

SUMMARY OF QUADRAT DATA

(a) Grasses and Sedges.									Totals
Symbol									
No. of Specimens									
Average height: leaves									
Average height: culms									
No. of culms									
Seed production									
Area occupied: sq. cm.									
Area occupied: %									

(b) Non-Grasses.									Totals
Symbol									
No. of specimens									
Average height: veg. growth									
Average height: flower stalks									
No. flower stalks									
Seed production									
Area occupied: sq. cm.									
Area occupied: %									

Relative Forage Value of Quadrat % **Carry-over**

Distance of Quadrat from nearest watering place

Other Factors affecting grazing

Remarks