STUDIES ON ALFALFA RUST (*UROMYCES STRIATUS* SCHROET.)

by

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INTRODUCTION

Alfalfa ranks high in importance among forage crops in the United States. It is generally conceded to be the best forage crop in existence for temperate parts of the world in regard both to yielding capacity and feeding value. It is very prominent in the drier farming regions and in the last few years has rapidly gained an important place in the north central states or corn belt region. Alfalfa is not only grown for its forage value for stock but it also has a place in crop rotations since it is capable of enriching the soil with nitrogen. Its habit of rapid growth is also of importance in any consideration of its value as a pasture plant. It is an old and stabilized crop, probably planted and cared for by man before history was written. The plant has proved its value and seems permanently established. By natural selection and breeding work by man resistant varieties have been found and no doubt the alfalfa crop of today is much more vigorous growing and disease-free than it has been at any other time in its history.

Any disease-producing organism that causes damage to such a crop deserves some study. Alfalfa rust is not the most important disease of the crop today, but in recent years, especially in 1936 and 1938, it has done more damage than realized. It has come to the attention of the Kansas farmer as well as plant pathologists because of its damage to the later cuttings, especially its vigorous attack on the crops grown for seed production. Alfalfa rust has been known to occur for many years but it seldom has been found to cause enough damage to warrant much attention.

Continued field examinations of nearly any crop will bring to light, from time to time, in obviously parasitic roles, fungi that may generally appear to be of no real harm to plants. Such parasites are of little importance to the general farmer and most pathologists, yet, with other minor pests they contribute measurably to the reduction of yield by disease. On such a crop as alfalfa, which has a value as a soil builder, they exert an indirect and tangible influence concerned with more than yield alone. In years when alfalfa rust is hardly more than noticed by the farmer and plant pathologist it often has a direct and detrimental effect on the vigor of a plant which is relied upon to improve the soil.

Weimer and Madson (23) presented the alfalfa disease problem very simply in 1932. They said, "Only in recent years have the diseases of alfalfa become sufficiently destructive to attract the interest of the grower. Certain leaf diseases have long been known, but they appear only at irregular intervals and apparently cause only a temporary setback to the plant, so they receive little attention. During the last few years, however, a decided change has taken place. In many localities, alfalfa stands no longer last from 10 to 15 years or more, but may be so depleted before the end of the second or third year as to become unprofitable. This rapid dying out of the alfalfa stands has aroused much interest in the subject of alfalfa diseases."

Hence, in view of the fact that very little is known about alfalfa rust, one of our common leaf and stem diseases, a general study was outlined to determine the importance and distribution of the disease, facts about its life history, how alfalfa strains may be tested for resistance under controlled conditions in the nursery, greenhouse and laboratory, and whether there is any difference in susceptibility of various strains and varieties of alfalfa to the pathogen.

REVIEW OF LITERATURE

Causal Organism of Alfalfa Rust

There is some discrepancy in the literature as to the classification of the organism causing rust of alfalfa. Some of the references list it as Uromyces medicaginis Pass. while others speak of it as U. striatus Schroet., the latter name holding the preference at present. Arthur (1), in 1912, called it U. medicaginis, but later (2) he listed it as U. striatus. While there is not complete agreement among authors upon the name of the organism, it will be referred to in this paper as U. striatus.

Arthur (2) stated that the first culture of the rust was by Schroeter who in 1884 reported sowing spores from aecia of Euphorbia cyparissias on Trifolium agrarium and obtaining the Uromyces. He noted that Trebaux in 1912 reported sowing aeciospores successfully from Euphorbia gerardiana and E. virgata on Medicago falcata, M. lupulina, and M. sativa, and later stated that he infected M. minima, M. murex and Trifolium arvense with aeciospores from E. virgata. From these results Trebaux assumed this proved that the assignment of Uromyces striatus to both Medicago and Trifolium as hosts was correct.

Arthur also observed that this species has not been reported in the United States on Trifolium although Kobel as well as Klinkowski and Lehmann (9) obtained infections by sowing aeciospores from E. cyparissias on species of Medicago and Trifolium. Klinkowski and Lehmann concluded, "It serves to mention that the alfalfa rust belongs to the host-changing rust fungi (alternate host--Euphorbia cyparissias)."

Arthur (1) made cultures with urediospores in 1910 and again in 1911, using spores from plants of *Medicago sativa* sown on *M. sativa*, *Trifolium pratense*, *T. medium* and *T. repens*, produced infection only on *Medicago*. Similar sets of sowings were made later with the same results. According to Arthur (2) teliospores are rarely found in American collections.

Cunningham (6) agreed with Arthur on the above points and discussed the confusion in literature concerning the relationship of the teleutosori on *Medicago sativa*, and aecidiosori on *Euphorbia cyparissias*. He also reported Schroeter's original work, and, in addition, stated that Jordi found that aecidiosori on this species occurred in the cycle of a morphologically identical *Uromyces* on a species of *Lotus*, and gave to it the name, *U. euphorbiae-corniculati*. The latter was considered by Grove to be a distinct species, *U. lotii* Blytt, although Arthur considered that name to be a synonym of *U. striatus*. Clinton (5) stated that the rust appears to be closely related to the red clover rust with which it was closely associated in one of the fields he observed. After reviewing all the evidence, Cunningham concluded that since all forms under discussion are morphologically identical, it seemed evident that they must belong to the same species.

Range or Geographical Distribution of Causal Organism

Alfalfa rust is common in the United States and is perhaps worldwide in distribution since it has been reported from every continent where alfalfa is grown. Its range, according to Arthur (2), is Massachusetts to South Dakota, southward to North Carolina and Texas, and from Oregon southward to Arizona and southern California; also in the West Indies, Mexico, South America, Europe, southern Asia, northern Africa, Japan and New Zealand. Nattrass (17) in 1933 reported *Uromyces striatus* on lucerne as new to the island of Cyprus.

Arthur (2) reported rust on different species of *Medicago* in the United States namely, *Medicago sativa* L. in Alabama, Arizona, California, Colorado, Indiana, Iowa, Kansas, Louisiana, Nebraska, Ohio, Oklahoma, Pennsylvania, South Dakota, Tennessee, Texas and Utah; *M. falcata* L. in South Dakota; *M. hispida* Gaertn. (*M. denticulata* Willd.) in California and Louisiana; *M. lupulina* L. in California, Delaware, Kentucky, Massachusetts, New York, North Carolina, Ohio, Oregon, Pennsylvania, West Virginia and Ontario; and *M. tuberculata* Willd. in Louisiana.

The following maps (Figs. 1-3) afford a better picture of the distribution of the disease in the United States. Figure 1, which is from the Plant Disease Reporter (20), gives all reports of rust on *Medicago sativa* and *M. lupulina* up to and including the year 1922.

Figure 2 includes all reports of the disease in the United States from 1917 to 1939 inclusive (Plant Disease Reporter, 20). This is by no means a complete record of the occurrence of the rust. It does, however, give a good idea of its prevalence and distribution in the states and lists the relative number of times that the disease has been reported to the disease survey.

Figure 3 indicates only the states in which the writer has had direct correspondence with plant pathologists in an effort to learn more about the occurrence, distribution and prevalence of the rust organism.

Fig. 1.--Reports of alfalfa rust in the United States on Medicago sativa and M. lupulina prior to 1922.

Fig. 2.--Occurrence of alfalfa rust in the United States during the years 1915-1938 as determined by reports of the Plant Disease Survey, United States Department of Agriculture.

Fig. 3.--Presence or absence of alfalfa rust in certain states of the United States and provinces of Canada as determined by reports obtained in correspondence with plant pathologists.

Economic Importance of Disease

Alfalfa rust is considered to be of very little economic importance in some regions. But in other areas it is considered by authorities as one of the most important leaf and stem diseases. Its damage in any one season, of course, depends to a great extent upon the prevailing climatic conditions, especially the amount of rainfall during the summer months and the later part of the growing season. This rust, in all cases, seems to do its greatest damage to the crop that is being saved for seed, as is well illustrated in Fig. 4. However, under favorable conditions, it may be conspicuous in the later hay cuttings in the western Mississippi valley. Figure 5 shows rusted stems in contrast to a noninfected stem.

From all indications the disease causes greater losses in the warmer, more humid parts of the United States than in the cooler areas. Brown and Streets (3) reported, "In Arizona, in irrigated fields, usually there is enough moisture for the development of the disease on the lower leaves of the alfalfa plants; in dense stands spotted leaves and stems may be abundant." They listed such injury as hypertrophy or overgrowth of plant parts causing a deformed appearance and infected seed pods that often produce a variety of sizes of seeds, some abnormally large, some normal, some small and dwarfed. Such abnormalities are certain to affect the economic value of both the hay and seed crop.

Melchers (14) said that rust is not as injurious to the alfalfa crop in Kansas as the two leaf spots, Pseudopeziza leaf spot and alfalfa leaf blotch. Over a 5 or 10 year average this is true; however, some attacks by the rust fungus in more recent years have unquestionably been important, depending on prevailing weather conditions.

EXPLANATION OF FIGURE 4

A. Stem from a heavily rusted plant showing severe defoliation and numerous uredia on internodes.

B. Stem from a rust-free plant of the same age showing retention of leaves and clean internodes.

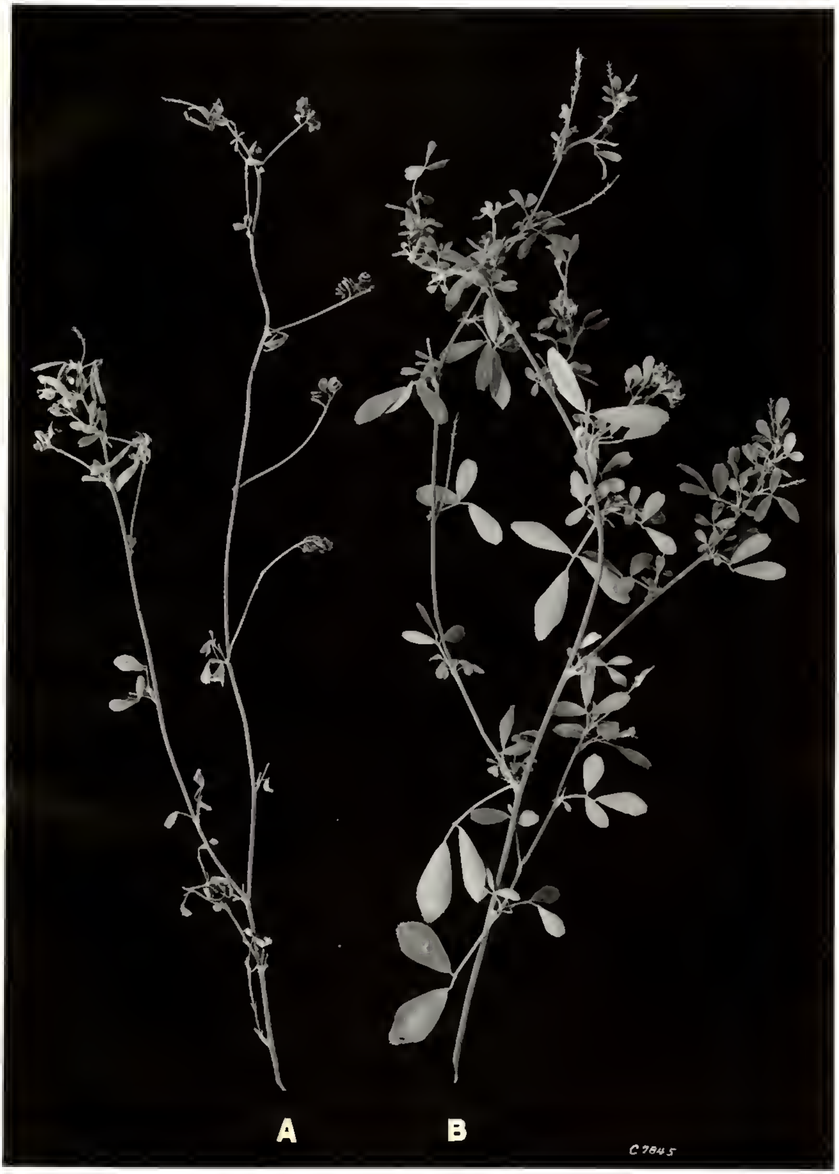

Fig. 4.--Effect of alfalfa rust upon the seed crop.

