PROBLEMS RELATED TO BREEDING ALFALFAS FOR RESISTANCE TO BLACK-STEM DISEASE

by

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TABLE OF CONTENTS

STATEMENT OF PROBLEM[1]

Alfalfa ranks high in importance among forage crops. Its prominence is due to its forage value and soil building characteristics and is emphasized by the fact that 35 million acres were devoted to hay production throughout the world in 1929 (8). A proportionately large acreage was devoted to seed production where conditions were favorable for it.

Many state experiment stations and the United States Department of Agriculture cooperating with state experiment stations have alfalfa breeding and improvement work in progress. One of the important problems with which they have to deal is disease resistance (20).

Diseases attack all parts of the alfalfa plant. Those which attack leaves and stems are especially important in wet seasons and in humid regions. Of all the leaf and stem diseases which attack alfalfa in Kansas and also in the eastern part of the United States, black-stem disease caused by Ascochyta imperfecta Peck has been outstanding.

It was the opinion of the workers at the Kansas Station

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[1]This study was made in collaboration with the Department of Botany and Plant Pathology, Kansas State College and the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

and the Division of Forage Crops and Diseases that this disease was of sufficient economic importance to justify a more comprehensive and detailed study than had hitherto been made.

The ultimate goal of such a study was to select, breed and develop strains of alfalfa which have resistance to black-stem disease. Before much of this kind of work could be done, however, certain pathological phases of the problem had to be learned. This included a knowledge of methods of securing suitable inoculum for laboratory testing and methods of making inoculations. It was also necessary to find ways of producing and controlling the disease under field conditions. The next step was to find out if varieties and plants within a variety varied in susceptibility. This was important because resistant strains could be produced only if this variation occurred.

This study has been devoted to working out the various problems and techniques in connection with breeding alfalfas for resistance to black-stem disease.

CAUSAL ORGANISM OF BLACK-STEM DISEASE

The literature on black-stem disease of alfalfa is limited and confusing. The scarcity of information on this disease may be due to a lack of appreciation of the damage it does. The more outstanding publications have made their

appearance within the last ten years. The most recent of these, an article by Toovey, Waterston and Brooks (19), has clarified much of the confusion concerning the organism involved.

Valleau and Fergus (21) in 1929 reported a disease of alfalfa, sweet clover and red clover in Kentucky which they called black-stem. The causal organism was not known but it was thought to be one of the fungi. In 1933 Johnson and Valleau (6) report further work on the disease and found that a different organism was involved on each of the three legumes. The black-stem of alfalfa was attributed to Phoma medicaginis Malbr. and Roum. Remsberg and Hungerford (11) reporting on black-stem of alfalfa in Idaho in 1936 considered the disease was the same as reported by Valleau and Fergus and Johnson and Valleau in Kentucky.

Toovey, Waterston and Brooks (19) of England sent for cultures reportedly causing black-stem in widely separated areas of the United States. They received cultures of Phoma medicaginis Malbr. and Roum. from Dr. F. R. Jones of Wisconsin and Dr. E. M. Johnson of Kentucky. In addition they received cultures of Ascochyta imperfecta Peck from Jones and from D. R. Sprague of Ohio. They made careful comparisons of these cultures with the British fungus causing black-stem. After observing their reaction to various tests

they concluded that all of these cultures were the same species of fungus. Dr. F. R. Jones to whom they sent cultures of the British fungus was of the opinion that all of these fungi were identical. Toovey et al. decided to refer to the fungus which causes the black-stem disease of alfalfa as Ascochyta imperfecta Peck.

Rosella (13) in 1929 described a disease of alfalfa in France which he attributed to Ascochyta medicaginis Fuck. and Corneli (2) in 1932 reported a disease of alfalfa in Italy caused by Phyllosticta medicaginis (Fuck.) Sacc. Concerning these, Toovey et al. state, "The symptoms of the diseases they describe are similar to those of the black-stem disease in Britain, and it may well be that the causative fungus is the same, viz. Ascochyta imperfecta."

Melchers (9) reported alfalfa stem lesions in Kansas in 1918 which he attributed to various organisms. He is now confident that some of these injuries were due to Ascochyta imperfecta.[2]

Richards (12) reported an outbreak of what he referred to as stem blight in Utah in 1933. He did not name the causal organism but his description of the disease was very similar to the signs and symptoms of the Ascochyta black-stem.

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[2]Personal conversation with Professor L. E. Melchers.

In this thesis, references made to papers dealing with Phoma medicaginis, Ascochyta medicaginis, Phyllosticta medicaginis, and the unnamed organism causing black-stem reported by Valleau and Fergus (21) and Richards (12) will be treated as the black-stem disease under consideration.

The first record of Ascochyta imperfecta on alfalfa was made in 1908 by Stewart, French and Wilson (17) of the New York Agricultural Experiment Station at Geneva. They referred to the disease as an Ascochyta and probably an undescribed species. In 1911 C. H. Peck, the New York State Botanist, named the organism and described the disease (10). Following is his original description of Ascochyta imperfecta:

> Spots variable, 4-12 mm. in diameter, amphigenous, orbicular, semicircular or subtriangular, the larger ones usually terminal or marginal, pale brown or smoky brown, not sharply defined; perithecia amphigenous, few, depressed, .3-.6 mm. broad, brown or blackish brown; spores variable, continuous or pseudouniseptate, oblong or subcylindric, obtuse, hyaline, 6-15 x 2.5-4 M.

GEOGRAPHICAL DISTRIBUTION AND ECONOMIC IMPORTANCE

The geographical distribution of black-stem is not entirely known. In America it has been reported in New York (17), Kentucky (6, 21), Kansas (9), Utah (12), Arizona (1), and Idaho (11). It undoubtedly occurs in other states where alfalfa is grown. In England Toovey, Waterston and Brooks (19) reported the disease had been found in Hertfordshire,

Suffolk, Norfolk and Bedfordshire. A similar disease was reported in Wales. Corneli (2) and Rosella (13) reported the disease from Italy and France, respectively. Gill (4) found a stem spot at White River, Transvaal, South Africa which he called a Phoma species. This might be the black-stem under consideration.

Johnson and Valleau (6) reported that during long wet springs black-stem kills back the early crop of shoots forcing new crown buds to push out from below the soil surface. This greatly reduced the growth of alfalfa and on poor soil during a favorable black-stem season infection was severe enough to destroy the stand.

Richards (12) in Utah reported a loss of 40 to 50 per cent of the yield during a severe outbreak following a cool moist spring. The disease assumes economic importance, according to Toovey, Waterston and Brooks (19), where alfalfa is grown on a large scale for manufacturing alfalfa meal. This is because the quality of the meal depends upon the color of the foliage and the leaf-stem ratio. Stewart, French and Wilson (17) in 1908, George Stewart (18) in 1926, and Brown and Street (1) in 1934 all reported the disease to be of minor importance.

SYMPTOMS OF BLACK-STEM DISEASE

The symptoms of black-stem disease as it occurs in the field and on artificially inoculated plants have been carefully noted and recorded. A detailed knowledge of symptoms was important for identification of the disease and for working out a method of making disease readings. The detailed description which follows should be of value to other investigators working with this or similar diseases.

Field Symptoms

The first appearance of black-stem disease on the stems of alfalfa was in the form of small black spots surrounded by more or less water-soaked appearing regions. These spots sometimes appeared to be slightly raised. As the disease progressed these spots grew and began to coalesce and finally the entire stem became blackened. The stem usually died when this condition had been reached.

Plate I shows a healthy stem on the left and progressing stages of diseased condition on the stems to the right. These diseased stems if allowed to lie in the field over winter develop numerous pycnidia by early spring. The appearance of pycnidia on overwintered stems is shown in Plate II.

EXPLANATION OF PLATE I

The stem on the left is free from disease. The other five stems show, from left to right, progressive stages of diseased condition. The first diseased stem shows numerous small black lesions. The second shows these lesions beginning to coalesce and the last three stems show increasing amounts of blackened stem tissue.

Plate I

EXPLANATION OF PLATE II

This plate shows numerous pycnidia on overwintered alfalfa stems. Each tiny black spot is the top of a flask-shaped pycnidia with an ostiole through which spores are released during the rainy spring season. The removal of stems such as these reduced the amount of disease on plots of alfalfa.

Plate II

The disease appeared on the leaves as small dark spots which may or may not be surrounded by chlorotic areas. The color of the spots varied from a sooty black to brown. As the disease progressed the spots coalesced to form irregular shaped darkened lesions. If the spots were numerous the leaf soon turned yellow and withered. Early and late stages of leaf spots are shown in Plate III. In the field repeated infection of leaves took place to give various aged lesions on a single leaf. The appearance of lesions of various ages on a single leaf are clearly shown in Plate IV.

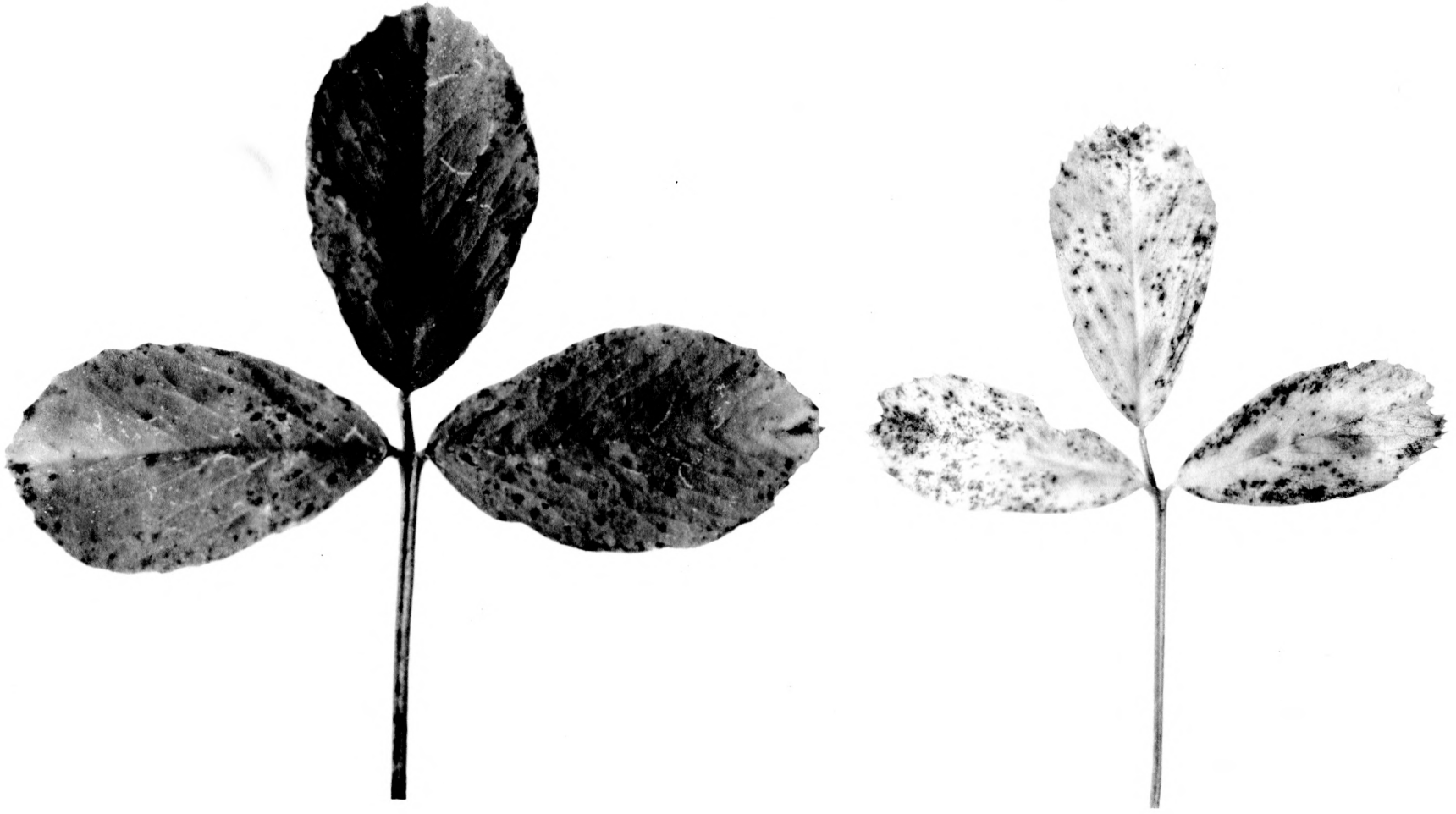
The petiole lesions were similar to those found on the stems. The petioles being small in diameter were more quickly girdled than stems. Girdling of a petiole often resulted in the death of the attached leaves. Petiole lesions are shown in the left figure of Plate III.

The general appearance of infected leaves and stems is illustrated in Plate V. The two stems on the left are healthy and the other four show varying degrees of infection. The height to which defoliation occurs on a stem is an important characteristic by which to estimate the severity of infection. This photograph shows the more severely infected stems to be defoliated to a greater extent. Some of the withered leaves and petioles which have not yet fallen can be seen on the stems to the right.

EXPLANATION OF PLATE III

The figure on the left shows early stages of leaf and petiole lesions. Some of the marginal leaf lesions already are beginning to coalesce. The figure on the right shows yellowed leaflets resulting from numerous infection points. In this case the number of spores which gained entrance to start infection was so great that the leaf began to die before the lesions had time to become very large.

Plate III



EXPLANATION OF PLATE IV

Lesions of various ages on a single leaflet are shown in this plate. This is characteristic of leaf lesions in the field as infection of a single leaf may take place from time to time when conditions are favorable. The oldest lesions which are the large ones shown in this picture are not so abundant and consequently the leaflets have not yet been killed.

Plate IV

EXPLANATION OF PLATE V

The two stems on the left are healthy while the other four show increasing degrees of severity. The more severely diseased stems are defoliated to a greater extent. The withered diseased leaves and petioles can be clearly seen in this picture.

Plate V

Greenhouse Symptoms

Artificially inoculated plants under very favorable conditions showed tiny darkened spots on the leaves about four days after inoculation. These spots could best be seen when the leaf was held to the light. They were first circular in shape and uniform in size. The marginal leaf spots grew more rapidly than those in the center of the leaf and very soon lost their regularity in shape. The lesions in the middle of the leaf also lost their regularity in shape as they became older. This was due mostly to coalescing of lesions or death of tissue between two rather large lesions.

The lesions continued to grow until the leaf became withered and fell. Plate VI shows the appearance of the lesions at various intervals of time after inoculation. The leaf to the left shows lesions appearing four days after inoculation. The second and third leaves show lesions nine days after inoculation and the fourth and fifth leaves show lesions 21 days after inoculation.

Alfalfa varieties, and to a lesser extent plants within a variety, varied in four important ways. They varied in regard to the number of spots present on a leaf, their rapidity of growth, their color, and the presence or absence of a chlorotic area around the lesion.

EXPLANATION OF PLATE VI

Lesions of various ages resulting from artificial inoculation are shown in this plate. The leaflets on the left show tiny lesions beginning to appear just four days after inoculation. Careful inspection reveals small chlorotic areas around these lesions. The spots on the second and third leaves show lesions nine days after inoculation, while the fourth and fifth leaves show the lesions 21 days after inoculation. Only leaves with few lesions will still remain attached to the plant 21 days after inoculation.

Plate VI

Plate VII illustrates how individual plants may vary in regard to number of spots. This photograph shows representative leaves of four plants inoculated at the same time and kept under identical conditions. The varieties represented from left to right are Turkestan 86696, Turkestan 19304, Grimm and Ladak. This photograph shows an extreme case of variation in number of spots on individual plants and is not in any way intended to show variety reaction to black-stem.

In some plants the lesions practically ceased growth when the plant was removed from the moist chamber. In others growth was very rapid and soon resulted in defoliation of the plant. Lesions varied in color from black to brown. Occasionally they were surrounded by a distinct chlorotic area while in other instances the brown or black lesion shaded directly into apparently green healthy tissue.

The lesion itself in some cases showed a decided zoning. This was not a consistent characteristic of certain plants as lesions of a single leaf varied in respect to this zoning characteristic.

Petiole lesions were very common. They were found to form a blackened streak along one edge of the entire petiole in some cases while in other instances they girdled the petiole in one restricted area causing it to break at this point. In the latter case the leaves soon withered and fell.

EXPLANATION OF PLATE VII

Each of the four groups of leaves are representative of the amount of disease on the plant from which they were taken. The varieties represented are from left to right Turkestan 86696, Turkestan 19304, Grimm and Ladak. This shows the extreme range of amount of disease on plants inoculated under identical conditions. It is not intended to show the variety ranking in susceptibility.

Plate VII

MATERIAL AND METHODS

Excellent facilities have been available for work on this problem. At the Agronomy farm there have been available for observational and experimental purposes 900 alfalfa rod rows representing over 400 strains and varieties. These included strains from India, Turkey, Afghanistan, New Zealand, Brazil, Russia, Persia, Argentina, Canada, Italy, China, Australia and Africa. In addition there has been the Uniform Alfalfa Nursery which contained 135 improved strains from many sections of the United States. There were also 54 square rod plots and 84 plots which are 1/163 acre in size. Three field scale plots containing the varieties Kaw, Kansas Common and Ladak were also available for experimental purposes. These plots were in strips 75 feet wide and extended throughout the length of the field.

Greenhouse and laboratory space and equipment belonging to both the Departments of Botany and Plant Pathology and Agronomy have been available for use in this study.

Methods of Making Disease Readings

It was extremely important in a study of this kind that an adequate disease reading method be adopted which would meet all the requirements to which it was to be put. This

problem became apparent at the very outset of the work and continued to be a problem throughout the study. Various methods were tried and found suitable for certain needs and inadequate for others.

Naturally, various systems could not be tried and compared until the disease made its appearance. When it did appear, however, it was time to make readings on the various varieties and experiments which had been planned, allowing little time for a comparative method study. For these reasons data are reported using various methods throughout the study. This may bring about some confusion if attempts are made to compare readings on separate experiments. It is hoped that the reader will make allowance for these difficulties.

Following is a detailed description of every method tried upon which data are reported. In the discussion at the end some comments will be made concerning the merits of these various methods.

Richards' Method. Richards (12) described a method of making black-stem readings of alfalfa by which he derived a coefficient of susceptibility. The method has been used in this study for making readings of diseased plots in the more advanced stages of the disease. A sample consisted of all the stems which were within a ring cast into the plot. The

method as he described it was to separate these diseased stems into six classes depending upon the degree of severity exhibited. The classes were very light, light, average, severe, very severe and dead. These classes were given numbers from one to six with one being the class of least severity. To arrive at a coefficient of susceptibility the number of stems in each diseased class was multiplied by the index number for the class and the sum total thus derived was divided by the number of diseased and disease-free stems collected. In the study to be reported the disease-free or zero index number was not used. Table 1 shows the disease readings on six square foot samples taken on each of two plots which differed in the amount of disease present.

Table 1. Black-stem readings taken by Richards' method on two plots of alfalfa.

Sample number	Disease class index number 1	2	3	4	5	6	Sample score
			Ladak - Cleaned				
1	81	8					1.090
2	71	9					1.113
3	86	7					1.075
4	61	16	2				1.253
5	61	17					1.218
6	68	30	1				1.323
						Mean	1.179
			Kansas Common - Fall Growth				
1		7	15	25	4	2	3.604
2	3	5	10	19	4		3.390
3	1	11	18	20	4		3.278
4		8	23	15	4		3.300
5	2	7	5	21	3		3.421
6	1	8	14	18	2		3.279
						Mean	3.379

This method was found to be inadequate for disease readings for mild infection or for early stages of the disease. This was because little stem infection was present under these conditions and the stems were a major consideration in making readings by Richards' method. It was difficult to determine by mere observation of the diseased and disease-free leaves on a stem, into what disease class the stem and attached leaves should be placed. Practically all the stems in a sample had some leaves which were diseased to a greater or less degree of severity and some which were

disease free. Obviously a detailed method needed to be devised which would indicate how many of these leaves were diseased and how severe the disease was on these leaves.

Readings for Mild Infection. A method suitable for making disease readings on plants during the early stages of the disease was devised. The method allowed for making finer distinctions than could be made by Richards' method. Since the procedure was much more detailed, it necessarily was more tedious and consequently more restricted in its use.

The readings were made on leaves and stems which were collected at random throughout the plot. These stems were cut off near the ground with a pocket knife. Each stem constituted a sample.

The primary leaves at the first six nodes from the bottom of the plant were classified into severity groups ranging from one to five inclusive. The higher numbers indicated more severe disease. To aid in classifying leaves a scale was prepared for making comparisons. This consisted of a glass-covered mount with representative leaves to illustrate the various degrees of infection.

The primary leaves referred to leaves on petioles to which stipules were attached. If no stipules were attached to what appeared to be a petiole the off shoot was

considered a branch and not a primary leaf. In the event a primary leaf was also present at the node it was classified. However, if none was present the leaf at that node was considered as fallen. Fallen leaves were placed in class five. This was based upon the assumption that falling was caused by the disease. Although some falling of leaves was probably due to other causes it was not believed that this would have an erroneous influence on the reading since falling due to causes other than the disease should be uniform on all plots.

The next step was to get a single figure on the one to five basis which indicated the condition of the leaves of that sample. For this purpose the following formula was used: $X = \frac{(C \times N) + \ldots + (C \times N)}{T}$

X = score for the leaves on a single stem

C = disease class number for leaves

N = number of leaves in the disease class

T = number of leaves on the stem which were classified

The leaf score for stem one on the Kansas Common - Cleaned plot in Table 1 was derived by substitution in this formula as follows: $X = \frac{(1 \times 2) + (2 \times 1) + (5 \times 3)}{6} = 3.17$

The stems were also classified on the one to five basis and this figure was added to the leaf score to give the sample a reading on the one to ten basis.

Table 2 illustrates the detailed method of disease readings for ten samples each on two plots which varied in amount of disease present.

Table 2. Detailed method of disease readings for slight infection on two plots of alfalfa.

Stem number	Disease class for leaves						Leaf score	Stem score	Sample score
	1	2	3	4	5 Attached	5 Fallen			
Kansas Common - Cleaned									
1	2	1				3	3.17	1	4.17
2	3	2	1				1.67	1	2.67
3	3	2				1	2.00	1	3.00
4		2			2	2	4.00	1	5.00
5	2	1			1	2	3.17	1	4.17
6	4					2	2.33	1	3.33
7	3			1	1	1	2.83	1	3.83
8		2			2	2	4.00	1	5.00
9	4					2	2.33	1	3.33
10	4			1		1	2.17	1	3.17
								Mean	3.77
Kansas Common - Fall Growth									
1					2	4	5.00	2	7.00
2					1	5	5.00	3	8.00
3				1	2	3	4.83	2	6.83
4				1	1	4	4.83	2	6.83
5		1			3	2	4.50	1	5.50
6	1	1			1	3	3.83	2	5.83
7					2	4	5.00	3	8.00
8						6	5.00	2	7.00
9			1	1	2	2	4.50	2	6.50
10				1	1	4	4.83	2	6.83
								Mean	6.83

Sample Size. A preliminary study was made to determine the number of samples necessary to get a plot reading within a desired degree of accuracy. For this purpose 120 samples were taken from one plot and readings made according to the method just described. The mean of the 120 samples was 6.63. The standard deviation was .8265 which gave a standard error of .0754 as derived from the formula. Standard error = $\frac{\text{standard deviation}}{\sqrt{\text{number}}}$

An error of .12 was arbitrarily chosen as satisfactory for these readings. Substituting .12 for .0754 in the above formula the number of samples could be reduced from 120 to 47.3. Fifty samples were taken from each plot in the work later to be reported.

A Simplified Method. The detailed method of disease readings as described above probably represents a high degree of accuracy but the procedure was quite laborious and time-consuming. A careful study was made of the data collected by this detailed method to see if a simplified procedure of this method could be worked out. It was found that classes two, three and four added nothing to the data since the number of leaves in those classes was practically the same regardless of the severity of the disease on the plots from which the samples were taken. The percentage of

leaves in class one varied inversely with the leaf reading. The number of leaves in class five varied directly with the leaf reading and appeared to be more closely related to it than did the number of leaves in class one.

Correlations were run between the number of leaves in class five and the leaf score on part of the data collected in the detailed manner. Table 3 shows the computation of this relationship. The X values are for the leaves in class five and the Y values are for the leaf score.

Table 3. Computation of correlation between the number of leaves in class five and leaf score.

$SX = 371$	$SY = 373.70$	$n = 90$
$\bar{x} = 4.12$	$\bar{y} = 4.15$	
$SX^2 = 1723$	$SY^2 = 1616.07$	$SXY = 1646.55$
$(SX)^2/n = 1529.34$	$(SY)^2/n = 1551.68$	$(SX)(SY)/n = 1540.47$
$Sx^2 = 193.66$	$Sy^2 = 64.39$	$Sxy = 106.08$

$$r = Sxy/\sqrt{(Sx^2)(Sy^2)} = 106.08/\sqrt{(193.66)\ (64.39)} = .9499$$

According to Fisher (3) a correlation of .2175 is the level of significance at the five per cent point and .2704 for the one per cent point for 88 degrees of freedom. The correlation is highly significant. Data will later be reported using this simplified procedure with certain

modifications. Instead of using one stem as a sample, the number of stems within one square foot constituted a sample. The number of nodes at which counts were made depended upon the amount of defoliation at the time the readings were made. For example, readings made when the disease was in its early stages might be made on the first six nodes while later as the disease progressed perhaps eight or even all the nodes on a stem might be taken into consideration. Readings made at two different times could not be compared if this were done.

Readings for Artificially Inoculated Plants. Disease readings on artificially inoculated plants in the greenhouse presented a different problem from disease reading in the field. Theoretically all leaves of an artificially inoculated plant regardless of position had equal chances of becoming infected. Therefore, all the leaves were considered in making the plant reading. Since the lesions on the leaves varied in respect to both number and size, consideration was given to both these factors. Stem lesions were also considered in making readings.

To aid in making readings of artificially inoculated plants, a scale was set up showing the range in the number of lesions on a leaf and another scale for the size of lesions. Still another scale was established showing the

range in severity of stem lesions. The amount of disease on the stems, however, was not sufficient to show the greatest amount of variation between plants. Both leaf and stem readings were made on the zero to five basis. The readings for number of lesions were averaged with the readings for size of lesions to give the final leaf score. The leaf score was then added to the stem score to give the plant a reading on the one to ten basis.