Fig. 6.—Comparison of heavily rusted alfalfa stems A and B with a rust-free stem C.

In California, Weimer and Madson (23) found that the disease occurred on alfalfa leaves and frequently on stems during a large part of the year, but was especially abundant during the summer. The leaves and stems of the seed crop were usually heavily infected and often caused serious injury. Rust, however, according to them, rarely did much damage to the hay crop.

Pammel and King (19), reporting on four new fungous diseases in Iowa in 1910, stated that rust showed up in July and spread very rapidly during the next two weeks, reaching the epidemic stage in early August. Alfalfa rust became epidemic again in the late summer of 1911 in the alfalfa plots on the college farm, especially on volunteer plants.

Clinton (5) stated that rust has been found in Connecticut alfalfa fields only twice and then it was so scarce that it caused no evident harm. Freeman (8) from Kansas likewise found that rust caused no serious loss in alfalfa fields in this country but that it was rather common. Dickson (7), in 1939, said rust occurred fairly commonly on alfalfa in the United States, especially late in the season, but little damage resulted.

In a report from Germany Klinkowski and Lehmann (9) stated that in periods of damp weather rust injury from *Uromyces striatus* was strongly manifested. Large losses caused by this fungus were hardly known. They reported that in certain regions of the United States seed alfalfa was often so greatly injured that a considerable cut in yield resulted.

Nivikov (18), in 1935, detected a derangement of metabolism in the leaves of alfalfa when infected with the rust *Uromyces striatus*. By chemical analyses he endeavored to determine the significance of alfalfa rust.

He gathered leaves at different dates from both healthy and diseased plants infected naturally. On the first date, the leaves of the diseased plants were about 50 percent infected. On the second date, just three weeks later, the leaves of the plants were 100 percent infected. These samples were analyzed for carbohydrates, monosaccharides, disaccharides, dextrin, starch, hemi-cellulose, cellulose and also for total nitrogen, protein nitrogen and nonprotein nitrogen.

The healthy and diseased leaves of the first gathered did not, as a whole, show a difference in carbohydrates, notwithstanding the fact that the diseased leaves expend more of them. This, he said, was due to the fact that the parasite can draw or mobilize reserve carbohydrates from other organs of the plant to the place where they are being especially rapidly consumed, in connection, for example, with spore formation. His analysis of the second gathering showed many features in common with the first but was quite different in some respects. The total carbohydrates was 14.5717 for the noninfected and 11.7939 for the infected.

His data on the content of nitrogenous substances in healthy leaves are of no less interest. The diseased leaves of the first gathering had less total nitrogen, as also less protein and nonprotein nitrogen, than the healthy one. By the time of the second gathering, the nitrogen content had increased in the healthy leaves and decreased in the infected ones, especially in the case of protein nitrogen. He remarked that it was hardly probable that nitrogen should be drawn from the diseased leaves into other organs of the plant. He then ventured the supposition that infection with rust created conditions in which combined nitrogen was converted into gaseous nitrogen, which in turn, escaped from the leaf.

Nivikov later stated that infection of any of the higher plants with rust led, not only to a decrease of the general production of dry substance but also to a yield poor in nitrogenous compounds. Consequently, the food value of plants infected with rust was notably diminished. He also remarked that this fact is especially important with regard to lucerne, which, when infected with rust, loses, to a certain extent, not only its food value, but inasmuch as its nitrogen content decreases, also one of the principal properties determining its place in crop rotation--that is to say, its capability of enriching the soil with nitrogen. In closing he concluded, "And so, as a cause of derangement of the physiological processes, the rust, Uromyces striatus, is a serious source of injury to lucerne and consequently ought to receive more attention than is ordinarily paid it."

For losses due to the organism and as an aid in determining the distribution of the disease, the Plant Disease Reporter (20) was very helpful. During the twenty-five years, 1916 to 1940 inclusive, alfalfa rust was found to be listed 23 times, either in the Supplements or the regular numbers of the Plant Disease Reporter. These reports, in a general way, gave a panorama of the occurrences of the disease in the United States. They, however, are not absolutely accurate since alfalfa rust undoubtedly must have appeared some place in the United States on years when it was not reported. During the years 1916, 1917, 1918, 1928, 1932, and 1938 no reports were sent to the disease survey. This might indicate that on these years rust was relatively unimportant or perhaps not even noticed, but notes recorded in files at the Kansas station by Dr. D. B. Creager in the summer of 1938 denoted that the disease appeared on July 7, one of the earliest recorded dates of occurrence of the disease.

According to reports, rust was most severe in the United States in the years 1915, 1921, 1922, 1929, 1930, 1933, 1934, and 1939. During 1922, the states of Pennsylvania, Illinois and Missouri reported the disease to the survey for the first time. It also occurred in Indiana in much greater quantities than usual that year, especially in the central and northern parts. In Missouri it was unusually abundant in the farm crop plots at the university, while workers reported that the outbreak of 1922 was the most severe that had ever been noted in Kansas. Considerable loss also was reported from Louisiana, where the disease is usually prominent, while in Arkansas much damage resulted from the shedding of leaves.

In a report of the plant diseases in Iowa in 1927 unusually severe infections of rust were recorded for some of the southern counties, causing considerable dwarfing of the leaves. Weimer (21) reported in 1929 that rust (U. medicaginis) was very prevalent in southern California in the fall and added that he had never seen the disease so destructive elsewhere. In some fields as high as 75 percent of the leaves were attacked and stem lesions were also abundant. Again, in 1933, Weimer (22) reported that rust was very severe in his plots in October when nearly 100 percent of the plants were affected and many leaves were lost.

The occurrence of heavy infections of alfalfa rust in Oklahoma were observed by Chester (4) in 1939. He noted the presence of the disease in many fields and added that only rarely had the organism been known to cause the defoliation frequently observed in that year. These observations were taken as early as July 18.

Epiphytotics seem to occur in localized areas in the United States nearly every year, depending on the prevailing weather conditions in the area. New Jersey, the Carolinas and Georgia, the Gulf states, especially Mississippi and Louisiana, and the part of Texas along the lower Rio Grande Valley seem to report rust very frequently. However, the locality in the United States where the greatest amount of damage is done year after year is in the fog belt along the coast in southern California. Extended rainy periods during the months of July, August, or September may lead to general epiphytotics in the inland states such as Kansas, Oklahoma, Missouri, Iowa, Illinois, Indiana and Arkansas.

Alfalfa rust also seems to manifest itself in the irrigated districts of the Southwest, mainly New Mexico, Arizona and parts of southern California.

In the 25 years, a total of 34 states and the territory of Hawaii reported alfalfa rust to the disease survey. Of the 34 states, California and Texas reported rust the greatest number of times.

In an effort to learn more about the prevalence, distribution and economic importance of alfalfa rust in the middle western states and also, perhaps, to obtain information on overwintering, and the date of first appearance, the writer sent letters of inquiry to plant pathologists or forage men from several of the states and Canada.[1/] In general, the informa-

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1/ The writer is indebted to the following persons for information on alfalfa rust supplied through correspondence: Dr. K. Starr Chester of Oklahoma A. and M. College; Dr. W. E. Maneval, University of Missouri; Dr. J. G. Brown, University of Arizona; Dr. A. A. Dunlap, Texas Agricultural Experiment Station; Dr. J. G. Dickson, University of Wisconsin; Dr. I. E. Melhus, Iowa State College; Dr. I. L. Forbes, Louisiana State University; Dr. J. J. Christensen, University of Minnesota; Dr. J. H. Jensen, University of Nebraska; Dr. G. B. Cummings, Purdue University; R. W. Lewis, Michigan State College; Dr. L. W. Durrell, Colorado State College; Dr. L. C. Snyder, South Dakota State College; Dr. J. M. Raeder, University of Idaho; H. E. Morris, Montana State College; Dr. V. H. Young, University of Arkansas; Dr. W. E. Brentzel, North Dakota Agricultural College; J. J. Bayles, Superintendent of the Texas substation at Balmorhea, Texas; and Dr. I. L. Connors, of the Central Experimental Farm at Ottawa, Ontario, Canada.

tion obtained was of very little value as far as an increased knowledge of the organism itself is concerned. It further proved that little is really known about the life history of the organism. Nevertheless, by means of correspondence, opinions were obtained on the importance of the rust in various areas, as well as valuable information concerning the date when the disease generally makes its appearance.

It was interesting to note that in some states alfalfa rust was unknown, while in others, pathologists were very much aware of its presence. According to information obtained by correspondence, rust has never been found in Idaho, North Dakota and Montana. Only one report was listed from Minnesota and South Dakota. It is not at all common in Colorado and investigators in Arkansas had almost no information to report. All these reports, however, do not coincide exactly with those of the Plant Disease Reporter. In 1921 the disease was reported from North Dakota and in 1929 *Uromyces medicaginis* was listed as occurring among the diseases of alfalfa in Montana. This latter report was the first time that it had been noticed in the state.

As a direct contrast to the above statements, Chester reported that *Uromyces striatus* was very prevalent in Oklahoma and he remarked that letters from growers nearly every year expressed concern at the trouble and requested control recommendations. He stated, "The rust is apparently our leading leaf disease and cause of defoliation and must be seriously affecting tonnage in many cases. This past year I have found it in every alfalfa field examined. In October, 1938, I observed a field near Norman so heavily rusted as to change the color of the field when viewed from a distance. I estimated 10 percent damage, but this may have been an underestimation."

Brown of Arizona reported that rust can be found practically throughout the year in some of their alfalfa growing districts but that more rust usually occurred in later cuttings as compared with the first cutting in spring. He remarked, "Ordinarily, the rust does not appear to cause great loss, but occasionally it is bad in certain fields. At times it is present only in a few spots per leaflet; again it is abundant. One field of alfalfa near Tucson, 18 acres in extent, was so badly affected by a combination of rust and girdle at the beginning of autumn that further cutting was abandoned and livestock turned in to graze."

Alfalfa rust caused occasional heavy losses in Missouri and Iowa reported that sometimes it becomes destructive. Growers from the irrigated land of western Texas have reported that the disease had reduced the crop in their fields more than half. Other states, Wisconsin, Nebraska, Indiana, and Michigan reported rust to be usually of minor importance.

It is also interesting to note here that rust, according to I. L. Conners, occurred and caused some injury in Canada and can be found as far north and east as Prince Edward Island. He listed six references to the disease in the Canadian Annual Reports since 1926.

HOST AND PARASITE RELATIONSHIPS

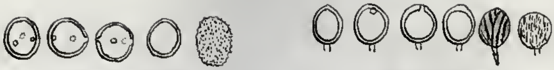
Etiology

Uromyces striatus is a true rust, belonging to the class Basidiomycetes, order Uredinales and family Pucciniaceae. It has the characteristic one-celled teliospores of the genus _Uromyces_, and except for the absence of the I and II stages in the United States this rust might be considered as typical for the genus. It is reported as heteroecious by several workers in Europe, however, with the aecial stage developing perenniel mycelium in <u>Euphorbia</u> <u>cyparissias</u> L. The details of the cycle have never been worked out in the United States; in fact, very little is known concerning it. It has been suggested that the fungus lives over winter in the leaves infected late in the fall and infection occurs from urediospores produced the following spring. This supposition is doubted because of the results of experiments reported herein.

Spore forms have been described by various investigators. However, after examining several descriptions, the work of Cunningham seemed most usable in this case, and hence has been adopted in spite of the fact that the original work was done in Europe and Arthur's work (2) on rusts is considered as authoritative in this country. Following is the description of the causal organism as given by Cunningham (7):

> O. Pycniosori hypophyllous, numerous, honey colored.
>
> I. Aecidiosori hypophyllous, scattered. Peridia cupulate, margins white, revolute, lacerate. Spores polygonal, 18-23 microns diameter; epispore densely verruculose, 1.5 microns thick, hyaline.
>
> II. Uredosori amphigenous, scattered, elliptical, to 1.5 mm. long, pulverulent, cinnamon brown, surrounded by the ruptured epidermis. Spores subglobose or elliptical, 20-25 x 12-20 microns, average 22 x 19 microns; epispore sparsely and finely echinulate, 2 microns thick, pallid brown; germ pores 4-8, scattered.
>
> III. Teleutosori similar to and immixed with the uredosori, chestnut brown. Spores obovate or subglobose, 18-25 x 15-18 microns, average 20 x 18 microns; apex rounded, not or scarcely thickened, base usually rounded, seldom attenuate; epispore 2 microns, covered either with fine longitudinal striae or with scattered irregular warts arranged in lines, cinnamon brown; pedicel deciduous, hyaline, to 30 x 6 microns; germ pore apical, crowned with a narrow hyaline papilla.

The markings of the teliospores are not always the same in different collections; for certain specimens they appear as finely scattered striae, in others as warts aggregated in lines or even scattered somewhat.



Urediospores Teliospores

Fig. 6.--Spores of Uromyces striatus on Medicago sativa.

Symptomatology

Signs and symptoms of alfalfa rust are very definite and are not easily mistaken for those of any of the other leaf and stem diseases. The first symptoms of rust infection on the alfalfa leaflet is evidenced by a slight yellowish or chlorotic spotting, a sort of flecked appearance as in the case of rust-infected wheat. Light-yellowish, raised pimples, due to the upward growth of groups of spores, then begin to appear on the lower surface of the leaflets and soon the epidermis is ruptured, disclosing a mass of reddish-brown to cinnamon-colored spores. These pustules (uredia) appear first on the under surfaces of the leaves but later they can be found on both sides of leaves and on the stems. Each spore mass, although relatively small, can be seen clearly without aid of a lens. After exposure the spores rub off on the fingers in the form of a rusty powder.

The uredial stage on the leaves is reddish brown with small, round uredia usually scattered over the leaf. The telia appear in the same lesions and in independent lesions later in the season and are darker brown in color. Leaf infection often reaches the stage when the infected areas lose their color and a necrosis of the tissue occurs, leading to leaf fall, especially if petiole lesions are very numerous. Infected leaves tend to curl and dry up readily after the pustules make their appearance. Secondary infections are common as evidenced by Fig. 7. Sometimes as many as three or four concentric rings of pustules have been noticed on the same leaflet. Figure 8 shows the chlorotic spotting often noticed on the upper surface of alfalfa leaflets infected with rust. Stem and petiole pustules vary greatly from those on the leaves. They are generally larger and more elongated, as shown by Fig. 9.

Fig. 7.--Leaflets of Turkestan 26696 alfalfa showing rust infection on the lower surface. (1.5 X)
Note the large normal uredia without chlorosis and the concentric rings of pustules showing secondary infection.

Fig. 8.—Leaflets of Kansas Common alfalfa showing rust infection on the upper surface of a moderately resistant type. (1.5 X) Note the small uredia surrounded by chlorotic areas and absence of secondary infection.

Fig. 9.—Heavily rusted stems of Turkestan alfalfa collected in the field. (2.5 X)
Note the large uredia on the internodes, stipules and leaflets.

EXPERIMENTAL RESULTS

Laboratory Experiments

Spore Germination Studies. Laboratory work with the problem was limited mainly to microscopic spore examination and germination studies. However, one inoculation device was also worked out and studied in the laboratory.

One of the foremost questions in the writer's mind concerning alfalfa rust was how the organism overwinters as far north as Kansas. If the alternate host is not needed for the propagation of the organism the urediospore must be the all-important spore in the life cycle. But can it overwinter in Kansas? And, if so, why does this spore not germinate in the spring and infect the early cuttings of the crop instead of waiting until late July or August when the disease makes its appearance?

With these questions in mind an experiment was set up to ascertain how long the urediospores remained viable under different environmental conditions. Samples of rusted plants collected in the field on different dates were saved for this purpose. Rusted plants were collected in the field, dried in plant presses, labeled, and placed in screen wire envelopes for storage or overwintering. In all cases the spores were allowed to remain on the host. More teliospores occurred in the later collections, therefore, they were used for teliospore germination studies. All material used in this test was collected on September 13, 1939, and placed in storage on October 4, 1939.

Four different indoor storage places were selected for the urediospores besides being stored under natural conditions outside. A wire envelope of rusted alfalfa stems and leaves was placed in a warm location in the Experiment Station Laboratory, another in a cooler place in the herbarium room in the basement of the Horticultural Building, a third in a cool fruit storage room in the basement of a dwelling house, and still another in the Horticultural cold storage room.

The temperature and relative humidity varied greatly in these different environments. Several readings were taken and the averages for the different environments are listed in Table 1.

Table 1. Temperature and relative moisture content of air in rooms used for storage of rusted alfalfa samples in 1939-40 as determined from readings on a Lloyd Hygrodeik.

Location	Hygrodeik readings Wet bulb Ave.	Dry bulb Ave.	Ave. Rel. humidity	Additional notes
Laboratory	57+	75	32	Temperatures rather high, moisture content of air low.
Herbarium room	61	81	28	Constant high temperatures, air dry, low relative humidity.
Basement of dwelling	No readings taken.			Conditions varying with temperature and humidity outside, never below freezing.
Horticultural cold storage room.	33+	35+	80	Constant cool temperatures with high relative humidity.

Readings taken between Jan. 28 and April 8, 1940.

In the case of the dwelling storage, the conditions varied so much with the external weather that average readings would have shown very little. A window to the small room was often opened in warmer weather but remained closed in cold weather. Temperatures were never below freezing, however.

Average dry bulb temperatures ranged from 35° F. in the Horticultural cold room, to 81° F. in the herbarium, while the average relative humidity range was from 32 in the herbarium room to 80 in the Horticultural storage room. These average readings were formulated from a series of readings taken between January 26 and April 8, 1940.

Samples of spores from each of these envelopes were examined in the laboratory under the microscope at fairly regular intervals. Van Tieghem cells were mounted and sealed on glass slides. A drop or two of water was placed within the cell, then a cover slip on which spores were suspended in a drop of water was inverted and placed on the upper border of the ring. The slides were then placed on moistened filter paper in Petri plates. The spores seemed to germinate best in this manner but some were placed directly on glass slides and germinated in moist Petri dishes. Generally, fair results were obtained but all technical work was done by the former method.

Estimated percentages of germination were determined by observing different fields under the microscope. Results of these examinations are shown in Table 2 and Fig. 10. Urediospores were first taken from storage for examination on November 10. All samples at that time were germinating approximately 100 percent. On December 7, however, there was a very sharp drop in the viability curves in all cases except that of the spores in the cold storage room. The latter spores were still germinating around 75 percent and it was not until one month later, on January 10, that they showed a sharp decline in germinating power. All spore samples tended to show a rather abrupt drop in germination for a 2-month period, then a leveling off with an ultimate decline in viability to the point where none of the spores germinated. In the case of the spores in the Experiment

Station laboratory, this point was reached on February 10, 1940, just three months after the first test. Spores in the herbarium room remained viable until March 28. None germinated after April 10. A very few of those stored in the basement room germinated on May 9, 225 days after collecting. On June 3, germination had stopped completely. Approximately 10 percent of the spores in the horticultural cold storage room were viable on May 9. On the fourteenth of the month the use of this room was discontinued and the spore material had to be removed. It is highly probable that some of these spores would have retained their viability for another month or more. These spores and those from collections made on September 22 and September 29, 1939, were tested for germination on November 5, 1940. They had been placed in a refrigerator soon after being removed from the cold room but no spores were viable at this late date.

Table 2. Percent of germination of urediospores under different environmental conditions in the laboratory.

Date of examination	Approximate percent of germination			
	Laboratory	Herbarium	Basement of dwelling	Horticultural cold room
November 10	50	50	70-75	75-80
December 7	7-10	30-35	20-25	70-75
January 10 and 12	1-2	5	10-12	45-50
February 10	0	1-2	10-12	15-20
February 28			8-10	12-15
March 28			6-8	15-16
April 10	0	0	5	15-20
May 9			1	10
June 3			0	

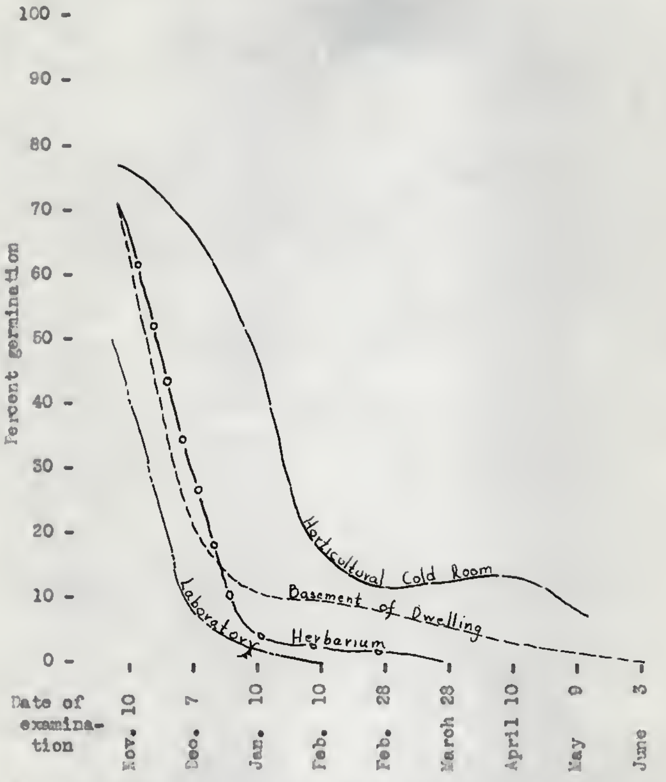


Fig. 10.--Viability curves showing decline in germination of urediospores of Uromyces striatus when stored under different environmental conditions.