The scale used for the leaves is shown in Plate VIII. The upper row of leaves shows the scale intervals zero to five for the number of lesions on the leaves. The lower row shows the same for size of the lesions.

BLACK-STEM CONTROL BY SANITARY MEASURES

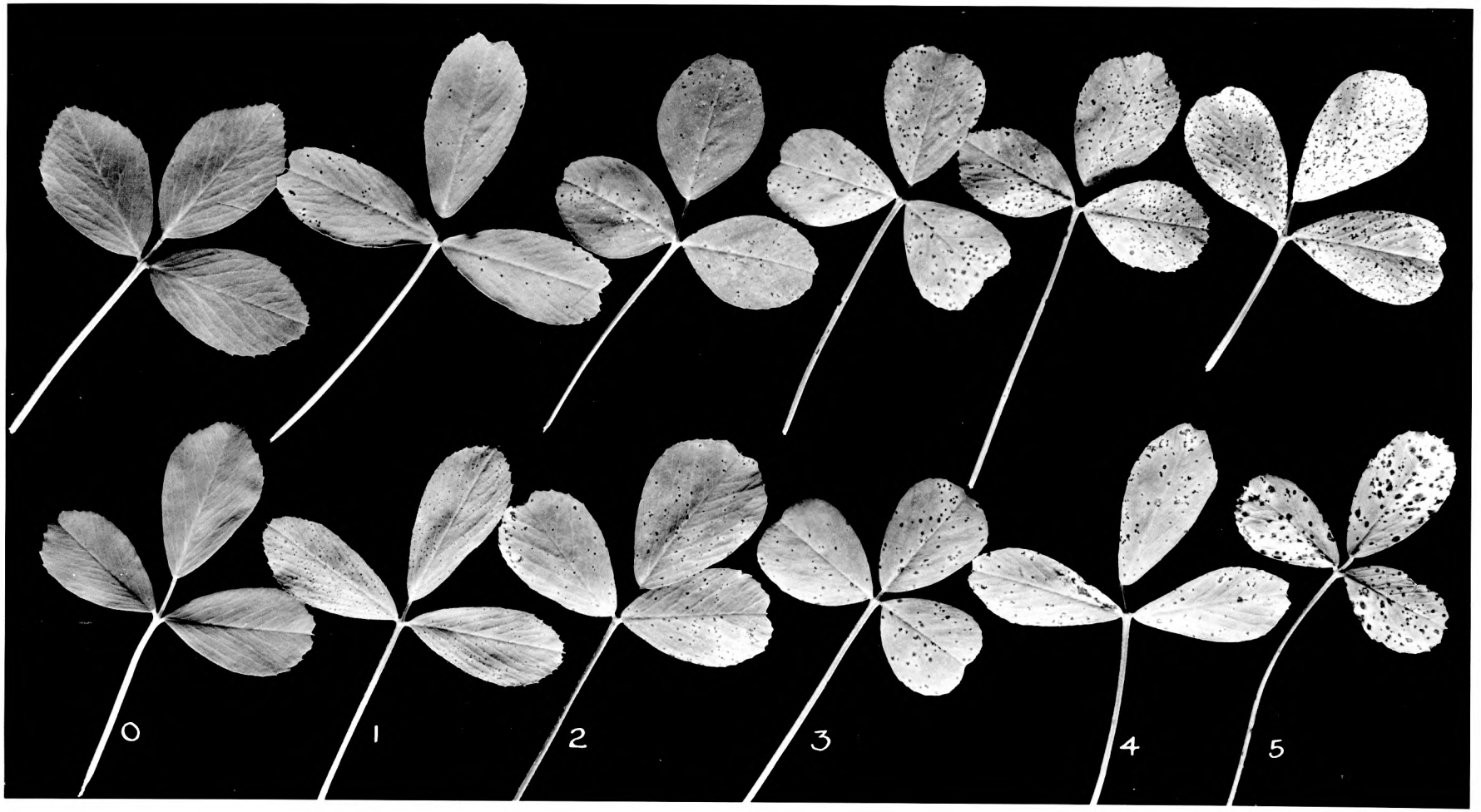
Toovey, Waterston and Brooks (19), Johnson and Valleau (6) and Brown and Street (1) suggested cutting the infected crop early as a means of control. Toovey et al. suggested that any measure which will remove dead shoots will reduce primary infection the following spring. Johnson and Valleau recommended winter grazing by sheep.

The length of time which spores remain viable in dead stems is illustrated by the work of Remsberg and Hungerford (11). They isolated the organism from an herbarium specimen in 1935 which had been kept since 1925.

EXPLANATION OF PLATE VIII

This plate was used for purposes of comparison in making leaf readings of artificially inoculated plants. The upper row of leaves shows the scale intervals zero to five for number of lesions. The lower row shows the same for size of lesions.

Plate VIII



In the present study an experiment was designed to determine if and to what extent black-stem disease could be controlled by sanitation measures.

Three treatments or amounts of crop residue were used in this experiment. The plots upon which the most crop residue was present were plots upon which the fourth cutting of the previous year had not been removed. These plots were designated as the fall growth plots. The untreated plots were plots which had the fourth cutting removed the previous year. The cleaned plots were burned over after spraying lightly with gasoline. This was done during the winter. In the spring before growth began these plots were swept with stable brooms to remove any material which may have washed or blown on the plots. These three treatments were used on each of the varieties Kansas Common, Kaw and Ladak. The plots were approximately 40 feet wide and 60 feet long. This experiment was conducted on a three-year-old alfalfa field where these three varieties were sown in strips 80 feet wide throughout the length of the field.

Plate IX shows the amount of crop residues on these three plots. In the foreground the amount of crop residue can be seen on the untreated plots. This represents the amount of growth which had taken place before frost after the fourth cutting had been removed. To the extreme left of the picture the fall growth plots can be seen. Here the

EXPLANATION OF PLATE IX

This plate shows the plots which varied in amount of crop residue. The crop residue in the foreground is representative of the amount present on the "untreated" plots. This is the growth which took place after the fourth cutting had been removed the previous season. On the extreme left is the "fall growth" plots. Here the fourth cutting had not been removed. The dark colored plots are the "cleaned" plots which have been burned over to remove dead stems and leaves.

Plate IX

fourth cutting had not been removed. The dark colored plots are those which had been burned to remove as much crop residue as possible.

The dark plot in the foreground and the next plot beyond it are the variety Kansas Common. The next dark plot with the one adjacent to it are the variety Kaw while the last burned plot and the plot beyond are Ladak.

Three sets of data were taken on these plots. The first set of readings was made on the plots on May 5, a time when all the disease present as near as could be determined was caused by the black-stem organism.

Readings were made according to the detailed method of disease readings for slight infection described in Material and Methods. Fifty single stem samples were taken from each of the nine plots. These data are presented statistically as analysis of variance in Table 4. Snedecor's (14) methods were followed in the statistical treatment of these data.

The variation between treatments was highly significant exceeding the one per cent level of significance. The variation between varieties exceeded the five per cent level of significance. The treatment x variety interaction also was highly significant.

The reason for this highly significant interaction is shown in the May 5 readings of Plate X. On the fall growth

Table 4. Analysis of variance of disease readings made May 5 on alfalfa plots varying in amount of crop residue.

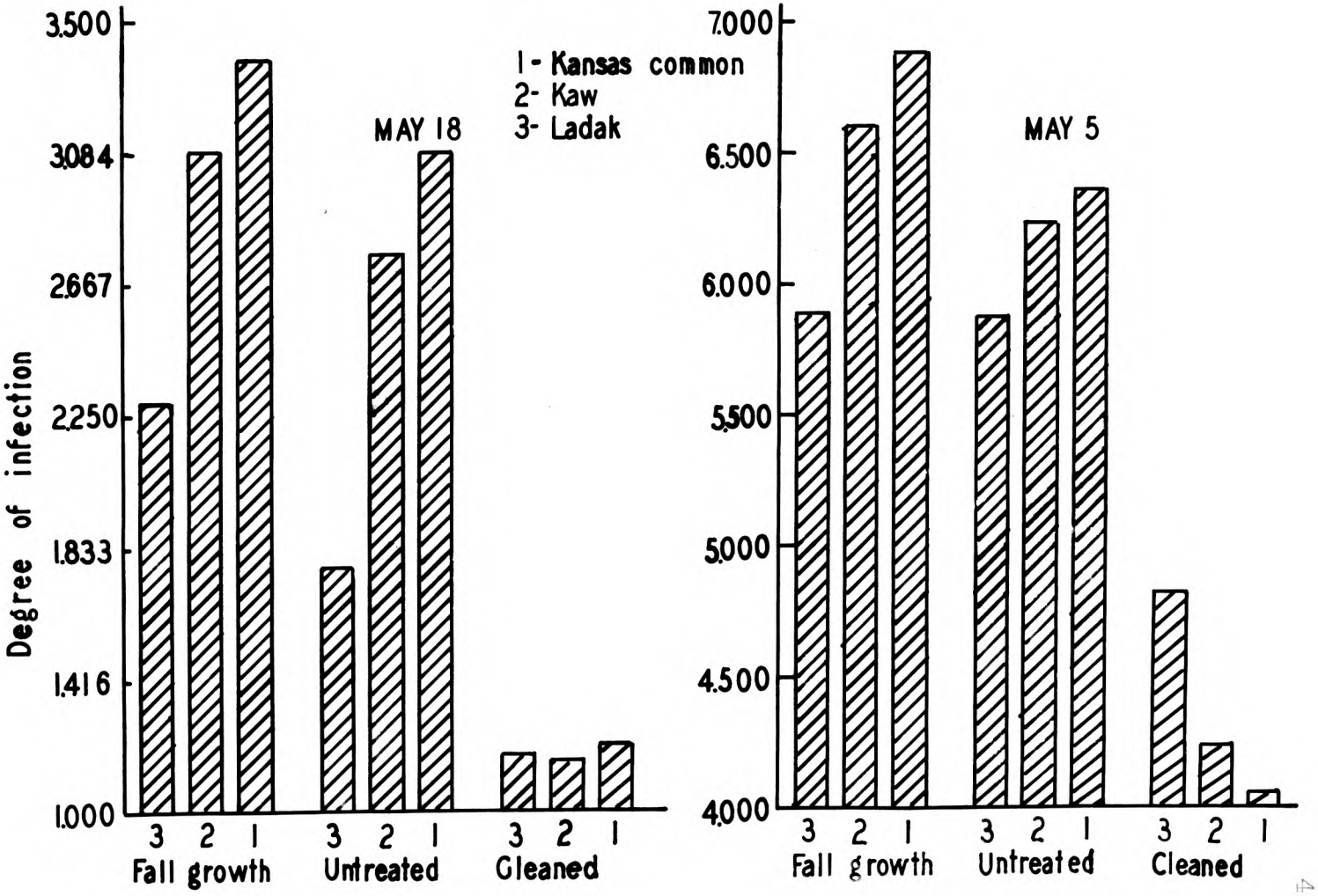
Source of variation	Degrees of freedom	Sum of squares	Mean square	F	Level of significance 1%	Level of significance 5%
Total	449	686.70	1.53			
Treatment	2	383.27	191.63	330.97	4.66	3.02
Variety	2	4.47	2.24	3.87	4.66	3.02
Treatment x variety	4	43.30	10.82	18.69	3.36	2.39
Sample	49)	32.98)	.67)			
Sample x treatment	98)	46.09)	.47)			
	)441	)255.67	).579			
Sample x variety	98)	57.31)	.58)			
Sample x treatment x variety	196)	119.29)	.61)			

EXPLANATION OF PLATE X

This plate shows the results of two sets of readings taken on plots varying in amount of crop residue. There were three varieties, Kansas Common, Kaw and Ladak, on each plot. Both the May 5 and the May 18 readings show the fall growth plots to be most severely infected and the cleaned plots least severely infected with the untreated plots as intermediate. The varieties stand in the order Kansas Common, Kaw and Ladak, listed in decreasing order of susceptibility.

Plate X

DISEASE READINGS ON ALFALFA PLOTS VARYING IN AMOUNT OF CROP RESIDUE



and untreated plots the varieties stand in the order Kansas Common, Kaw and Ladak, listed in decreasing order of susceptibility. On the cleaned plot the varieties stand in the reverse order.

The explanation for this may have to do with a combination of weather factors and peculiarities of the varieties. The presence of some disease on the cleaned plots indicated not all of the source of inoculum had been removed from the cleaned plot or it had been brought in later. Since there were no standing diseased stems or stubs on the cleaned plots the inoculum had to be on the surface of the soil perhaps as tiny bits of leaves or stems. Dissemination of spores from this material probably took place from splattering raindrops after striking this diseased material. During this infection period which extended well over the month of April, the three varieties varied in height. Kansas Common was the tallest and Ladak was shortest with Kaw being intermediate. On the cleaned plots where all the inoculum needed to come from the surface of the soil the shortest variety possibly had the best opportunity of being well splattered with spores. It was more difficult for splattering to take place high upon the taller varieties.