Fresh spores, when taken from the living plant, generally showed 100 percent germination. This was evidenced several times by taking spores from artificially inoculated plants in the greenhouse and running a test with the other spores.

Maneval (12), in his studies on the viability of urediospores of rusts, has kept spores under various conditions, both in the open and indoors. He collected them late in the fall and, after drying, kept them in a cool room, 5-15^{o} C., most of the time. At intervals germination tests were made by floating the spores on distilled water and incubating them at room temperature for one or two days. He experimented with several different rust species but his results of Uromyces striatus on Medicago sativa only are of interest here. Maneval states, "Urediospores of this rust were collected on November 11 and kept in a cool room. No tests were made until March 23 when a fairly large proportion of spores germinated. Subsequent tests on March 31, April 13 and 20, and May 7 gave positive results, but no spores germinated in a final test on June 9." Urediospores were also collected on October 9 of the following year and kept at room temperature until November 14, after which they were stored in a cool room. He reported, "A high percentage of spores were germinating on November 14. Subsequent tests resulted as follows: December 12, fairly high percent; January 4, 10-15 percent; February 1, few spores; February 27, numerous spores; March 29, plus 1 percent; April 17, negative. Spores of this rust therefore remain viable for at least 173-178 days after collection."

In the present studies, it was found that the urediospores, under the right conditions, will remain viable for at least 233 days. It is believed that even longer periods might be obtained since at the end of this time

10 percent of the spores were germinating. The closing of the cold room on May 14 necessitated the termination of the experiment.

Length of viability, therefore, seems to depend mainly upon a temperature relation. Moisture conditions no doubt are likewise important. The environmental conditions in the Horticultural cold storage room were no doubt near optimum. Those in the dwelling house basement were so variable that it is difficult to report definite results, but some of the spores retained their viability for more than 200 days. The temperature in the herbarium was constant, but too high, and there was probably a lack of moisture. The high fluctuating temperatures in the Experiment Station laboratory and also a definitely low moisture content in the air at all times led to the rapid decline in germinating power of the spores.

Maneval's conclusions as to the overwintering of urediospores out of doors are sometimes doubtful, since mycelium may live through the winter and spores present in the spring may have developed during the winter or early spring. In all probability, however, this does not happen in the case of alfalfa rust in Kansas since the winters are too severe for mycelium to survive and no spores have been found to develop in the spring. Maneval made collections of urediospores on March 3 and April 2, but all efforts to germinate them failed.

About 35 percent of the urediospores were still germinating in the Manhattan collections stored outside on December 6. It must be remembered, however, that hardly any cold weather had occurred up to this time in 1939. Only three or four spores were found germinating on February 15 and the last signs of viability was on March 21 when only one urediospore was found to produce a germ tube.

This diseased material was collected in October and placed in screen wire envelopes. Some were placed flat on the ground, others were laid on dead weeds and another was hung on a low limb of a bush about a foot from the ground in order to have comparable conditions to those surrounding old alfalfa stubble as it overwinters.

It not only was very desirable to conduct germination studies with the urediospores, but the teliospore also offered another phase of interesting study. Hence, the purpose or function of the teliospore, if any, in the life cycle of the organism in Kansas was another problem connected with this study. Was this stage actually necessary for the yearly occurrence of the disease or was the urediospore the only functional stage in the life cycle? Experiments such as those with the urediospores were set up in order to obtain more information concerning these questions.

Rusted material, collected in late October, 1939, was placed in screen wire envelopes, labeled and left outside to overwinter under natural conditions. From February 15, 1940, at different intervals, these spores were scraped from the leaflets and tested for germination. No teliospores were ever found to be germinating. It was decided after running several duplicate tests for a month to try treating the spores in a different manner in the hope that germination could be obtained. The different methods used were as follows: (1) An alternate wetting and drying of the spores on slides in alternate periods of light and darkness (day and night) for three to four weeks, (2) placing spores in hanging drops in refrigerator pan at a temperature of 24° F. and alternating with warm temperatures in laboratory for a period of three to four weeks, (3) placing spores on slides in droplets in refrigerator at an average temperature of 42° F. and alternating with warm, exposed condition in laboratory for some period of time, and (4) placing rusted stems and leaves in refrigerator pan and leaving them there, except for the various times they were removed for examination. At no time were any of the teliospores seen to be germinating. An alternate wetting and drying under room conditions were also tried but with negative results.

A 5-percent solution of potassium dihydrogen phosphate (KH_2PO_4) was made and spores collected September 13 and October 12, 1939, and overwintered out of doors were suspended in it for germination tests. The mounts were examined microscopically after 12, 24, 36, and 60 hours but there was no signs of germination in either the uredio- or teliospores.

Teliospores from the collections stored out of doors were tested in duplicate on February 15, March 1, March 11, March 21, April 8, April 12, April 21, May 9, and May 21. On no occasion did any of them show signs of germinating. Two to three urediospores were germinating, however, on February 15, another was found on March 21 and two more were found in all the mounts tested on April 21.

The pustules seem to disappear, to dry up as the leaves and stems weather and disintegrate. By the first of May it was very difficult to find pustules containing spores, even on the stems. Saprophytic molds and other fungi tend to destroy the stems when left lying on the ground. One of the packets of rusted material, however, had been brought inside about the first of March and had been hung outside a north window. This remained fresh and clean, free from saprophytic growth, and could be used as a source of inoculum until late in the year. The best policy, it seems, for overwintering material would be to hang or suspend the material about 4-6 inches from the ground. There, it would really be under more natural conditions since the old stems stand throughout the winter, and in this way the material would escape a considerable amount of saprophytic growth and subsequent rotting.

Maneval (11), in 1922, also conducted some experiments on the germination of teliospores of rusts. In general, he found that teliospores of rusts germinate only after a more or less definite resting period, usually extending from summer or autumn of one year to the time of leaf development of the host the following year. It is generally necessary for the teliospores of heteroecious rusts to pass the winter in the open. He claimed that light, moisture, temperature and some chemicals may affect the germination of these spores. Generally a practically saturated atmosphere was necessary for germination and he and other workers found that germination was especially favored by prolonged floating on water and by alternate wetting (immersion in water) and drying. In various later tests (13), teliospores of certain rusts germinated better in a solution of potassium dihydrogen phosphate than in distilled water. No one, it seems, has reported improvement in germination by using complex nutrient solutions, however. In conclusion, he stated (11), "Teliospores of certain rusts having a more or less definite rest period may germinate to some extent in December or earlier. As the season advances there is a marked increase in the percentage of spores that will germinate and a decrease in the time necessary for germination to begin."

Inoculations. Germinating spore material in the laboratory suggested a means of inoculation, other than by the methods used with potted plants in the greenhouse. After some experimentation it was found that excised alfalfa leaves would live from three to four weeks in moist Petri dishes, especially if the petiole and a small portion of the stem were left attached. This discovery led the writer to believe that leaves could be inoculated in Petri dishes and resistance studies made just the same as with potted plants in the greenhouse.

The first experiment was performed with only three dishes and leaves of a variety known to be very susceptible from field observations and greenhouse tests. Filter papers were placed in sterile Petri plates and moistened well with distilled water. A clean, well-formed, nondiseased leaf was placed in the dish and each leaflet pressed down in order that it was in contact with the paper. Spores then were scraped from rusted leaves with a scalpel and placed on the leaves in the Petri dishes. A droplet of water was left on a few of the leaflets and more distilled water was added to the filter paper with a dropper as it was needed. Thus it was easy to keep a very high humidity in the dishes at all times.

After nine days the leaflets were noticed to be turning a yellowish-green color around the inoculated spots and small protuberances, looking very much like small pustules seemed to be appearing. Two days later the leaves were examined with the binocular microscope and small but definite pustules could be seen, especially on the dorsal surfaces. This was definite proof that rust could be cultured on semi-excised leaves in the laboratory.

At the end of 25 days the last notes were taken on these leaves and they were then discarded. After three weeks the leaflets were turning yellow and some were becoming detached from the petiole. New leaflets were sent out from the node, and small, white, hairy roots were growing from the end of the cut petioles and stems. A few of the leaflets were being attacked by molds and other fungi by this time, especially if they had been injured badly or had been broken from the petiole. Others were still green except for the spot where the rust organism had made its attack. Here, the leaflet first turned a yellowish color, later changing to a dark brown to black.

Before discarding this experiment, more extensive and thorough tests were started. Eighteen dishes were lined with moistened filter paper as before, and cuttings of all six of the common varieties used in the field test were prepared. Three to nine leaflets could be placed in each dish, depending on the size of the leaf.

Fresh spores from infected plants in the greenhouse were applied to the leaflets by means of a small camel's hair brush after they were pressed well against the moist filter paper. Both dorsal and ventral sides of different leaflets were exposed in order to test the differences, if any, in the power the organism had to infect the different surfaces. Often, when the brush was wet, it left a film of water over the leaf surface. This, according to all findings, should have been ideal conditions for rust infection if the oxygen supply was not too limited.

By the end of the fifth day the leaflets were showing a definite mottling and yellowing in the areas where spores were inoculated and on the seventh day the first pustules were bursting from the surface. This incubation period was at least two days shorter than it was found to be in the greenhouse where it was from eight to nine days before any signs of the organism appeared.

The pustules were just as numerous on the upper surface as the lower or it at least seemed so from observations with the naked eye and binocular lens. This should be true if alfalfa rust infection occurs through stomata as do other members of the Uredinales. Miller (15) reported that the average number of stomata in thousands per square inch of leaf surface is 109 for the upper surface and 89 for the lower, or a ratio L/U equal to 0.816. This difference, however, would not be easily discernible by a general observation of the total number of pustules appearing on the respective surfaces.

Upon examination under the binocular the pustules were seen to be very numerous, especially along the midrib where small droplets of water seemed to collect due to surface tension. They were, however, scattered over the whole leaflet. The pustules appeared very erumpent and the brownish spores piled up into small pillar-shaped structures. Nothing like this had ever been observed on leaves collected in the field or greenhouse. Undoubtedly, the difference in the appearance of the pustules was due mainly to the environment. The leaves in the Petri dishes were undisturbed, not moved about by air currents, man, insects, etc.; hence, instead of the spores being spread, they were piled up into small columns after the rupturing of the epidermis. The heavy infection, as stated before, was often largely confined to the area along the midribs of the leaflets where droplets of water containing spores seemed to converge. Figure 11 shows the small rust pustules as they appeared on a leaflet taken from a Petri dish. Figure 7 showed the pustules as they appeared on leaves in the greenhouse.

Gradations from large erumpent pustules to those just beginning to burst through the epidermis could be found on the same leaflet. The immature pustules appeared as light-colored, boil-shaped lesions, and spores could be seen just beginning to form.

Fig. 11.— Enlarged view of under surface of a leaflet of Turkestan alfalfa showing uredia of rust resulting from inoculation of an excised leaf in a Petri dish in the laboratory. (Approx. 20 X)

After 23 days rust readings were taken on six varieties. In the laboratory, as in the greenhouse, these readings were based on their relative susceptibility to rust, and given a numerical value of 0 to 5, the low number always representing the most resistant types. The following are the readings taken on this test:

1. Ladak, 2, leaves did not live as long as other varieties; some rust, but not to the extent of the Turkestans. Leaves turned yellow.
2. Kansas 308, 2.5, pustules much fewer in number, but, in general, larger than other Kansas Common selection. One specimen, especially, was not rusted to any great extent. Leaves not infected were still as green and vigorous looking as they were when placed in the dish 23 days earlier.
3. Kansas Common 205, 3, moderately rusted, pustules numerous but rather small.
4. Grimm, 3, plants definitely rusted, just as badly on one surface as the other.
5. Turksetan 19304, 5, very badly rusted on both surfaces.
6. Turkestan 86696, 5, rusted seriously on both surfaces, pustules very numerous and larger than usual when in such close proximity to each other.

A still more inclusive test was made at a later date in which ten different varieties were used. The same camel's hair brush method of inoculation was found to be the most effective in getting good infection. Drops of water were added to the Petri dishes every day. It was learned that if the dishes were placed in the light near a north window and covered with an inverted glass jar they would not only retain their moisture longer but would also remain green for a greater length of time.

The leaf cuttings were not kept so long this time as in the previous case. Small pustules again began appearing on the seventh day, always two to three days ahead of the greenhouse potted plant inoculations.

Following are the summarized notes taken on this group:

1. Medicago ruthenica, .5, very slight infection on two leaflets, molds and other fungous rotting organisms quickly killed the cuttings.
2. Medicago falcata, .5, very little infection.
3. Semipalatinsk, 1, slight infection.
4. Kansas Common, 1.5, infected, but not heavily.
5. Ladak, 2.5, good infection on one leaf, others relatively free.
6. Hairy Peruvian, 3, moderately rusted.
7. Kansas 308, 3.5, infection good, definite chlorotic yellowing of leaves, especially around pustules.
8. Grimm, 4, infection very good, cuttings remained alive well.
9. Turkestan 86696, 4.5, larger pustules than all previous.
10. Turkestan 19304, 5, very good infection but saprophytic fungi destroyed all evidence after an early reading.

Another interesting fact was observed with this last experiment. Rust pustules definitely were found to occur on both surfaces of a leaf, even at the same time. This was observed in the three varieties, Kansas 308, Ladak and Turkestan 86696. Leaflets that happened to stand on edge in the dish due to a bend in the petiole were found to have rust pustules on both surfaces, but they were always larger and more prominent on the upper side.

In one case the leaflet was standing in an almost vertical position and the rust pustules on the two surfaces were found to be almost identical in size and shape. The mycelium, therefore, seems to fruit freely on either leaf surface, depending either upon the intensity of light or the oxygen supply, or perhaps a combination of these two factors. More work should be done on this phase to determine positively to what it is due.

In connection with all these leaf experiments extra check dishes of susceptible Turkostans were inoculated. These were examined at twelve, twenty-four and forty-eight hour intervals in an effort to find germ tubes entering tho stomates. It was found to be practically impossible to strip off pieces of the epidermis of alfalfa leaves, except possibly for thin strips of tissue along the veins. Hence, only very small strips of macerated leaf tissue could be mounted for examinations under the microscope. Many spores could be found scattered over the field, some on the tissue and others floating in the medium. The urediospores, when viable and under ideal conditions, began germinating almost at once. Several spores were found to be germinating but no germ tubes were found to be actually entering the stomata.

One exceptionally good mount showed a cross section of a small sorus. Definite uredinial stalks could be seen. The urediospores still were attached to two or three of them. These stalks appeared to be two-celled and hyaline in nature. No mention ever has been made of this fact in the literature, or in the descriptions of the sorus and spores. The spores undoubtedly break off very early, however, and the stalks disintegrate. In the case of the teliospores, the stalks are hyaline but more resistant, and they often were found connected to teliospores floating in water.

Greenhouse Studies

Methods and Results of Inoculation. The work in the greenhouse consisted of searches for a procedure or method of inoculation by which alfalfa rust infection comparable to that in the field could be obtained. After a satisfactory method was finally worked out a varietal testing program and a search for rust-resistant types was begun.

It soon was evident that some method of obtaining rust infection with potted alfalfa in the greenhouse was necessary to supplement readings made in the field. Many problems hampered the progress of the work after the plants were potted and growing in the greenhouse.

Facilities for experimental rust work were very adequate due to intensive wheat rust investigations that are under way at the Kansas Agricultural Experiment Station. The large metal moist chamber and small cylinder-type moist chambers used in wheat rust work were used extensively in the alfalfa rust experiments. In addition a detachable frame box-like moist chamber was erected for inoculation work. A type of closely-woven cloth called Solar-Weave was stretched and tacked over wooden frames. These were set up over a well-watered sand bed in the greenhouse and served excellently as a moist chamber since they would hold as many as thirty-six five-inch pots of plants at one inoculation.

The first experiments were very simple, involving only the use of a scalepl, a large bell jar for a moist chamber, and viable urediospore material collected from plants in the field. Potted plants of the right size were selected in order that they would fit under the large bell jars that were placed over water-soaked sand. These plants, two selections of Turkestan and one of Kansas Common, were sprayed with an atomizer. The leaves to be inoculated were then rubbed lightly between the thumb and fingers to distribute a surface film of moisture, and urediospores scraped from infected leaves gathered in the field were placed on the under surface of some of the leaflets with a scalspl. The bell jars were placed over the plants and left for a little more than two days. The plants then were moved to another, more humid greenhouse where the conditions seemed to be more favorable for rust development.

These plants were not examined until 15 days after inoculation when small rust pustules were noted to be appearing on the inoculated leaves. They were left in the greenhouse for some time thereafter and the infected leaves began turning brown, parts of them dying. After 35 days several of the infected leaves had fallen. Many secondary infections around the primary pustules were noted before they fell, however.

This hand method of inoculation worked very well for a small number of pots but it was a very slow and tedious process. Spores were scraped from infected leaves and placed in a drop of water on a glass slide. Then by using a needle flattened for the purpose the spores could be applied to both sides of the alfalfa leaflets. Some method was needed, however, whereby a uniform infection could be obtained with a large number of plants in order that a comparative study of the rust on different varieties could be made.

A so-called dusting method of inoculation was tried. It consisted mainly in running dry rust-infected leaves through a very fine meat grinder. This action made a dust in which urediospores were present in considerable quantity, as was evidenced by a microscopical examination of the particles. The potted plants were placed in either the frame or metal moist chamber and atomized with a fine spray of water by means of a specially constructed spray nozzle attached to a garden hose. After the mist had settled upon the plants, the dust inoculum was sifted gently through a cheesecloth over the leaves. This method seemed to work nicely, the dust settling in a very even pattern over the leaves.

After the plants were left in the moist chamber for 48 hours they were observed to be very damp and a mold-like growth was forming on many of the old stems. The plant material that was dusted on the plants with the spores had soured and gave off an odor characteristic of moldy hay. Infection with the first set of plants seemed very slow, rust not showing up until the thirteenth day. Possibly the coating of moldy plant material had something to do with the long incubation period since with some of the later experiments no infection at all was obtained because of an excessive amount of inoculum being sifted on the leaves. The shorter days of mid-winter marked by shorter periods of light also could have been acting as a retarding agent. This method was not consistently effective, sometimes no infection was obtained after inoculation, while on other occasions heavily infected leaves resulted.

A semi-spore-suspension method of inoculation was worked out a little later. Dry, rust-infected leaves were placed in a small jar or bottle, covered with distilled water, and allowed to soak for about two hours. Small glass beads then were added and the bottle shaken vigorously for several minutes. This loosened the spores from the leaves and resulted in a heavy spore suspension. The mixture was strained through a cheesecloth, with the idea that many of the spores would be loosened and pass through the cheesecloth with the water leaving the plant material and beads behind. This inoculum then was placed in an atomizer and sprayed on the leaves and stems of plants. This method worked, not to perfection, but could be classed as one step better than the dust method. However, large pieces of the leaves managed to get through the cheesecloth and tended to clog the atomizer. Infection never was uniform, and if the plants were left in the moist chamber very long other organisms caused trouble, especially those causing blackstem of alfalfa (Ascochyta imperfecta Peck) and bacterial spot (Pseudomonas alfalfae Jones). It was found advisable not to leave the plants in the moist chamber more than 48 hours. This length of time proved to be well adapted for rust infection.

Modifications of this method generally ended with somewhat the same results. Rust-infected leaves were ground in a mortar and shaken vigorously in water before being strained through the cheesecloth into the atomizer.

In a similar method trimmed portions of leaflets containing only the pustules were tried. These pieces were placed in a small bottle, a little distilled water was added and well shaken. The water, containing the spores in suspension, then was decanted into a small atomizer bottle and the mixture sprayed on the potted plants.

Another method of inoculation consisted of brushing potted seedling plants that had been atomized with water over infected alfalfa plants. Good infection was obtained but the method was found to be impractical when large plants and increased numbers had to be used.

Another and somewhat doubtful method of inoculation was attempted. Four pots of susceptible plants of different ages were used in testing the usefulness of a hypodermic needle in inoculating alfalfa plants with rust spores. A spore suspension was made by scraping spores from infected leaves and placing them in distilled water. Alfalfa stems were found to be hollow (to a certain extent) but only a small amount of a solution could be forced into the stem even though extra punctures were made between the nodes to allow the escape of air as inoculum was injected. The plants were very carefully inoculated in this manner and placed in a moist chamber for about 48 hours.

Final notes were taken one month later. Some stem infection was evident, but very little, if any, was due to the injections of spores into the stem. The large pustules on the stems were found mainly around the nodes where the water seemed to collect in droplets; infection probably taking place through stomata on the stems. The stipules and small leaves at the nodes also were heavily infected with pustules, presumably arising from spores in inoculum running down the stem. All infections on one entire plant appeared to be due to accidental distribution of water over the plant when the hypodermic bulb and needle were rinsed.

The hypodermic injection method of inoculation apparently is impractical when working with alfalfa. It certainly is not so useful as it is with wheat rust inoculations. The alfalfa stems are too woody and the pith (hollow area) is too small.

The conclusion was finally reached that the easiest and quickest way to collect the urediospores was to scrape them from the fresh leaves with a flattened dissecting needle that had been bent at the point and place them in suspension in distilled water. The tops of rusted plants were cut off and wrapped in oiled paper or the infected leaves were clipped and placed in covered dishes. In this way, they remained fresh and usable for a month or more after being stored in a refrigerator.