On the untreated and fall growth plots, however, this would not be such a factor. Here the new green shoots were

growing among the previous years diseased stems and stubble. Infection could take place by splashing of rain drops from diseased stems to the new shoots, not having to rely upon inoculum from the soil surface.

On May 18 another set of readings was made using Richard's method as described in Material and Methods. This method was particularly suitable at this time for three reasons. The disease had progressed to the stage where considerable stem infection had taken place and the plot and variety differences could be read in this way. Considerable defoliation on all plots at this time made consideration of leaves less reliable for finding plot and variety differences. The third consideration was that yellow leaf blotch Pyrenopeziza medicaginis Fuckel was making its appearance and causing some discoloration and defoliation of leaves. This disease, being wind disseminated, (7) would tend to equalize the leaf readings on all plots.

Table 5 shows the analysis of variance of the May 18 readings made by Richard's method. Six square foot samples were taken from each plot.

These readings, made at a different date and by a different method than the first readings, show practically the same results. In this case the treatments, the varieties and the treatment x variety interactions all were highly significant

Table 5. Analysis of variance of disease readings made May 18 on alfalfa plots varying in amount of crop residue.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	Level of significance 1%	Level of significance 5%
Total	53	41.04	7.74			
Treatment	2	29.98	14.99	416.38	5.12	3.21
Variety	2	6.34	3.17	88.06	5.12	3.21
Treatment x variety	4	3.09	.77	21.39	3.78	2.58
Sample	5	.32	.06			
Sample x treatment	10	.50	.05			
Sample x variety	10	.39	.04			
Sample x treatment x variety	20	.42	.02			
	45	1.63	.036			

exceeding the one per cent point. This fact added strength to results of the first readings and indicated these two methods of disease readings could be used for early and late stages of the disease without introducing any great error.

Reference to the May 18 readings in Plate X shows that the reversed order of variety rank on the cleaned plots as shown in the May 5 readings had now been removed. This probably was due to the fact that conditions which may have caused the reversal in the previous readings had now been removed. The height of the varieties at this time was about the same. The leaves upon which the variety ratings were made on May 5 had now mostly fallen and the disease showing at this time was for a large part that of repeated infection, some of which had taken place when the height of the varieties had become more nearly the same.

Effect of Black-stem on Leaf-stem Ratio

The leaf-stem ratio was determined on these plots at the same time the last set of disease readings was taken. Five square foot samples were taken from each plot. These samples were hand-picked. The leaf portion was considered to be the leaves with attached petioles and the terminal bud. The leaves and stems were dried in an oven and the weights recorded on a moisture-free basis. Table 6 shows the proportion of leaves and stems on each sample.

Table 6. Proportion of leaves and stems on plots of alfalfa varying in amounts of crop residue.

Variety	Sample number	Cleaned		Untreated		Fall growth	
		Leaves	Stems	Leaves	Stems	Leaves	Stems
		Oven-dry weight in grams					
Kansas Common	1	56.1	43.9	52.1	47.9	48.2	51.8
	2	55.6	44.4	48.5	51.5	47.4	52.6
	3	52.0	48.0	50.1	49.9	49.1	50.9
	4	50.8	49.2	50.0	50.0	47.5	52.5
	5	53.0	47.0	49.6	50.4	42.9	57.1
	Mean	53.5	46.5	50.1	49.9	47.0	53.0
Kaw	1	50.8	49.2	49.0	51.0	46.9	53.1
	2	53.0	47.0	48.1	51.9	43.3	56.7
	3	51.8	48.2	54.1	45.9	44.0	56.0
	4	50.4	49.6	50.9	49.1	42.3	57.7
	5	54.7	45.3	50.1	49.9	41.9	58.1
	Mean	52.1	47.9	50.4	49.6	43.7	56.3
Ladak	1	52.9	47.1	48.6	51.4	40.9	59.1
	2	46.9	53.1	45.8	54.2	43.6	56.4
	3	53.4	46.6	42.7	57.3	42.1	57.9
	4	48.7	51.3	43.8	56.2	44.1	55.9
	5	47.5	52.5	46.2	53.8	41.8	58.2
	Mean	49.9	50.1	45.4	54.6	42.5	57.5

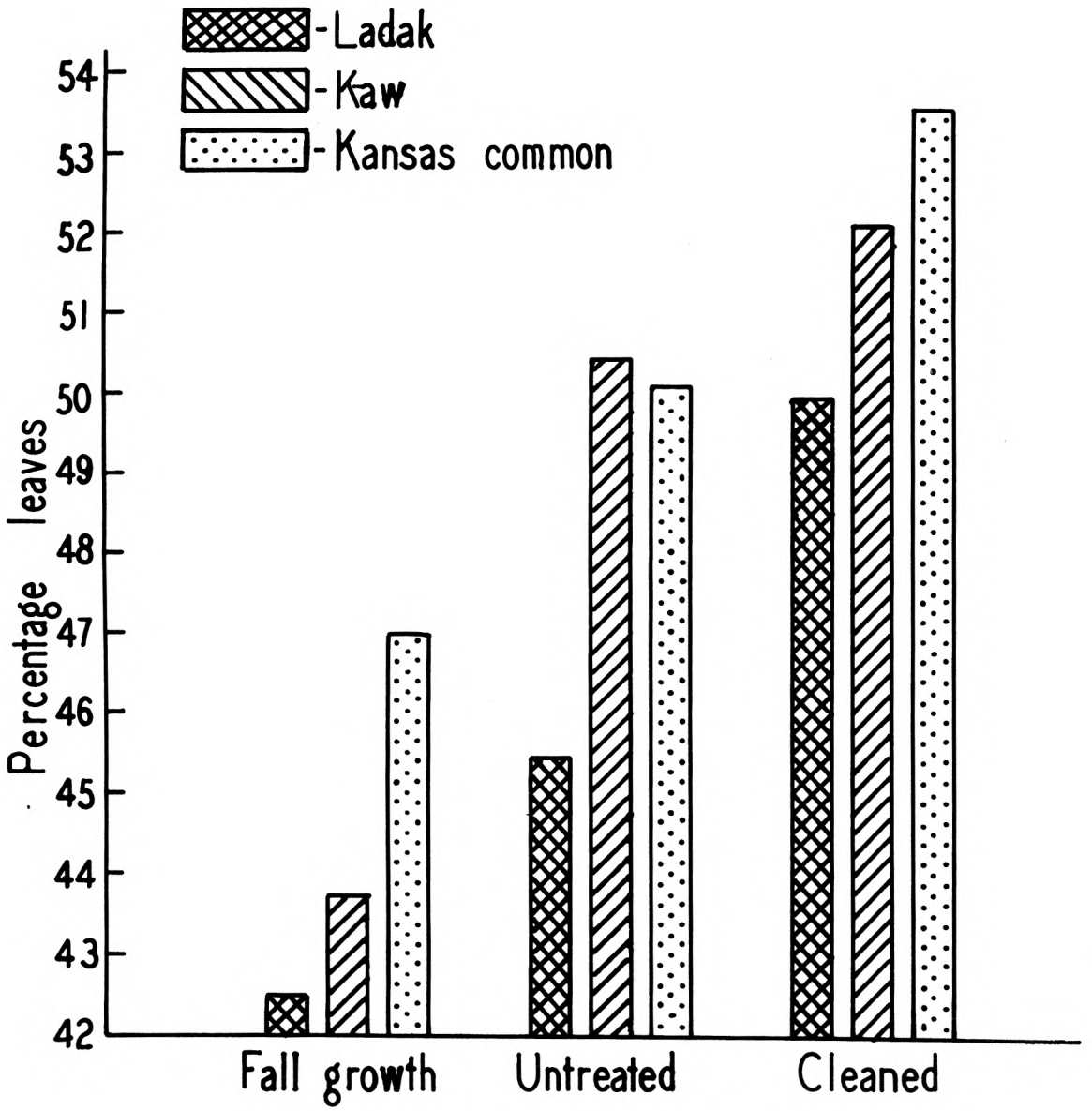
This table shows there is considerable variation between samples within a plot. It would be necessary to increase the number of samples taken to prove beyond doubt the relative ranking of these varieties on the three plots. However, a striking consistency is shown for variety ranking as well as for the treatments when only averages are considered. This is best illustrated in Plate XI. One inconsistency is shown in the relative ranking of Kansas Common and Kaw on the untreated plots. It is believed that if more samples

EXPLANATION OF PLATE XI

This plate shows the percentage of leaves on alfalfa plots varying in amount of crop residue. The cleaned plots have the highest percentage of leaves and the fall growth the lowest percentage of leaves with the untreated plots intermediate. The varieties listed in decreasing percentage of leaves are Kansas Common, Kaw and Ladak. These determinations were made on the first cutting in 1939.

Plate XI

PERCENT OF LEAVES ON ALFALFA FROM PLOTS VARYING IN AMOUNT OF CROP RESIDUE



had been taken the Kansas Common variety would have shown a higher percentage of leaves on this plot the same as on the other two plots.

The variation in the percentage of leaves was not very great. The range of the averages on all plots was from 42.5 per cent of leaves on the Ladak fall growth plot to 53.5 on the Kansas Common cleaned plot. The greatest range of average percentage of leaves within a variety was from 52.1 per cent on the Kaw cleaned plot to 43.7 per cent on the Kaw fall growth plot. This small difference would be expected when it is considered that 1939 was a poor season for blackstem and even the most severe damage was not thought to be of much consequence. However, when these data are calculated as the percentage of leaves lost considering the cleaned plots as 100 per cent, the loss of leaves appeared to be of some importance. Table 7 shows the percentage of leaves calculated on this basis.

Table 7. Percentage of leaves on plots of alfalfa varying in amount of crop residue calculated on the basis of 100 per cent on cleaned plots.

Variety	Treatment		
	Cleaned	Untreated	Fall growth
Kansas Common	100	93.6	87.9
Kaw	100	96.7	83.9
Ladak	100	91.0	85.2
Mean	100	93.8	85.7

Sotala (15) reported that in 10 experiments, 67 to 83 per cent of the total protein of the alfalfa plant was contained in the leaves, 71 to 85 per cent of the calcium and 46 to 79 per cent of the phosphorus. He figured the coefficients of apparent digestibility for the crude protein as 51.1 for stems and 77.4 for the leaves. This indicates that a loss of 15 per cent of the leaves is certainly of considerable economic importance.

LABORATORY STUDIES WITH *ASCOCHYTA IMPERFECTA* PECK

Isolation Studies

The fungus *Ascochyta imperfecta* Peck was isolated from stems of alfalfa plants gathered from the field in the fall of 1938. The method of making isolations was as follows: Small pieces of stem 1/8 to 1/16 inch in length were cut

from areas in which the diseased tissue appeared. These pieces were dipped into 95 per cent alcohol and then quickly into a solution of calcium hypochlorite. The calcium hypochlorite surface sterilized the tissue while the alcohol dipping facilitated wetting by the calcium hypochlorite. The pieces of tissue were allowed to stand in the calcium hypochlorite solution for 15 to 30 minutes. They were then removed one at a time with a sterile needle and placed in a test tube with potato-dextrose agar.

The fungus isolated was identical in all respects to laboratory cultures collected by Dr. D. B. Creager, Assistant Professor in the Department of Botany, and labeled Ascochyta imperfecta Peck. Numerous isolations made by Dr. Creager previous to this time indicated that this organism was responsible for the diseased condition of leaves and stems which has been called black-stem.

Purification of the Organism

Out of 15 cultures isolated, two were selected for purification. A number of transfers were made from each of these cultures. All the transferred cultures were similar to each other as well as to the original two cultures.

One of these transferred cultures was selected for further study after sufficient time had elapsed to allow

fruiting to take place. A very dilute suspension of spores was prepared from this culture. Transfers were made from this suspension to petri dishes containing potato-dextrose agar. These petri dishes were observed at frequent intervals under the microscope for germination and mycelium growth. Two dishes were found which had hyphae tips far enough apart to enable them to be removed by careful manipulation with a needle under the microscope. Both hyphae tips continued growth after being transferred to test tubes. Five transfers were made from each of these two tips. After they were allowed to grow for a time one group of five was selected and 10 transfers were made for each of these cultures. All of these cultures appeared to be identical and it was assumed that the equivalent of a single spore culture had been obtained. All laboratory studies of Ascochyta imperfecta Peck reported in this thesis were done with the purified fungus.

Effect of Temperature on Growth Rate

The optimum temperature for growth of Ascochyta imperfecta Peck is important information in making artificial inoculations. An experiment was undertaken to determine optimum temperature as indicated by rate of spread of mycelium. The fungus was grown in petri dishes on potato-dextrose agar at nine different temperatures. The temperatures

used were 9, 12, 15, 18, 21, 24, 27, 30 and 33 degrees Centigrade.