A highly successful and most used method of inoculation proved finally to be a very simple process. The urediospores were scraped from the leaves in the manner previously described and placed in suspension in distilled water in the glass container of a small fly sprayer. When enough rust spores were present to give the mixture a rusty, clouded appearance, it was shaken well and sprayed onto potted plants that had been placed in a moist chamber. Then a very fine spray of water was turned on the plants, but caution had to be taken to remove it at the right point just before droplets began to form on the surface of the leaves. If the spray was not removed the droplets were observed to run off the edges of the leaflets carrying the rust spores with them or to result in infection only around the edges or along the midrib where the spores seemed to concentrate. A very even and uniform spore covering could be obtained under the right conditions. Proof of this fact was established by spraying a group of slides, then examining them under the microscope for the presence of urediospores. It was noted that the spores were very evenly distributed upon the slides. Therefore, a comparable or somewhat similar condition should result from spraying the plants. After inoculating the plants, the insides and the top of the moist chamber were sprayed, and, in the case of the metal chamber, water was allowed to run slowly over the outer surface for 48 hours, thus keeping the relative humidity around 100 for the entire period that the plants were in the inoculation chamber.

After the pots were removed they were placed on sand beds on the floor of the greenhouse. The sand was soaked daily and the plants were kept well watered. Generally after eight to ten days flecks and small rust pustules began to show on the leaves. The very small immature rust spots, in most cases, could be identified by a chlorotic ring showing up first, with a minute brownish fleck in the center. Soon the small pustule would bulge through the epidermis, grow quickly, and when nearly mature would break the cuticle and expose the mass of reddish-brown spores. Often two or three rings of secondary pustules could be seen surrounding the original pustule before final notes were taken.

Inoculation work in the greenhouse was carried out under varying conditions, depending on the type of moist chamber used in the experiment. After removing the plants from the moist chamber they were placed in sand beds on the floor of the greenhouse until final rust notes were taken. Table 3 shows the average temperature and relative humidity to which the plants were exposed during their 48 hours in the inoculation chamber and the period afterwards in the greenhouse. All temperature readings and relative humidity determinations were made through the use of a Lloyd's hygrodeik during the months of February and March, 1940. Good results were obtained when either moist chamber was used.

There seemed to be no definite nor consistent correlation between the length of the infection period and the type or severity of infection. Table 4 shows three representative varieties and the relative coefficient of infection given each for different infection periods. Some readings were taken at the end of 17 days while with other groups of pots 44 days elapsed before readings were taken. A high reading, in nearly all cases, was given on June 11 after the group of plants had been left for 44 days before notes were taken. The organism, by that time, had begun ti injure the plants considerably and it was natural to give them higher readings. Just as accurate notes on the severity of the attack could be taken much sooner, however. The best length of time seemed to be around four weeks.

Table 3. Temperature and relative humidity records obtained in moist chambers and over greenhouse beds in connection with inoculation studies of alfalfa rust. Manhattan, Kans., 1940.

Place and time	Conditions	Average dry bulb temperature	Average wet bulb temperature	Average relative humidity
Frame moist chamber, at time of inoculation.	Sand well soaked with water, spores atomized on plants, then atomized with fine spray of water.	75	74+	97
Frame moist chamber, 24 hours after inoculation.	Covering had been placed over frame at time of inoculation.	80+	76+	83
48 hours after inoculation.	No water added to plants or sand, cover remaining.	81	74	72
48 hours after inoculation.	Spray added to plants at end of 24 hours and sand soaked. Cover remaining.	77	75+	92
Metal moist chamber, 1 hour after inoculation.	Inside of chamber sprayed, plants atomized, then spores either dusted on or atomized. Constant stream of water flowing over outside of chamber.	69+	69+	100
Metal moist chamber, 24 hours after inoculation.	Stream of water over outside of chamber, none added on inside.	68	67+	98
In metal can with glass plate cover.	Instrument placed on pots in chamber, sand well soaked with water.	69+	68+	94
Over sand in beds, 1 hour after watering.	Instrument placed about 6 inches above sand.	69	64+	76
Over relatively dry sand 12-24 hours after watering.	Instrument placed as above.	71+	64-	67

Table 4. Data on the relation of length of infection period to the severity of rust infection on three strains of alfalfa grown in the greenhouse.

Date of readings		Number of readings	Length of infection period Days	Coefficient of infection
			Turkestan 19304	
March	3	12	27	2.50
	5	18	26	2.83
	12	12	33	4.71
	26	12	27	3.71
April	2	39	31	3.04
	11	64	17	3.60
	25	27	33	4.02
May	4	60	26	1.72
	16	24	28	3.50
June	1	66	35	3.20
	11	15	44	4.33
	19	69	36	4.05
July	17	66	36	3.44
			Kansas Common 1-205	
March	3	12	27	1.38
	5	18	26	2.31
	12	12	33	1.42
	26	27	27	2.54
April	2	12	31	2.04
	25	12	33	3.46
May	4	21	23	1.24
	16	24	28	2.85
June	1	27	35	3.00
	11	15	44	2.40
	19	12	36	3.67
July	17	30	38	2.75

Table 4. (Continued)

Date of readings	Number of readings	Length of infection period	Coefficient of infection
		Days	
		Medicago falcata	
March 3	12	27	1.17
12	12	33	0.79
April 2	6	31	1.92
May 16	24	28	1.23
June 11	15	44	3.13
19	6	36	2.83

Miscellaneous Greenhouse Experiments. During the fall of 1939 the most susceptible plants in the field were marked and staked. After all of the field notes had been taken, these rusted plants were dug, transplanted into pots and brought to the greenhouse. They consisted mostly of Turkestans but a few plants of the Kansas Common and Grimm varieties were chosen. These plants served mainly as the source from which the first rust inoculum supply was obtained. Later, however, they were included in the varietal tests.

Tests in the greenhouse were run to note any differences between the susceptibility of seedling plants and that of older, more mature plants. Small seedlings about three inches tall were inoculated with a spore suspension and a very good infection was obtained. The infected leaves of seedlings seem to die and disappear much more rapidly than those of older plants. Even though they wilt sooner, the seedlings continue to live. New leaves are produced after the old ones die and fall, thus it seemed to do them very little harm, unless it would be a premature defoliation causing stunting. However, it was found impossible to build up an inoculum supply on seedlings, since the pustules werealways small and not very erumpent. Infection on susceptible seedling plants seemed to be obtained with the same ease as that on mature plants. Seedlings apparently carry no resistance that is not carried by mature plants.

Susceptible plants in the greenhouse served as a means of testing the viability of urediospores in various collections. For example, four potted plants of Turkestan 86696 were used in a test on May 14 to ascertain whether urediospores collected in September of the previous year still were viable. These spores had been collected in the field and stored in the

Horticultural cold storage room during the winter and spring. On May 14, the use of this room was discontinued so the spores had to be removed. At this time, 244 days after collecting, a germination test in the laboratory showed about 10 percent of the spores to be germinating.

It was questionable whether spores of this age would result in infection on the host. Susceptible host plants therefore were inoculated with spores from the stored material. Two methods of inoculation were employed, the hand method and the spray method. In the first named, spores were scraped from leaves and put in suspension in a droplet of water on a slide. They were then applied to leaves with a flattened inoculating needle. One pot was atomized with distilled water and placed under a bell jar for about two days; the other was treated in a similar manner except that it was placed in a metal cylinder moist chamber covered with a glass plate for the same length of time. Two other pots were inoculated by the spray method and placed in like containers for the same length of time.

Some infection was obtained on all plants, possibly better on the hand inoculated plants, but not as well distributed. The results of this test show that urediospores will remain viable for a period of eight months or more and still cause infection, but only if they are stored under the right conditions.

Another and somewhat similar test was conducted with susceptible plants in the greenhouse. In this case, spores were used that had been collected on October 6 and over-wintered out of doors. The spores were sprayed on several alfalfa plants and three pots of Euphorbia cyparissias L. Some of the plants were removed from the moist chamber after 48 hours, others were left 72 hours and still another group was allowed to remain about 96 hours before they were removed.

Final notes were taken 27 days after inoculation but there was no sign of infection on either the alfalfa or the Euphorbia plants. The results here lead the writer to believe that the urediospores do not overwinter in Kansas and cause infection the following year. Results of germination tests in the laboratory also help to substantiate this belief.

It was noted in all experiments with potted alfalfa plants in the greenhouse that there was a mottling of the leaves. This often appeared as a yellowing, reddening or browning of the tissues due to some unknown factor. Rusted plants tended to show a greater degree of this coloring, yet after comparing checks and inoculated plants no positive conclusion could be reached as to whether the rust in an indirect way brought about the discolorations. It would not be beyond reason, however, to assume that the rust might cause a concentration of carbohydrates in portions of the leaf and thereby bring about this coloring effect.

Blackstem, caused by Ascochyta imperfecta Peck, on a few occasions threatened to be a serious hindrance to the proper progress of the greenhouse experiments. The time that the plants were allowed to remain in the moist chamber and the method of inoculation proved to be the governing factors. Any period over 48 hours in the moist chamber was found to be favorable for the development of blackstem, especially when the modified spore suspension methods were used. In some cases, this disease nearly destroyed the plants before rust had time to make its appearance.

This was well illustrated by a series of experiments in which the plants were inoculated by the following method. Infected leaves were taken to the laboratory, rubbed and ground in a mortar until the spores were loosened or rubbed off. The leaf particles then were shaken in a bottle of distilled water for several minutes. The mixture was strained through a cheesecloth into the bulb of a sprayer. This light brownish mixture resembled a very heavy spore suspension. Soil particles, plant refuse, and other organic matter made up some of the material in suspension, but upon examination many rust spores were also found. This material was atomized on plants in a moist chamber after which a fine mist of water was added. The plants were sprayed again with water the second day to retain the relative humidity and then left in the moist chamber for approximately 90 hours.

Upon examination at the end of this period both seedling and grown plants were found to be heavily infected with blackstem in a very even and uniform pattern, just as it would look when sprayed with the conidia or infecting spores of Ascochyta imperfecta. Just why and how this infection should occur in such great quantities was indeed a question. The leaves from which the rust inoculum had been obtained did not show any signs of blackstem but undoubtedly the conidia or mycelial strands had been applied to the plants in the inoculum distributed by the sprayer.

Thus, blackstem loomed as a threat to the work in the greenhouse unless care was taken with the methods of inoculation and incubation to prevent it. Two solutions to the problem were found; one, a different, much better, and more sterile method of inoculation and secondly, a shorter period in the moist chamber. With these conditions, very little harm was done by the blackstem organism.

Some mistaken identity of this rust in the literature led to a further study of the problem. Schroeter, in 1884, stated that aecidiosori on Euphorbia cyparissias occurred in the cycle of an Uromyces morphologically identical with Uromyces striatus on a species of Trifolium (2). Pammel and King (19) in 1912 also related that Schroeter took teliospores from red clover to infect the Euphorbia. Treboux, according to Arthur (2), infected Trifolium arvense with aeciospores from a species of Euphorbia, and added that this proved that the assignment of Uromyces striatus to both Medicago and Trifolium as hosts was correct. This species has not yet been reported in the United States on Trifolium, however.

As noted heretofore, Euphorbia cyparissias has been reported as the alternate host of the alfalfa rust organism in Europe, but, according to Arthur (2), such an occurrence has never been found in this country. After checking the distribution of the aecial host plant, it was found to be reported in Kansas but not in abundance. The writer had plants sent from northern Michigan during the summer of 1939 and planted them in pots in order that they might be used in future study.

Potted Euphorbia plants often had been placed in the moist chambers with the alfalfa plants when they were being inoculated. Nothing had ever been expected from this, since it was found that teliospores failed to germinate, but there was always a chance of one of the teliospores germinating under the proper environmental conditions to produce infection.

After failing to obtain teliospore germination during the spring, (From February 15, 1940, when the first test was made), hopes of proving that Euphorbia cyparissias was the alternate host of alfalfa rust, Uromyces striatus, were about gone. But on May 9 some of the teliospore material that had been lying on the ground all winter in a screen wire container was brought into the greenhouse. Even though much saprophytic growth had begun to destroy the stems a few of the pustules were scraped off and the spores put in a distilled water suspension in a small atomizer. This suspension was sprayed on a potted Euphorbia plant and some detached leaves that were placed on moistened filter paper in a petri dish. The pot and dish were then placed on one of the moistened sand beds and covered with a large bell jar.

The sand was watered daily, thus maintaining a high humidity underneath the jar. Temperatures were moderate in the greenhouse, ranging from 50 to 70 degrees. These environmental conditions proved to be near ideal for the plant for rapid advance of the new growth of the plant soon was noted.

On May 25, a few minute, light-colored flecks were noticed on the detached leaves in the Petri dish. No close examination was made since they could easily have been mistaken for the mottlings due to thrip and red spider injury. Two days later, however, the spots seemed to be somewhat larger and more distinct, so they were examined in the laboratory under binoculars. Definite, raised, yellowish areas were found which greatly resembled rust pustules before they had burst through the epidermis. The potted plant under the bell jar then was immediately examined and also found to be infected. The spots here were even larger and more numerous than on the leaves in Petri dishes. Upon examination under the binocular they appeared to be rust pustules, greatly resembling aecia found on other plants. However, they were as yet very immature, some just broken through the epidermis and all were very small. All appeared on the upper surface of the leaves, were light yellow to orange in color and mostly oval to elliptical in shape.

On June 3, seven days later, the leaves were examined again. The raised, pustule-like areas were not much further advanced. The under surfaces were examined in the search for pycnia but none were found. They should, according to previous findings, be found on the opposite side of the leaf from the aecia and should appear before them. Could it be that these structures are the pycnia? Perhaps this was so - and the aecia would no doubt appear on the under surface in the future. With this in mind, a small camel's hair brush was moistened and the questionable pycnia were brushed to mix the plus and minus spermatia, which, in the case of wheat rust, seems to be necessary for the formation of aecia. The potted plant was then watered well and placed under the bell jar.

Further brushings proved futile and all efforts to produce aecia were in vain. Examinations of the leaves led to no further information concerning the exact identity of the lesions. Other inoculations were made with spores collected at different dates and overwintered out-of-doors but no other infection was obtained. It will be necessary, therefore, to do more research on the problem before the life history of the alfalfa rust organism can be completed in the United States, that is, if Euphorbia cyparissias is the alternate host. It is the opinion of the writer, however, that this could be done by subjecting the spores and host to more varied environmental conditions.

After experimenting with many plants during the inoculation work in the greenhouse, a very definite conclusion was reached. For success, one must use strong, rapidly growing plants. Even if weak plants become infected, the fungus rarely will come to a satisfactory fruition. All growth of the rust, stopped with the death of the host and thus it was found that alfalfa rust, like all the other known rusts, cannot be considered apart from its host. The vigor of the parasite seemed to be proportional to the vigor of the host and observations showed that a starved or dried-up host plant meant necessarily a starved or dying rust.

Clinton (5) stated in 1934 that alfalfa rust appeared to be closely related to the red clover rust with which it was closely associated in one of the fields observed. Arthur (1) in 1910 and again in 1911 made cultures with urediospores, using spores from _Medicago sativa_, sown on _M. sativa_, _Trifolium praterse_, _T. medium_, and _T. repens_, and produced infection only on _Medicago_. A somewhat similar test was made in this study in the greenhouse during the spring of 1940. Two varieties of Medicago, _M. sativa_ and _M. falcata_ were used. With these in the test were two species of sweet clover, _Melilotus officinalis_ and _M. alba_, and two species of clover; namely red clover, _Trifolium pratense_, and alsike clover, _T. hybridum_. Ten small potted plants and two pots of larger and more mature plants of each variety were inoculated with a spore suspension and allowed to remain in the moist chamber for 48 hours. The results were very striking and very much in agreement with those obtained by Arthur. On the tenth day, flecks began to show on the two species of alfalfa but no trace of rust was found on the other plants. Final notes taken on the thirty-sixth day found the alfalfa plants covered with rust pustules but there was no evidence of infection on any of the sweet clovers, red, or alsike clovers. Not even small flecks appeared so it seems evident that infection did not even occur, or, if it did, the fungus was unable to make any growth within the tissues of these plants. Therefore, it is the opinion of the writer that

Uromyces striatus will not attack these other legumes, and undoubtedly clover rust is completely different from alfalfa rust, with which it has been confused.

Relative Susceptibility of Varieties and Species of Alfalfa in the Greenhouse. The greatest problem confronting the writer after a satisfactory method of inoculation had been worked out concerned a means of evaluating the severity of rust infection on alfalfa plants in the greenhouse. In other words, some method of taking accurate notes on infected plants was necessary before a comparison of the susceptibility of alfalfa varieties and species could be made.

Chlorosis, in a way, showed resistance in alfalfa, necrosis being the resulting effect as evidenced by both field and greenhouse observations. In general, with an increase in resistance, there was a decrease in the number and size of the uredia and an increase in the proportion of necrotic areas without uredia. In the most highly resistant variety only faint flecks occurred.

A successful method of evaluation was finally derived after much study and deliberation over the problem. Notes were taken, on an average, about two weeks after the plants were inoculated. Following each inoculation the set of potted plants was placed in moist sand beds on the floor of the greenhouse. Rust pustules generally began to appear after nine to twelve days. Three weeks later the final rust notes were taken.

In taking notes, a scale, 0-5, was adopted in order to place the rust readings on a numerical basis, rather than noting resistance and susceptibility as such. Some such system of taking notes was necessary before susceptible and resistant varieties could be compared. The rust readings, then were classed under three general criteria; namely, coefficient of severity, type of infection and length of infection period. Each plant examined was given a reading under all three criteria, each being based upon the same 0-5 scale. By this system, a very susceptible plant might have a total coefficient of infection of 15, while a resistant plant could easily have a total reading of less than 5.

The term coefficient of severity is nearly self-explanatory, meaning the severity of the rust attack as determined by the size and number of the lesions on the infected leaves as well as the number of leaves infected. The type of infection varied with different plants as well as with different species and varieties. This reading was based upon the size of the pustules, how erumpent they were, and whether any resistance was shown by chlorotic ringing; in short, it denoted the response of the plant. The third criterion, length of infection, concerned the apparent stage of development of the rust at the time the readings were made. The higher readings, in a true sense, mean that the rust had a shorter incubation period. It often appeared one to three days sooner after inoculation, and therefore caused more damage to the plant. Such was the case with Turkestan and Hairy Peruvian.

To summarize, then, the coefficient of severity is practically synonymous with degree of infection. Type of infection corresponds to the response of the plant to rust infection, i.e., the general effect on plants as manifested by chlorosis, erumpence, etc. The length of infection concerns mainly the time element in relation to infection.

Generally, a high reading in the severity column was accompanied by correspondingly high readings in the other two columns, but this need not necessarily be the case. Often, even though the severity of infection ranked highly, the type of response of the plant was such that a low reading was recorded. Likewise, the rust on all varieties did not develop at the same rate after infection had taken place, hence, varied readings were recorded in the column considering the length of the infection period.

The total coefficient of infection was derived by finding the sum of these three criteria of rust infection. By dividing the total coefficient by the total number of readings a figure was obtained that denoted the average coefficient of infection or the so-called rust reading of the plant. This method is considered by the writer to give as accurate data on the plant and varietal reaction as any method known. The average of all the readings taken for the plants of any given species or variety gave the rust reading for that particular species or variety.

These data were greatly condensed from the original plant notes and put in table form in order to compare the reactions of one variety or species with another. Table 5 shows the results of the tests run with a diploid species of alfalfa, Medicago ruthenica Trautv., and a strain of Medicago falcata L. called Semipalatinsk, F.C.22613. Both proved to be very resistant to rust. On only one occasion did any of the plants of M. ruthenica show any symptoms of infection and at that time it was questionable, since no pustules appeared. Only slight flecks were evident, a symptom commonly observed in highly resistant forms. It is recognized that the number of plants used in the greenhouse was small, but observations in the laboratory and field justify the opinion that this species is practically immune. In fact, this species was found to be the most highly resistant form in all greenhouse tests.

Table 5. Response of *Medicago ruthenica* and Semipalatinsk to inoculations with *Uromyces striatus* in the greenhouse. Manhattan, Kans., 1940.

Date of readings	Number of plants	Number of readings	Coefficient of severity	Type of infection	Length of infection	Coefficient of infection	
						Total	Average
			Medicago ruthenica				
June 11	5	15	0.0	0.0	0.0	0.0	0.0
July 17	5	15	0.0[2/]	0.0	0.0	0.0	0.0
Total	10	30	0.0	0.0	0.0	0.0	0.0
Average			0.0	0.0	0.0		0.0
			Semipalatinsk				
March 3	4	12	1.0	2.5	3.0	6.5	0.54
12	4	12	0.0	1.5	1.5	3.0	0.25
April 2	1	3	0.5	0.5	1.0	2.0	0.67
May 16	8	24	9.5	10.0	8.0	27.5	1.15
30	2	6	4.5	3.5	5.0	13.0	2.17
June 11	5	15	1.5	2.0	3.0	6.5	0.43
Total	24	72	17.0	20.0	21.5	58.5	5.21
Average			0.71	0.83	0.90		0.81

2/ Reading questionable, slight symptoms of infection but no pustules appeared.

Semipalatinsk had a final rust reading of 0.81 and showed a very high degree of resistance in nearly all plants. Very few pustules appeared, but chlorotic areas and small flecks on the leaves denoted infection by rust. Both this strain and the previous species show considerable promise from the standpoint of rust resistance but neither has been used extensively in breeding work because of their undesirable agronomic characters. In the greenhouse tests, Semipalatinsk proved to be highly resistant, but slightly less so than M. ruthenica as shown in Table 5.

Ladak showed the most resistance of any of the agronomically important varieties. Pustules on this variety were generally very small and non-erumpent. Often no more than a minute, necrotic flecking could be seen as evidence of infection. Ladak plants varied in their reaction to rust as well as in agronomic characters, as shown by tests of selections that will be discussed later. In general, however, they all showed considerable resistance. Results of rust tests with Ladak are given in Table 6 and show the average coefficient of infection to be 1.63. In comparison with susceptible Turkestan it was found that Ladak carried about twice as much resistance as this commonly grown variety. On the basis of all the rust tests discussed herein, Ladak must be considered to possess the highest degree of resistance of any of the commercial varieties.