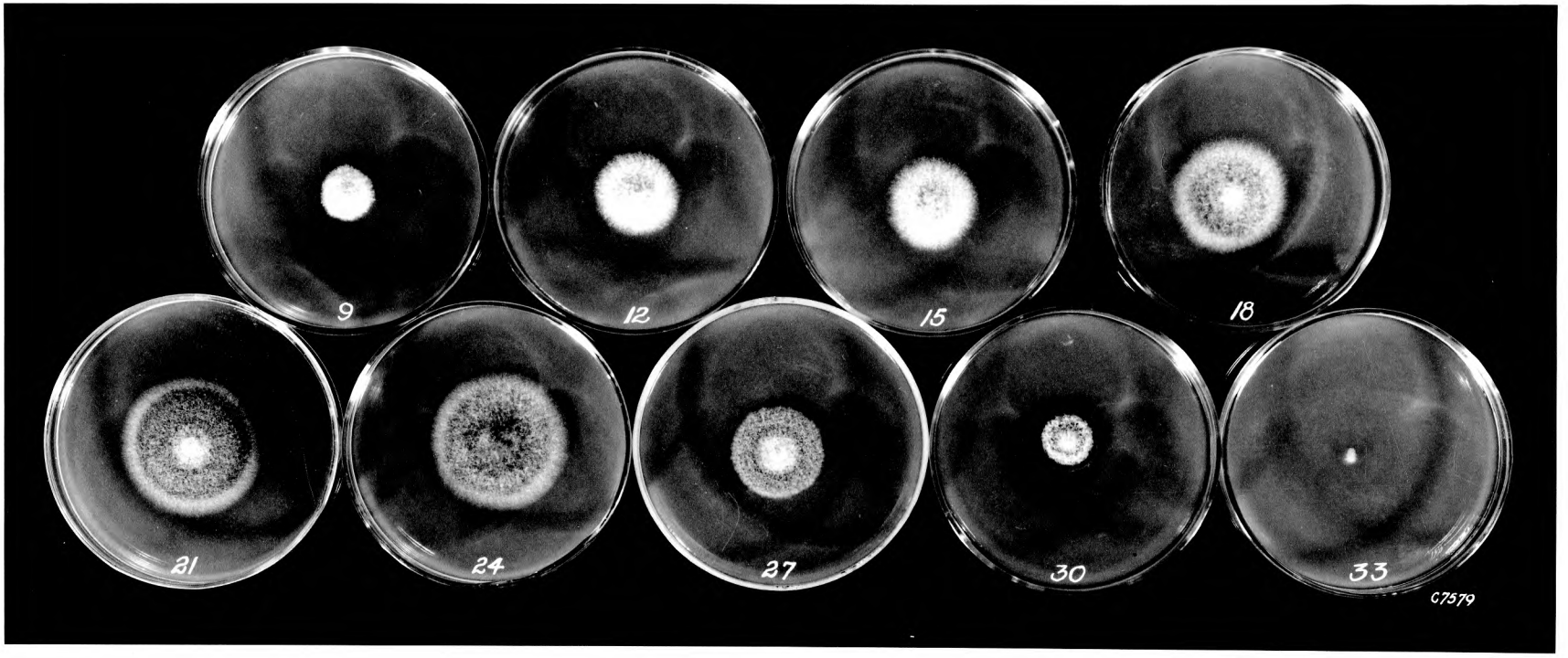
A preliminary test was made to determine the best technique. In two subsequent tests upon which data are reported the following procedure was used: About 75 petri dish cultures were poured and allowed to harden so that the agar was of a uniform depth throughout the dish. These dishes were allowed to stand for two or three days to allow any contamination from the pouring to begin growth. One loop from a spore suspension was then placed in the center of each uncontaminated dish. These dishes were then allowed to stand in the laboratory until a small whitish mycelium mass in the center of the dish indicated that germination had taken place and growth had begun. The dishes were again sorted to remove any further contamination or cultures in which the newly germinated spore suspension was abnormal in size or appearance. The dishes were then numbered and placed in the temperature chambers, six dishes being placed in each chamber. Measurements were made on all six dishes but only dishes one to five inclusive were reported, number six being used only in case a contamination interferred with accurate readings on one of the first five dishes.

Plate XII shows one culture selected from each of the nine temperatures at the time of the fourth measurement.

EXPLANATION OF PLATE XII

The rate of growth of *Ascochyta imperfecta* Peck at various temperatures is shown in this plate. These are petri dish cultures with the fungus growing on potato-dextrose agar. The number on the bottom of each culture is the Centigrade temperature.

Plate XII



At the cooler temperatures the outline of the growth was more irregular and less dense giving the edge of the growth a somewhat frayed appearance. As viewed from the top side, the 9, 12 and 15 degree cultures had a white cottony appearance. A darkened center of fruiting region could be seen showing through the whittish mycelium.

At the higher temperatures the edge of the growth became more definite, not showing the frayed characteristics of the cooler temperatures. Another striking thing was that at the higher temperatures the amount of whitish margin where no fruiting has yet taken place became less. At the 30 degree temperature practically no whitish margin could be seen. The 18 to 24 degree temperature cultures were olive green in appearance. The 27 degree culture was still darker while the 30 and 33 degree cultures were dark and light tan, respectively.

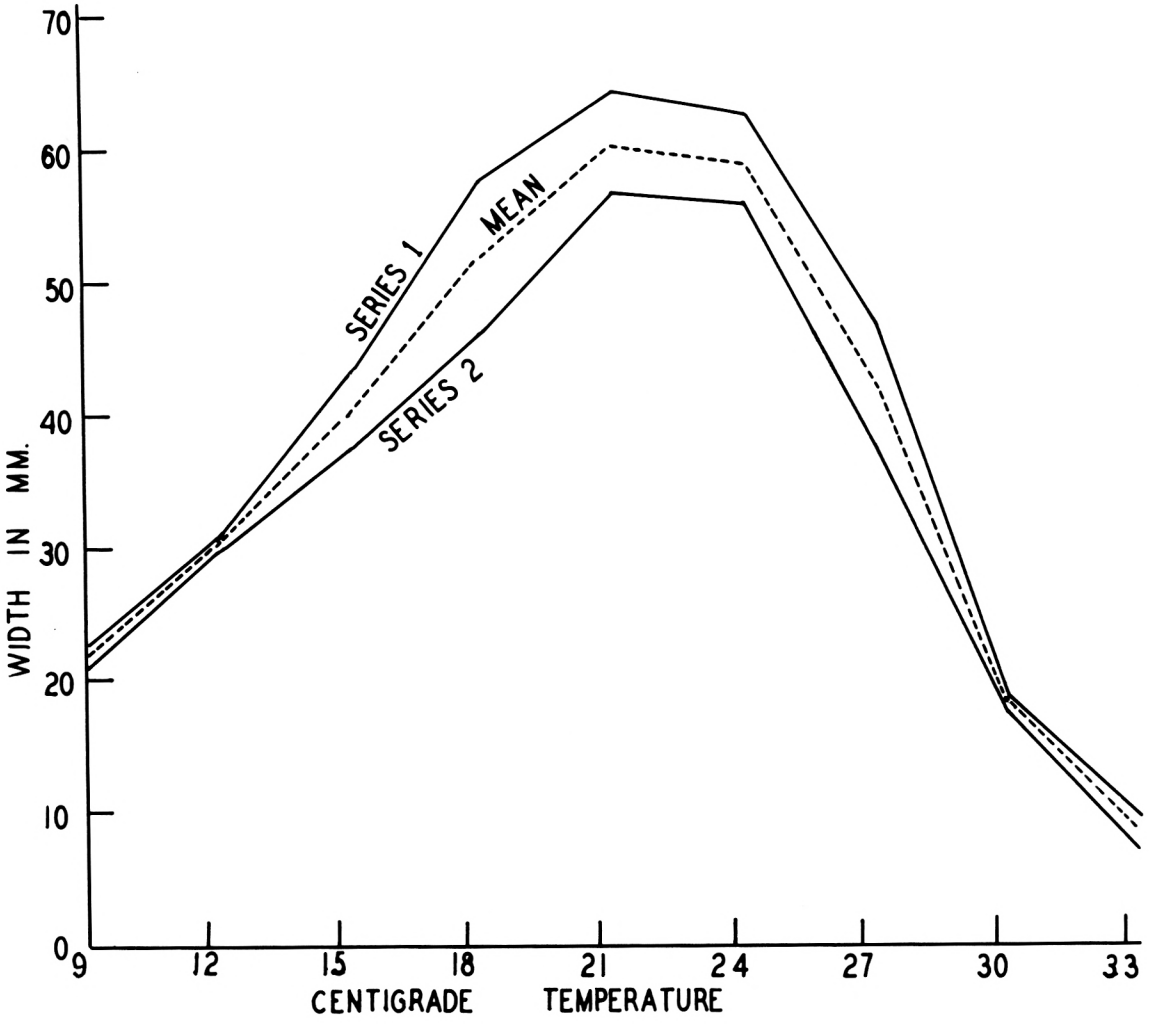
Table 8 shows the diameter in millimeters of each of the five cultures in the nine different temperatures for two series. Series I showed a more rapid and a more uniform growth than did Series II. In Series I the greatest variation in width of cultures between the five dishes within a temperature was three millimeters. In Series II the variation ran as high as nine millimeters. A graphical representation of these data is shown in Plate XIII. The curves are

EXPLANATION OF PLATE XIII

The effect of temperature upon growth of Ascochyta imperfecta Peck is shown in this plate. Readings were made by measuring the width of growth of the fungus in petri dish cultures. The optimum temperature is about 22 degrees Centigrade.

Plate XIII

EFFECT OF TEMPERATURE UPON GROWTH OF ASCOCHYTA IMPERFECTA PECK



quite normal looking with perhaps a more rapid falling off for the high temperatures than there is at the low temperatures. Series I gives a more normal looking curve than Series II. The trends of the two curves are very similar, both showing the optimum to be at about 22° Centigrade.

Table 8. Effect of temperature upon growth of Ascochyta imperfecta as indicated by rate of spread.

Culture number	Width in millimeters at Centigrade temperatures								
	9°	12°	15°	18°	21°	24°	27°	30°	33°
				Series I					
1	22	29	45	55	64	62	46	18	10
2	23	30	43	58	63	63	46	19	9
3	23	32	42	58	65	62	48	18	9
4	22	30	42	58	65	62	47	18	9
5	23	31	42	58	64	62	46	18	10
Mean	22.6	30.4	42.8	57.4	64.2	62.2	46.6	18.2	9.4
				Series II					
1	18	27	35	49	55	53	33	14	5
2	24	28	39	47	56	58	36	20	5
3	24	33	40	44	58	58	42	22	5
4	21	31	37	48	55	53	40	15	11
5	23	30	36	43	59	57	37	17	10
Mean	21	29.8	37.4	46.2	56.6	55.8	37.6	17.6	7.2

Source of Inoculum for Artificial Inoculations

Sprague (16) grew the fungus for inoculation studies on sweet clover stems, potato-dextrose agar, and pea soup agar. Three methods were tried in this study for growing

the fungus for inoculation purposes. The fungus was grown on potato-dextrose agar, on alfalfa stems and on sweet clover stems. A definite test has not been made to compare these three methods but general observation of spores and the results of inoculations indicate that the sweet clover stems have been most satisfactory.

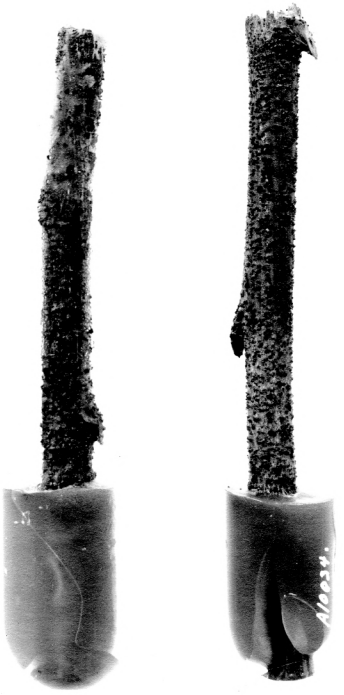
The sweet clover stem cultures were prepared in the following manner. The large stems of second year sweet clover were gathered and cut into pieces about three inches long. These were allowed to dry before using. When cultures were to be prepared the required number of stems were boiled to soften them. They were then dropped into test tubes containing about three-fourths inch of plain water agar. The test tubes were plugged and autoclaved at 15 pounds for 20 minutes. When the tube and contents had cooled a loop of spores from a pure culture was drawn along the edge of the stem and the cultures set aside until fruiting had taken place. Cultures were kept in the refrigerator for extended periods of time. Spores from six months old cultures were found to be perfectly viable.

Plate XIV shows numerous pycnidia growing upon sweet clover stems such as were used in making inoculations. The left figure shows the stem within the test tube and the right figure shows it after it had been removed. The pycnidia can

EXPLANATION OF PLATE XIV

Two cultures similar to the ones used for making inoculations are shown in this photograph. The purified fungus is shown growing on sterile sweet clover stems in test tubes with a small amount of water agar in the bottom. The fungus produced pycnidia abundantly under these conditions. The figure on the left shows the pycnidia on a sweet clover stem within a test tube while on the right the stem has been removed from the test tube.

Plate XIV



be seen as numerous small black projections over the entire surface of the stems. The agar at the bottom of the test tube slowly gives up its water to keep the stem moist.

Fruiting in Relation to Temperature. An experiment was set up in hopes of finding out if the temperature best suited for pycnidia formation of Ascochyta imperfecta Peck was the same as the optimum temperature for mycelium growth. Alfalfa leaves of uniform size and age from a single plant were used upon which to grow the fungus. Three of these leaves were placed on each of 27 slides. These slides were placed in small moist chambers made of petri dishes lined with moist blotting paper. They were then autoclaved at 15 pounds for 20 minutes. After the leaves had been allowed to cool a suspension of spores was prepared and one loop of spores was drawn across each leaf. In 36 hours the spores had germinated to form a whitish mass of mycelium over the surface of the leaf but no pycnidial formation was observed. The dishes were placed in the temperature chambers described in the study on the effect of temperature on mycelium growth. Three of the petri dishes were placed in each of nine temperatures ranging from 9° to 33° C. After six days they were removed for observation.

Pycnidia were observed throughout the range of temperatures. Temperatures 21, 24 and 27 degrees had the most

numerous pycnidia. It was impossible to distinguish between 21, 24 and 27 degrees as to which temperature was most satisfactory but leaves in the 27 degree temperature appeared to have more numerous and smaller pycnidia. A conservative estimate of the number of pycnidia on one leaf at the 27 degree temperature was 1,500. The leaves in the 24 degree temperature seemed to have quite large pycnidia. There was a very marked falling off in number of pycnidia in the 18 and 30 degree temperatures. A large number of pycnidia were observed even at the 12 degree temperature but they were rather immature and transparent while at the 21 to 27 degree series they were very black in color.

The results of this experiment fit in quite closely with the observations made on the petri dish cultures. The tendency seemed to be for fruiting to take place more readily at the higher temperatures. Although the pycnidia were more abundant at temperatures higher than the optimum for mycelium growth, the fruiting bodies were not as large as they were at the optimum temperature.

The fact that fruiting took place almost throughout the range of temperatures indicated that there was not a delicate relationship between temperature and fruiting. For this reason cultures which were prepared to be used in making inoculations were allowed to stand at room temperatures until

abundant fruiting had taken place. They were then placed in a refrigerator until they were to be used.

Methods of Inoculation. Other investigators have used various methods of making artificial inoculations. Johnson and Valleau (6) crushed agar cultures in water and rubbed this decoction on the stems and leaves of the plant with a sterile swab made of a stick on the end of which was wrapped a piece of cheese cloth. Sprague (16), Remsberg and Hungerford (11) and Toovey, Waterston and Brooks (19) sprayed spore suspensions on healthy plants. Remsberg and Hungerford (11) transferred the organism on agar to healthy tissue. Toovey et al. (19) placed wet spore saturated cotton wads on sterile alfalfa stems. This was covered with tinfoil to prevent drying.

All inoculations made and reported on in this paper have been made by spraying spore suspensions on healthy plants. The amount and concentration of the spore suspension to use on a given number of plants can be determined by making a few trials. The spores were sprayed on the plants with an atomizer with a 220 cc. bottle. Air pressure was used instead of the rubber bulb to manipulate the atomizer.

Proof of Pathogenicity. A group of artificially inoculated plants became infected and showed the typical symptoms of black-stem disease which have been described.

Isolations were made from both leaf lesions and stem lesions according to the methods previously mentioned. The outcome of 15 leaf and 15 stem lesion isolations is shown in Table 8a.

The consistency of occurrence of Ascochyta imperfecta Peck in these cultures is ample proof of the pathogenicity of the artificially inoculated plants.

Host Range. The host range of Ascochyta imperfecta is somewhat limited. Sprague (16) who made a host range study of leguminous Ascochytae doubts if any host other than alfalfa and perhaps bur clover would carry the disease. By artificial inoculations under ideal conditions he was able to secure infection about 20 per cent of the time on Melilotus indica (L.) All., Melilotus officinalis (L.) Lam., Trifolium hybridum L., and Trifolium pratense L. He stated it rarely could be determined as Ascochyta injury except through reisolation. Previously Horsfall (5) found the disease reported by Valleau and Fergus occurred destructively on red clover in New York. The work of Sprague makes it improbable that Horsfall was concerned with Ascochyta imperfecta.

Remsberg and Hungerford (11) successfully inoculated Grimm and Common alfalfas and yellow blossomed sweet clover. Toovey, Waterston and Brooks (19) were able to secure slight infection on Vicia sativa L. and typical lesions on

Table 8a. Proof of pathogenicity of artificially inoculated plants.

Kind of tissue	Culture number	60 min.	Culture number	30 min.	Culture number	15 min.
Stem	1	Black-stem	1	Black-stem	1	Black-stem
	2	No growth	2	Black-stem	2	Black-stem
	3	No growth	3	Black-stem	3	No growth
	4	Black-stem	4	No growth	4	Black-stem
	5	No growth	5	No growth	5	Black-stem
Leaf	1	Black-stem	1	No growth	1	Black-stem
	2	Black-stem	2	No growth	2	Black-stem
	3	Black-stem	3	No growth	3	Black-stem
	4	Black-stem	4	No growth	4	Black-stem
	5	No growth	5	No growth	5	Black-stem

Trifolium pratense L., Medicago sativa L. and Medicago lupulina L.

Johnson and Valleau (6) successfully inoculated alfalfa, sweet clover and red clover with what they refer to as Phoma medicaginis Malbr. et Roum.

In the work here at Kansas two new species have been added to this list. Approximately 50 plants each of two strains of Medicago falcata L. and 50 plants of the species Medicago ruthenica Trautv. have been successfully inoculated with Ascochyta imperfecta. The one strain of Medicago falcata designated as Semipalatinsk was collected by N. E. Hansen, Brookings, South Dakota while traveling in western Siberia in 1908. Concerning this strain, Mr. H. L. Westover, Senior Agronomist in the Division of Forage Crops and Diseases, Bureau of Plant Industry, writes: "The original introduction was pure falcata, but so far as I know, any seed that is offered as Semipalatinsk at the present time is a hybrid that has resulted from natural causes."

The species M. ruthenica and the strain of M. falcata designated as Semipalatinsk appeared to be more resistant to black-stem than the other strain of M. falcata and some of the common alfalfas.

Moist Chamber for Artificial Inoculation. Artificially inoculated plants were kept in a moist chamber for several

days following inoculation. An inexpensive chamber suitable for this purpose was constructed of canvas stretched over all sides of a wooden frame. The chamber was six feet long, four feet wide and five feet high to the peak of the roof. The roof was an ordinary gable type with a half pitch. The canvas at the one end of the chamber was left hanging loose from the top as a flap which could be rolled up when plants were to be placed in or removed from the chamber. This chamber was set inside a galvanized tin tray which was about six inches longer and wider than the chamber. The tray was four inches high and was equipped with an outlet to the drain. Water was allowed to run down the roof and all sides of the chamber. It was supplied from small holes drilled into a pipe which extended along the peak of the roof and across the two ends. A raised wooden platform inside the chamber kept the plants out of the water which collected in the tray.

This type of chamber was suitable in meeting the requirements for temperature and humidity for infection to take place. The relative humidity ranged from 90 to 100 per cent and the temperature from 65° to 70° F.

The chamber held 60 large alfalfa plants in seven-inch pots.

Duration of Infection Period. The length of infection

period used by Sprague (16) varied from three to five days. A study was made to determine the most satisfactory length of infection period to use under the conditions of making inoculations for this study. This test was made in May and June when greenhouse temperatures were quite high.

For this study potted plants about 12 inches tall were used. All of these plants were cuttings from a single plant. In this way genetic variation between plants would be eliminated as a source of error. Twenty-five of these plants were inoculated and placed in the moist chamber. An additional five uninoculated plants served as checks.

Five of these plants were removed from the chamber every 24 hours until on the fifth day when the last five together with the uninoculated checks were removed. Two series of plants were run. Table 9 is a summary of the disease readings made on the two series of this experiment. Readings were made by the method adapted for greenhouse readings of artificially inoculated plants.

It was found later, however, that inoculations made during the winter months did not require such a long infection period. In these inoculation studies the length of infection period varied with conditions. The duration of this period probably will have to be determined by a preliminary experiment before a series of plants is inoculated. Temperature is possibly one of the most important factors.

Table 9. The effect of length of time in the humidity chamber upon the degree of infection of alfalfa by Ascochyta imperfecta Peck.

Plant numbers	Treatment	Leaf	Stem	Total
		Series I		
1- 5	check	0	0	0
6-10	1 day	0	0	0
11-15	2 "	3	.8	3.8
16-20	3 "	3.4	.6	4.0
21-25	4 "	4.2	.6	4.8
26-30	5 "	4.4	.2	4.6
		Series II		
1- 5	check	0	0	0
6-10	1 day	-	-	-
11-15	2 "	.8	0	.8
16-20	3 "	2.4	.2	2.6
21-25	4 "	4.0	.8	4.8
26-30	5 "	4.2	.6	4.8

Effect of Leaf Age Upon Infection. The effect of leaf age upon infection of alfalfa by black-stem disease is important in this study. If, for example, the older leaves of an alfalfa plant became infected more readily than the younger leaves, two possible sources of error in readings could be made. In the field, the rapidity of recovery after cutting of various varieties differs to a considerable extent. Turkestan 19304 shows a very rapid recovery after cutting while Ladak tends to lie dormant for several days before any new growth makes its appearance. If a rainy damp

period favorable for infection occurred shortly after a crop had been harvested it is possible that the growth on the Turkestan variety would be several days older than the growth on the Ladak variety. This would be disadvantageous to the Turkestan variety if the older leaves became infected more readily. More serious infection on the Turkestan alfalfas would substantiate this idea.

This same problem presents itself in making greenhouse inoculations. The problem in this case is whether or not plants to be compared in a black-stem test should be cut back at the same time in order for the top growth to be the same age.

An experiment was designed to determine what effect leaf age had upon ease of infection of alfalfa plants by black-stem disease. Six good sized alfalfa plants growing in seven-inch pots were selected for use in this experiment. Three of the plants were the variety Turkestan 19304 and three were the variety Ladak. At the beginning of the experiment all six plants were cut back to within one-half inch above the level of the soil in the pot. For the next 23 days the growth of six stems in each pot was plotted on paper. The first node to appear on each of the three stems was marked by inserting through the stem a tiny needle such as is used in mounting small insects. Each day all the

plants were observed for the appearance of new leaves. At the end of 23 days the age of every leaf on all six plants was known.

The plants were then carefully inoculated and when sufficient time had elapsed for infection to take place, each leaf was scored from zero to five depending upon its severity of infection. The leaves for each plant were then grouped into the age classes 0-4, 5-9, 10-14, 15-19 and 20-23. The number of leaves in each of these age classes and the average infection of the leaves in each class were determined. Table 10 gives a summary of the data obtained in this experiment.

There is no relationship between age of leaves and ease of infection according to the data obtained from this experiment.

Table 10. The effect of age of foliage upon degree of infection of alfalfa by black-stem.

Plant No.		No. leaves	0-4 days	No. leaves	5-9 days	No. leaves	10-14 days	No. leaves	15-19 days	No. leaves	20-23 days
Turkestan	1	7	2.14	7	2.29	12	2.00	11	2.00	9	2.11
"	2	12	5.00	16	4.81	16	4.69	22	5.00	2	5.00
"	3	16	4.69	13	4.69	18	4.61	24	4.88	0	-
Ladak	1	9	3.88	8	4.13	12	3.08	11	2.82	2	2.50
"	2	9	3.22	9	2.88	12	2.42	15	4.33	0	-
"	3	10	2.70	10	2.30	12	2.50	8	3.13	0	-
Average		10.5	3.61	10.5	3.52	13.7	3.22	15.2	3.69	4.3	3.20

ALFALFA VARIETY VARIATIONS IN SUSCEPTIBILITY

Not much is known of varietal susceptibility. Johnson and Valleau (6) indicated that resistance to black-stem injury was inherent in some varieties of alfalfa. Toovey, Waterston and Brooks (19) gave the results of black-stem readings on alfalfa strains in which "the strains were placed in the following order of decreasing susceptibility in 1935: 1. Medanos, 2. English grown and Grimm, 3. Provence, Malborough and Hungarian." Richards (12) made detailed readings on 44 varieties during a severe infection in 1933. The disease was least severe in Ladak and most severe in a French introduction. Introductions from Russia and Turkestan were severely infected while Grimm and Hardigan were less damaged.

Variety Reaction to Field Infection

Variety Differences in Field Plots. Some of the preliminary problems to breeding black-stem resistant alfalfas have been worked out. The next important problem was to find if varieties and plants within a variety varied in their resistance to the disease. An attempt was made to find if this variation occurred equally and consistently in the field and in the greenhouse.

A comparison was made of two varieties, Turkestan 84397 and Kansas Common, growing in plots at the Agronomy farm. Ten square foot samples were taken from each plot. Leaf readings were made according to the simplified method derived from the detailed procedure as described in Material and Methods. Stem readings were made according to the zero to five method but were then converted to percentage so they could be averaged with the leaf scores.

The average percentage of infection on the 10 samples of Turkestan 84397 was 65.1 while the average of the 10 Kansas Common samples was 43.2. The analysis of variance of these readings is shown in Table 11.

Table 11. Analysis of variance of disease readings on two varieties of alfalfa.

Source of variation	d.f.	Sum of squares	Mean square	F	Level of significance 1%	Level of significance 5%
Total	19	2768.87				
Between varieties	1	2417.80	2417.80	124.29	8.28	4.41
Within varieties	18	351.07	19.50			

The variation between varieties is highly significant exceeding the one per cent level of significance. This is a good indication that genetic variations in susceptibility

do occur.

Three sets of data involving three variety comparisons showed significant variations between varieties. Each of the three sets of readings was made by a different method. The first set of data shown in Table 4 indicated a significant variation between varieties within the five per cent level of significance. These data were taken by the detailed method adapted to light infection on the varieties Kansas Common, Kaw and Ladak.

The average disease score for the 150 samples taken on the three plots of each variety was Kansas Common 5.775, Kaw 5.696 and Ladak 5.536.

Table 5 shows data from these same varieties taken according to Richard's method of disease readings. These readings which were taken later than the above ones show a level of significance between varieties exceeding the one per cent level of significance. In this case the variety score and ranking for the three varieties was Kansas Common 2.560, Kaw 2.341 and Ladak 1.743. For a graphical representation of these variety variations reference should again be made to Plate X.

A third set of data involving three varieties was taken using the simplified method of disease readings to which previous mention has been made. The varieties involved were Kansas Common, Turkestan and a variety of unknown origin.

Ten square foot samples were taken from each plot.

Table 12 shows the analysis of variance of disease readings on these three varieties.

Table 12. Analysis of variance of disease readings on three varieties of alfalfa.

Source of variation	d.f.	Sum of squares	Mean square	F	Level of significance 1%	Level of significance 5%
Total	29	397.21				
Between varieties	2	150.98	75.49	8.27	5.49	3.35
Within varieties	27	246.23	9.12			

The variation between varieties is highly significant. The average percentage infection on the three varieties was 41.2 per cent for Turkestan, 37.3 per cent for the variety of unknown origin and 35.9 per cent for Kansas Common.

Field Testing of Seedlings. It is important in a disease resistant study to have field data to supplement greenhouse data. The purpose of this experiment was to determine if infection of spring transplanted plants would take place if the proper conditions were supplied.

Seed of the five varieties, Kansas Common, Turkestan 86696, Turkestan 19304, Ladak and Kansas Common Sel. 1-3018, were planted in flats in the greenhouse in mid-winter. When

they were of sufficient size they were transplanted into individual plant squares which were about two inches square and four inches deep. These plant squares were made of asphalt felt paper and were set side by side in flats. These were transplanted to the field on April 1. Each of the five varieties was represented by two rows of 500 plants each. The plants were spaced from three to four inches apart in the row.

These plants were transplanted to an old alfalfa field on which the fourth cutting the previous year had not been cut. This furnished an abundant supply of inoculum. Preparation for transplanting was made by spading rows through the plot at three foot intervals. After transplanting, old disease stems and leaves from the previous year were gathered from another plot and were scattered down the rows where the spading had removed this material.

This experiment was not entirely successful. The spring was unusually dry especially after the newly transplanted plants became large enough to become infected.

On May 27, however, enough infection appeared to warrant taking notes on the rows. Readings were taken on five successive plants every three feet on each of the rows. This gave readings on 115 plants per row and 230 plants for each variety. Kansas Common row one was on the edge of the

plot and apparently this position was less favorable for infection than the other plots. It is thought that the reading on this plot is less than it should be. Table 13 gives the average readings of the 115 plants in each row.

Table 13. Average of 115 readings on each of two rows of five alfalfa varieties.

Row	Kansas Common	Ladak	1-3018	Turkestan 86696	Turkestan 19304
1	3.304	3.992	4.355	4.246	5.601
2	4.304	3.789	4.449	4.688	5.130
Ave.	3.804	3.890	4.402	4.467	5.365

Not much importance should be attached to these results. This test was not considered to be an entire failure, however, because with certain changes it was thought that this method could be employed in making field readings of new strains of alfalfa. Suggestions will be made later for improvement of this method.

Variety Variations from Artificial Inoculations

A comprehensive variety test of artificially inoculated plants was conducted which involved 45 plants each of 10 varieties. The purpose of this experiment was first, to test the reliability of readings on artificially inoculated plants and second, to determine if plant selections could

be made in this way. This method, if it should prove to be successful, would greatly reduce the amount of time necessary to test strains and would eliminate the hazards of not having field conditions favorable for the disease.

These 10 varieties, Turkestan 86696, Turkestan 19304, Ladak, Kansas Common, Kansas Common Sel. 1-3018, Grimm, Hairy Peruvian 22486, Medicago falcata F.C. 30114, Medicago ruthenica F.P.I. 190365, and Semipalatinsk F.C. 22613 were planted in flats in the greenhouse on May 17. Seed of some of these species and varieties was furnished by H. L. Westover, Senior Agronomist with the Division of Forage Crops and Diseases. The two strains of falcata used in the test were considered to be partially hybridized with common alfalfas.

The seedlings of these 10 varieties were transplanted to rows in the irrigated nursery on June 22. On October 2, five plants from each variety were dug, transplanted into seven-inch pots and brought into the greenhouse. Five days later a second group of 50 plants was brought into the greenhouse. This procedure was repeated every five days until 450 plants had been potted.

By November 28 the first group of plants was of sufficient size to be inoculated. The inoculations were also made at regular intervals so that the foliage on each group

of plants was approximately the same age when the plants were inoculated. The length of time each group of plants was left in the moist chamber following inoculation varied from three to four days depending upon the length of time required for the plants to become diseased.

The readings were made according to the method described in Material and Methods for making disease readings on artificially inoculated plants. The disease scale shown in Plate VIII was used for comparison purposes whenever readings were made. Two readings were made on each plant. The first was made eight to 10 days after inoculation and the second five days later. Table 14 gives a summary of the two disease readings made on 450 plants of 10 varieties.

The disease score for the entire plant was reported on the zero to 10 basis. The range in plant scores for the second reading varied from 2.378 for Medicago ruthenica to 6.189 for Hairy Peruvian. Only the second readings will be considered in making references to disease readings or variety rank since they were believed to give a more accurate value of the diseased condition of the plant.

Under the conditions of this experiment the 10 varieties could be conveniently grouped into six classes each differing significantly from the class next to it or nearly so. These classes are: 1. Medicago ruthenica, 2. Semipalatinsk and Ladak, 3. Grimm and Medicago falcata,

Table 14. Summary of disease readings made on 450 artificially inoculated plants.

Variety	Plant Score Readings				Stem Score Readings				Leaf Score Readings				Size of Lesions Readings				No. of Lesions Readings			
	1st	Rank	2nd	Rank	1st	Rank	2nd	Rank	1st	Rank	2nd	Rank	1st	Rank	2nd	Rank	1st	Rank	2nd	Rank
Medicago ruthenica	1.767	1	2.378	1	.356	1	.422	1	1.411	1	1.956	1	1.422	1	2.111	1	1.400	1	1.800	1
Semipalatinsk	2.533	2	3.389	2	.489	3	.578	3	2.044	2	2.811	2	1.689	4	3.178	2	2.400	2	2.444	2
Ladak	2.544	3	3.478	3	.467	2	.533	2	2.078	3	2.944	3	1.644	2-3	3.333	3	2.511	3	2.556	3
Grimm	2.867	4	3.933	4	.689	5	.778	4	2.178	4	3.156	4	1.644	2-3	3.444	4-5	2.711	4	2.867	4
Medicago falcata	3.067	5	4.078	5	.778	7	.911	7	2.289	5	3.167	5	1.756	6	3.444	4-5	2.822	5	2.889	5
Turkestan 19304	3.155	6	4.356	6	.578	4	.800	5	2.578	7	3.556	7-8	1.711	5	3.578	6	3.444	9	3.533	9
Turkestan 86696	3.344	7	4.367	7	.733	6	.867	6	2.611	8	3.500	6	1.956	8	3.711	7	3.267	7	3.289	7
Kan. Common Sel. 1-3018	3.511	8	4.933	9	1.000	9	1.356	9	2.511	6	3.578	9	1.933	7	3.933	9	3.089	6	3.222	6
Kansas Common	3.555	9	4.711	8	.888	8	1.156	8	2.667	9	3.556	7-8	2.000	9	3.800	8	3.333	8	3.311	8
Hairy Peruvian	4.811	10	6.189	10	1.689	10	2.022	10	3.122	10	4.167	10	2.422	10	4.444	10	3.822	10	3.889	10
5% level of significance for difference between variety means	.295				.207				.165				.191				.248			

4. Turkestan 19304 and Turkestan 86696, 5. Kansas Common and Kansas Common Sel. 1-3018 and 6. Hairy Peruvian. Medicago falcata of class three and Turkestan 19304 of class four fall slightly short of the required .295 for the five per cent level of significance for variety means.

It should be noted at this point that Kansas Common and Kansas Common Sel. 1-3018 rank below the Turkestans. The reverse was true of the Kansas Common and Turkestans compared in the varietal reaction to field infection previously discussed. Many years of notes taken at Kansas showed that the Turkestan varieties were consistently more susceptible to black-stem than Kansas Common. It is not known whether greenhouse conditions, physiological forms of the disease, or other factors were responsible for this unusual relative ranking of these varieties when artificially inoculated. Obviously here is a point which requires further study.

The analysis of variance for the plant score of artificially inoculated plants in Table 15, shows the variation between varieties to be highly significant.

Table 15. Analysis of variance for plant score on 450 artificially inoculated plants.

Source of variation	d.f.	Sum of squares	Mean square	F	Level of significance 1%	Level of significance 5%
Total	899	2622.45	2.917			
Between varieties	9	672.99	74.777	47.996	2.43	1.89
" dates	8	321.66	40.207	25.807	2.53	1.95
" readings	1	255.47	255.47	163.973	6.66	3.85
Error	881	1372.33	1.558			

Variety Variations in Stem Score. The stem score and leaf score contributed equally to making up the plant score. The stem and leaf scores were each made on the zero to five basis and then added to give the plant score. The range in stem scores was from .422 for Medicago ruthenica to 2.022 for Hairy Peruvian. When the varieties were listed in increasing order of severity of disease, there were only two adjacent varieties whose readings are farther apart than .207 which was the five per cent level of significance for difference between variety means. These two varieties were Kansas Common Sel. 1-3018 and Hairy Peruvian. The exceedingly low readings of the stem scores and the small range from the lowest ranking variety to the highest ranking variety would lead one to believe that the stem scores were not of much value. It should be noted, however, that the variety ranking for stem score differed considerably from the

plant score ranking. Reference will be made to this fact under another topic. When all the varieties were considered as a group the variation between varieties was highly significant as indicated by the analysis of variance for stem score shown in Table 16.

Table 16. Analysis of variance for stem score on 450 artificially inoculated plants.

Source of variation	d.f.	Sum of squares	Mean square	F	Level of significance 1%	Level of significance 5%
Total	899	923.93	1.028			
Between varieties	9	146.30	16.255	21.083	2.43	1.89
" dates	8	91.14	11.392	14.776	2.53	1.95
" readings	1	6.93	6.93	8.988	6.66	3.85
Error	881	679.56	.771			

Favorable conditions following inoculation will produce more severe stem infection and consequently provide a more suitable basis for scoring the stems of the various varieties. If this is done, however, the leaf infection will be so severe that variety and plant variations in leaf score will be obliterated. At present it seems more suitable to provide conditions under which plant and variety variations in leaf infection can best be determined. Increasing the length of time the plants were left in the moist chamber following inoculation produced a more severe diseased condition.

Variety Variations in Leaf Score. The variety range in leaf score as shown in Table 14 was from 1.956 for Medicago ruthenica to 4.167 for Hairy Peruvian. The five per cent level of significance between variety means of .165 roughly placed the 10 varieties into five classes of severity. These were: 1. Medicago ruthenica, 2. Semipalatinsk and Ladak, 3. Grimm and Medicago falcata, 4. Turkestan 86696, Turkestan 19304, Kansas Common and Kansas Common Sel. 1-3018, and 5. Hairy Peruvian. The analysis of variance for leaf score shown in Table 17 indicates that variation between varieties is highly significant.

Table 17. Analysis of variance for leaf score on 450 artificially inoculated plants.

Source of variation	d.f.	Sum of squares	Mean square	F	Level of significance 1%	5%
Total	899	941.52	1.047			
Between varieties	9	224.79	24.977	50.766	2.43	1.89
" dates	8	105.03	13.129	26.685	2.53	1.95
" readings	1	178.23	178.23	362.256	6.66	3.85
Error	881	433.47	.492			

Variety Variations in Leaf and Stem Score. Comparison of the rankings of the varieties for leaf score and stem score illustrate the importance of considering both stems and leaves in making readings. An example of this is the relative ranking of the varieties Medicago falcata and

Turkestan 19304 for leaf score and stem score. Turkestan 19304 has a lower stem score than Medicago falcata. The difference is not statistically significant, however. When leaf scores are considered Medicago falcata has a statistically significant lower score than the Turkestan variety. This may mean that the factors for resistance to stem infection were not the same as the factors for resistance to leaf infection. This condition was repeatedly noticed when the readings were taken on the individual plants. This does not offer definite proof that factors for resistance to stem infection may be independent of factors for resistance to leaf infection but it does offer some definite clues upon which to work.

Variety Variations in Size of Lesions. As has previously been mentioned, the leaf score was obtained by giving equal consideration to size of lesions and number of lesions. The plants were scored on the zero to five basis for each of these factors and averaged to get the zero to five leaf score. There were two reasons for considering these two factors independently. First, a plant with few but very large lesions would be damaged as severely as a plant with more numerous but small lesions and, second, preliminary inoculations had shown that plants varied considerably in respect to size and to number of lesions.

The zero to five score for size of lesions ranged from 2.111 on Medicago ruthenica to 4.444 on Hairy Peruvian. The five per cent level of significance for difference between variety means in this case was .191. The number one ranking variety was significantly lower than the number two ranking variety and the number 10 ranking variety was significantly higher than the number nine ranking variety. None of the other varieties were significantly higher or lower than the one next to it. The analysis of variance for size of lesions is shown in Table 18. The variation between varieties is highly significant.

Table 18. Analysis of variance for size of lesions on 450 artificially inoculated plants.

Source of variation	d.f.	Sum of squares	Mean square	F	Level of significance 1%	Level of significance 5%
Total	899	1529.60	1.701			
Between varieties	9	149.07	16.563	25.210	2.43	1.89
" dates	8	167.10	20.887	31.791	2.53	1.95
" readings	1	635.04	635.04	966.575	6.66	3.85
Error	881	578.39	.657			

Variety Variations in Number of Lesions. The zero to five score for number of lesions on the leaves ranged from 1.800 on Medicago ruthenica to 3.889 on Hairy Peruvian. The five per cent level of significance for difference between variety means was .248. This placed the varieties into the

following disease classes: 1. Medicago ruthenica, 2. Semipalatinsk and Ladak, 3. Grimm and Medicago falcata, 4. Kansas Common Sel. 1-3018, Turkestan 86696 and Kansas Common, 5. Turkestan 19304, and 6. Hairy Peruvian. It may be questionable whether Turkestan 19304 should be placed in a class of its own or included in class four. It does differ significantly from the Kansas Common Sel. 1-3018 in class four.

The analysis of variance for number of lesions is shown in Table 19.

Table 19. Analysis of variance for number of lesions on 450 artificially inoculated plants.

Source of variation	d.f.	Sum of squares	Mean square	F	Level of significance 1%	Level of significance 5%
Total	899	1822.59	2.027			
Between varieties	9	333.37	37.041	33.552	2.43	1.89
" dates	8	514.48	64.310	58.252	2.53	1.95
" readings	1	2.25	2.250	2.038	6.66	3.85
Error	881	972.49	1.104			

The variation between varieties is highly significant.

Variety Variations in Size and Number of Lesions. Comparison of the ranking of the various varieties for size of lesions and for number of lesions indicated that the earlier stated supposition of plants varying in respect to these two factors may have had some foundation. For example Turkestan

19304 ranked sixth in size of lesions and ninth for number of lesions. Kansas Common Sel. 1-3018, however, ranked ninth for size of lesions and sixth for number of lesions. The variation between the two varieties was significant for both size and number of lesions.

This gives some indication that there may be two factors for resistance of leaves to black-stem disease which are independent of each other. The one factor restricts the entrance of the organism while the other inhibits its growth once it is inside. The independence of these two factors was particularly noticeable on individual plants even within a variety. Selections have been made for plants having large and small lesions and few and many lesions so that further study of this can be made. This work does not prove the existence of two independent factors for resistance but gives important clues for further study.

Variation Between Dates. Reference to the analysis of variance Tables 15 to 19, inclusive, show the variation between dates to be highly significant in every case. This means the nine groups of plants which were inoculated and put in the moist chamber did not become infected to the same degree of severity. One reason which can be given for this is fluctuations in greenhouse temperatures. Until some way has been found to decrease this variation between dates,

comparisons can only be made between plants which have been inoculated at the same time.

Variation Between Readings. The analysis of variance Tables 15 to 19, inclusive, show the variation between readings to be highly significant in every case except Table 19 for number of lesions. This indicates that practically all the lesions which are to appear have shown up at the time of the first reading. The F value for the leaf score is exceedingly large. This is due mainly to increased size of lesions from the first reading to the second. The F value for between readings for stem score is much smaller showing that the stem lesions do not develop as readily as the leaf lesions do after the plants have been removed from the moist chamber.

Plant Variation Within Varieties

Fairly definite proof that there is variation in blackstem susceptibility between varieties of alfalfa has been established. If any one variety is to be improved without crossing it with another less susceptible variety, variation in susceptibility within that variety must be present. Examination of the original data show rather wide variation in readings within a variety. For example, Kansas Common ranged from 1.5 to 9.0, Turkestan 86696 from 2.0 to 8.0 and

Ladak from 1.5 to 8.0. This range is made up largely of variation between dates. A measure of variability as expressed by the coefficient of variability would show the response of a variety to the conditions under which infection took place. This might be genetic. The most variable varieties would be the best place to select resistant plants. Table 20 gives the coefficients of variability for size of lesions and number of lesions for the 10 varieties.

Table 20. Variability in respect to size and number of lesions within 10 varieties of alfalfa.

Variety	Mean disease score		Standard deviation		Coefficient of variation in %	
	Size lesions	Number lesions	Size lesions	Number lesions	Size lesions	Number lesions
Hairy Peruvian	4.444	3.889	.7567	1.005	17.0	26.9
Kan. Common Sel. 1-3018	3.933	3.222	.9268	1.480	23.6	45.9
Grimm	3.444	2.867	.8420	1.358	24.4	47.4
Turkestan 86696	3.711	3.289	.9682	1.376	26.1	41.8
Kansas Common	3.800	3.311	1.0130	1.395	26.7	42.1
Ladak	3.333	2.556	.9045	1.391	27.1	54.4
Turkestan 19304	3.578	3.533	1.0130	1.289	28.3	36.5
Medicago falcata	3.444	2.889	1.1790	1.418	34.2	49.1
Semi-palatinsk	3.178	2.444	1.1730	1.120	36.9	45.8
Medicago ruthenica	2.111	1.800	1.0490	1.035	49.7	57.5

For size of lesions Hairy Peruvian shows the least variability. Kansas Common Sel. 1-3018, Grimm, Turkestan 86696, Kansas Common, Ladak and Turkestan 19304 follow in increasing order of variability with only a small range from low to high. Medicago falcata and Semipalatinsk are considerably more variable. This fact is considered to be significant because of the hybrid nature of these two varieties which has already been mentioned. Medicago ruthenica tops the list in variability for size of lesions.

The coefficients of variation for number of lesions also rank Hairy Peruvian as least variable and Medicago ruthenica as most variable. The next five varieties listed in decreasing order of variability are Ladak, Medicago falcata, Grimm, Kansas Common Sel. 1-3018, and Semipalatinsk.

DISCUSSION

At the present time it appears that two general methods of making black-stem disease readings of field plots can be employed. For early stages of the disease a record of the percentage of leaves of a sample which would be placed in disease class five, the class of greatest severity of the disease, seems most satisfactory. As the disease becomes more severe Richards' methods of deriving a coefficient of susceptibility seems most satisfactory.

Control of black-stem disease by sanitation may be worthy of consideration in regions where the disease is severe. This could be accomplished by winter grazing, especially to sheep. It is rather doubtful if burning could be successfully done. Usually there is not sufficient stubble and other crop residue for the fire to carry itself through the field unless it is very dry. If it is very dry there may be danger of heat injury to the plants. Removal of the last cutting in the fall at a late date, just before frost, prevents new growth from remaining on the field to serve as a source of primary inoculum the following spring. This practice should not be resorted to if the cover is needed for protection from winterkilling.

A good method for field testing of new strains, selections, or hybrids is very important in this work. It not only would serve as a check on the greenhouse readings but would allow for a more comprehensive test than could be done in the greenhouse. A method which was tried and described under the heading, Field Testing of Seedlings, was not entirely successful. It is believed, however, that some modification of this method would make it quite suitable. A border of five or six feet wide of alfalfa drilled around the plot would prevent border effect on the plants which are to be tested. If possible the plants to be tested should be started in the disease nursery in the fall so the

early spring growth would have the opportunity of becoming infected. A good supply of diseased stems and leaves should be evenly spread over the nursery to supply ample source of inoculum. An overhead sprinkling system should be installed to supply humid conditions and cause splashing of spores on the new shoots should rains fail to come at the proper time. Turning on the sprinklers a few minutes every night over a period of time would probably supply ideal conditions for infection to take place.

It is doubtful if the variety ranking with respect to susceptibility indicated by artificial inoculations are the same as would occur under field testing of these varieties. This is evidenced by the data on the Turkestans and Kansas Common varieties. In the field the Turkestans appear to be much more severely infected than Kansas Common. The reverse was true of the artificially inoculated plants. It seems unlikely that the plant characters causing resistance in the field should be much different from those causing resistance in the greenhouse. All greenhouse inoculations, however, were made with a single purified culture. Possibly physiological forms exist which react differently on the various varieties. The greenhouse conditions under which the plants develop could have a different reaction on different varieties and thus be responsible for this discrepancy.

The data on the 450 artificially inoculated plants

indicate that there may be at least three genetic factors concerned with resistance of alfalfas to black-stem disease. The factor for resistance to stem infection might be different from the factors for leaf resistance. Leaf resistance may be due to two factors, one which restricts the entry of the fungus and the other which restricts its growth once the fungus has gained its entrance. Plants have been selected for each of these types of resistance so that further work on the genetics of black-stem resistance may be carried on.

No work has been done to determine what plant characters affect resistance or susceptibility but one important observation has been made. Some plants of Ladak found to show resistance have smooth glossy leaves. A fine mist or spray of water directed on these leaves gathers in large drops and rolls off the lower edges of the leaves. A mist or spray of water directed on highly susceptible Hairy Peruvian plants remains as numerous small droplets over the entire surface of the leaves. Resistance may be due to the ability of a plant to shed spore-laden rain drops.

SUMMARY AND CONCLUSIONS

The causal organism of black-stem disease has been variously reported in the literature. The work of Toovey,

Waterston and Brooks indicated that black-stem of alfalfa reportedly caused by Phoma medicaginis, Ascochyta medicaginis, and Phyllosticta medicaginis may all be caused by Ascochyta imperfecta.

The many isolated places where the disease had been reported both in the United States and abroad indicated that the disease may be present wherever alfalfa is grown. Its importance varied with the weather conditions which prevail during the spring season and with the use to which the alfalfa was to be put.

The appearance of the disease on the stems, leaves and petioles of plants in the field are described. The symptoms of the disease on artificially inoculated plants are discussed with special reference to the development of the disease over a period of time.

Several methods of making disease readings have been used. A method of disease readings described by B. L. Richards for deriving a coefficient of susceptibility was used for making disease readings of severely infected plants. The method was very successful. A more detailed method of disease readings for field plots involved the classification of all or part of the leaves and the stem into zero to five classes for severity. Although this method probably represents a high degree of accuracy, it is much too tedious and time-consuming to be practical.

A sample size study was made to determine the number of stems to collect from a plot to give the desired degree of accuracy for plot readings. In this case approximately 50 samples were required. This test will probably have to be made from year to year to fit the particular conditions involved.

It was found from the data collected in the detailed method that a .95 correlation existed between the leaf scores and the percentage of the total number of leaves which were listed under class five. This correlation was highly significant.

Disease readings of artificially inoculated plants were made by giving the plant a zero to five score for relative number of lesions on the leaves and also a zero to five score for size of lesions. All lesions on the leaves as a whole were considered in making this estimate. A photograph was used as a standard for each of the classes. Stems were likewise scored on the zero to five basis.

Sanitary measures proved quite satisfactory in controlling black-stem disease on field plots. Removal of crop residue reduced the disease in proportion to the amount of crop residue removed. The percentage of leaves on the plants growing on plots varying in severity of diseased condition were in proportion to the severity of the disease. The percentage of leaves on the more severely diseased

plots was enough lower than the percentage of leaves on the less severely diseased plots to be of considerable economic importance.

Laboratory studies with Ascochyta imperfecta Peck were conducted with fungus which was isolated from diseased plants found in the field in the fall of 1938. This fungus was purified by the single hyphae tip method.

The optimum temperature for growth of Ascochyta imperfecta was found to be about 22° C. This fungus grows readily on potato-dextrose agar, alfalfa stems and sweet clover stems. The sweet clover stem cultures were thought to be most satisfactory because of their large size and abundance with which fruiting took place. Pycnidia seem to be more numerous but smaller when cultures were grown at temperatures above the optimum for growth. Cultures to be used for inoculation purposes were kept near optimum temperature for growth because the pycnidia were larger at this temperature.

Inoculations were made by spraying plants with spore suspensions prepared from test tube cultures of the fungus growing on sweet clover stems.

Proof of pathogenicity was established by isolating Ascochyta imperfecta Peck from artificially inoculated plants. Medicago falcata L. and Medicago ruthenica Trautv.

have been added to the host range which was established by other workers.

The duration of infection period varied with the time of year. For most of the inoculation work the plants were left in the moist chamber for three or four days. This gave optimum infection for finding plant variations for susceptibility. Leaf age was found to have no effect upon the severity with which the leaves became infected.

Disease readings of various varieties grown in the field under ordinary conditions have shown that statistically significant variety variations do occur. This includes several varieties, several tests and several methods of making readings.

Artificial inoculations of 45 plants each of 10 varieties were made. Analysis of variance showed variety variations in susceptibility highly significant. In deriving the plant scores, separate readings were made for stem score, leaf score, size of lesions and number of lesions. Variety variations were highly significant for each of these four characters. The variety ranking for each of these characters did not follow in the same order in all cases.

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