Another species, Medicago falcata, also was tested with rust. The results of tests with 25 potted plants are shown in Table 7. Plants with different degrees of resistance were observed in this species, the total coefficient of infection varying from 1 to 13.5 for different individual potted plants. Some plants proved to be somewhat resistant and probably would show promise if carried into further studies.

Table 6. Results of greenhouse inoculations of Ladak alfalfa with *Uromyces striatus*. Manhattan, Kans., 1940.

Date of readings	Number of plants	Number of readings	Coefficient of severity	Type of infection	Length of infection	Coefficient of infection Total	Coefficient of infection Average
March 3	4	12	2.5	6.5	7.5	16.5	1.38
5	6	18	10.0	10.5	11.5	32.0	1.78
12	4	12	6.0	7.5	7.0	20.5	1.71
26	1	3	2.0	0.5	1.0	3.5	1.17
April 2	6	18	4.5	5.0	7.5	17.0	0.94
11	8	24	7.0	6.5	7.5	21.0	0.88
May 9	6	18	12.0	12.0	12.5	36.5	2.03
16	8	24	13.5	15.0	14.5	43.0	1.79
June 1	1	3	2.0	1.0	1.5	4.5	1.50
11	5	15	8.0	12.0	12.0	32.0	2.13
19	14	42	28.5	26.5	25.5	80.5	1.92
Total	63	169	96.0	103.0	108.0	307.0	17.23
Average			1.52	1.63	1.71		1.63

Table 7. Results of inoculations of *Medicago falcata* with the rust organism, *Uromyces striatus* in the greenhouse. Manhattan, Kans., 1940.

Date of readings	Number of plants	Number of readings	Coefficient of severity	Type of infection	Length of infection	Coefficient of infection	
						Total	Average
March 3	4	12	4.0	5.0	5.0	14.0	1.17
12	4	12	1.5	4.5	3.5	9.5	0.79
April 2	2	6	4.0	3.5	4.0	11.5	1.92
May 16	8	24	11.0	8.5	10.0	29.5	1.23
June 11	5	15	12.5	18.0	16.5	47.0	3.13
19	2	6	6.0	5.0	6.0	17.0	2.83
Total	25	75	39.0	44.5	45.0	128.5	11.07
Average			1.56	1.78	1.80		1.71

The three Kansas Common selections of alfalfa used in these tests showed about the same rust reaction. Ordinary Kansas Common, or that used by the Kansas farmers, had a reading of 2.42, a wilt-resistant selection 1-2018 was nearly identical with a reading of 2.47, while Kansas Common 1-205 was only slightly higher. Detailed results of these tests are given in Tables 8, 9 and 10, respectively. These results show that the ordinary Kansas Common is just as desirable as the selections, as far as rust is concerned. The results of all rust tests indicate that Kansas Common, as a variety, has only a moderate degree of rust resistance, being less resistant than Ladak and considerably more resistant than Turkestan.

Grimm alfalfa ranked eighth among the species and varieties tested in the greenhouse, rating just above the Kansas Common strains. More uniformity existed among these plants than with any other variety unless it was the Turkestans, which were uniformly susceptible to rust. A total of 231 readings were taken on Grimm plants, with a resulting average coefficient of infection of 2.75. Detailed results are shown in Table 11 showing that Grimm is slightly more susceptible than Kansas Common. The variety should be considered a rust-susceptible one.

Turkestan types, including Hardistan, proved to be very susceptible to rust. Turkestan 86696 ranked next to Hardistan in the greenhouse tests with a rust reading of 3.28, and it was closely followed by another selection, Turkestan 19304, which had an average coefficient of infection of 3.34. Both were very susceptible to rust, and, in general, many large, erumpent pustules were produced on all plants. For this reason this variety was used to increase the supply of rust inoculum. Large stem pustules were often quite numerous. Detailed data of tests with 326

Table 8. Response of unselected Kansas Common alfalfa to rust inoculations in the greenhouse. Manhattan, Kans., 1940.

Date of readings	Number of plants	Number of readings	Coefficient of severity	Type of infection	Length of infection	Coefficient of infection	
						Total	Average
March 5	4	12	9.5	8.5	7.0	25.0	2.08
12	4	12	9.0	7.5	10.0	26.5	2.21
April 2	2	6	7.0	6.0	6.5	19.5	3.25
May 16	8	24	19.5	16.0	17.0	52.5	2.19
June 11	5	15	11.5	14.0	14.5	40.0	2.67
19	2	6	7.0	5.5	5.5	18.0	3.00
Total	25	75	63.5	57.5	60.5	181.5	15.40
Average			2.54	2.30	2.42		2.42

Table 9. Response of Kansas Common Selection 1-2018 to inoculations with *Uromyces striatus* in the greenhouse. Manhattan, Kans., 1940.

Date of readings	Number of plants	Number of readings	Coefficient of severity	Type of infection	Length of infection	Coefficient of infection Total	Coefficient of infection Average
March 3	4	12	4.0	6.5	6.0	16.5	1.38
5	6	18	15.0	13.0	13.5	41.5	2.31
12	4	12	4.5	6.5	6.0	17.0	1.42
26	9	27	23.0	23.5	22.0	68.5	2.54
April 2	4	12	7.5	8.0	9.0	24.5	2.04
25	4	12	13.5	13.5	14.5	41.5	3.46
May 4	7	21	4.5	10.0	11.5	26.0	1.24
16	8	24	23.5	23.5	21.5	68.5	2.85
June 1	9	27	25.0	28.5	27.5	81.0	3.00
11	5	15	10.0	11.5	14.5	36.0	2.40
19	4	12	15.5	14.0	14.5	44.0	3.67
July 17	10	30	27.0	28.0	27.5	82.5	2.75
Total	74	222	173.0	186.5	188.0	547.5	29.06
Average			2.34	2.62	2.54		2.47

Table 10. Response of Kansas Common Selection 1-205 to inoculations with *Uromyces striatus* in the greenhouse. Manhattan, Kans., 1940.

Date of readings	Number of plants	Number of readings	Coefficient of severity	Type of infection	Length of infection	Coefficient of infection Total	Coefficient of infection Average
March 5	6	18	15.0	12.5	11.5	39.0	2.17
26	4	12	10.0	8.0	9.0	27.0	2.25
April 2	2	6	3.0	3.5	4.0	10.5	1.75
11	4	12	11.0	11.0	10.0	32.0	2.67
25	4	12	9.0	7.0	10.0	26.0	2.17
May 4	2	6	5.0	5.5	5.5	16.0	2.67
9	6	18	8.5	14.0	13.0	35.5	1.97
June 1	4	12	11.0	13.5	14.5	39.0	3.25
19	6	18	23.5	23.0	22.5	69.0	3.83
July 17	6	18	17.5	16.5	15.0	49.0	2.72
Total	44	132	113.5	114.5	115.0	343.0	25.45
Average			2.58	2.60	2.61		2.60

Table 11. Results of greenhouse inoculations of Grimm alfalfa with <u>Uromyces</u> <u>striatus</u>. Manhattan, Kans., 1940.

Date of readings	Number of plants	Number of readings	Coefficient of severity	Type of infection	Length of infection	Coefficient of infection: Total	Coefficient of infection: Average
March 3	4	12	4.5	8.0	8.0	20.5	1.71
5	6	18	18.0	15.0	15.0	48.0	2.67
12	4	12	8.5	8.5	8.0	25.0	2.03
28	12	36	32.0	28.0	32.0	92.0	2.56
April 2	3	9	11.0	10.0	9.0	30.0	3.33
11	5	15	16.5	14.0	13.0	43.5	2.90
May 4	2	6	4.5	4.5	5.5	14.5	2.42
9	6	18	14.0	14.0	14.0	42.0	2.33
16	8	24	23.0	19.5	20.0	62.5	2.60
June 1	12	36	34.0	33.5	33.5	101.0	2.81
11	5	15	16.0	14.5	17.0	47.5	3.17
19	8	24	30.0	30.0	28.0	88.0	3.67
July 17	2	6	7.5	6.5	7.0	21.0	3.50
Total	77	231	219.5	206.0	210.0	635.5	35.70
Average			2.85	2.68	2.73		2.75

plants of this variety are given in Tables 12 and 13. Field observations as well as greenhouse tests indicated that Turkestan types were the most susceptible of the common alfalfas, excepting Hairy Peruvian.

Only 10 tests were made with the Hardistan variety, but the average coefficient of infection of 3.23 corresponds closely to the readings made with the Turkestans from which Hardistan supposedly originated. Such a reaction might help prove its origin. Results of the tests run with this variety are included in Table 14 with those of Hairy Peruvian.

The most susceptible of all the plants tested in the greenhouse proved to be the variety Hairy Peruvian. Rust seemed to make its appearance on this variety earlier than on any of the other species or varieties and also caused more damage to the plants. Stem pustules were large and very common. Results of greenhouse studies with this variety are shown in Table 14.

A summary of all greenhouse tests is shown in Table 15. All the varieties used in greenhouse study are listed here in the order of severity as determined by artificial inoculations. Inoculations were made in the greenhouse whenever the plants reached approximately the right size. No actual plant notes were taken until February 5, 1940, after a successful and practical method of inoculation had been devised. Between that date and the first week in June, after which the greenhouse temperature ranged too high for rust work, a total of 2109 rust readings were taken in the greenhouse. Varying numbers of plants of each variety were used in these tests. Only ten plants of <u>Medicago ruthenica</u> were available, whereas a total of 168 plants of Turkestan 19304 were carried in experiments. The table shows that a total of 703 plants were tested in all. The rust readings varied from 0.00 for <u>Medicago ruthenica</u> to 3.56 for Hairy Peruvian.

Table 12. Results of greenhouse inoculations of Turkestan 86696 with *Uromyces striatus*. Manhattan, Kans., 1940.

Date of readings	Number of plants	Number of readings	Coefficient of severity	Type of infection	Length of infection	Coefficient of infection	
						Total	Average
March 3	4	12	5.5	8.5	11.5	25.5	2.13
5	6	18	23.0	18.5	19.5	61.0	3.39
12	3	9	7.0	8.0	7.0	22.0	2.44
28	5	15	17.5	15.0	14.0	46.5	3.10
April 2	12	36	25.5	27.5	27.0	80.0	2.22
11	21	63	79.0	82.0	83.0	244.0	3.87
25	11	33	47.0	49.0	47.0	143.0	4.33
May 4	22	66	32.5	48.0	49.5	130.0	1.97
16	7	21	25.5	26.0	25.5	77.0	3.67
June 1	21	63	62.5	70.5	70.0	203.0	3.22
11	5	15	22.5	22.5	25.0	70.0	4.67
19	17	51	73.0	73.5	71.0	217.5	4.26
July 17	24	72	79.0	76.5	77.5	233.0	3.24
Total	158	474	499.5	525.5	527.5	1552.5	42.51
Average			3.16	3.33	3.34		3.28

Table 13. Results of greenhouse inoculations of Turkestan 19304 with *Uromyces striatus*. Manhattan, Kans., 1940.

Date of readings	Number of plants	Number of readings	Coefficient of severity	Type of infection	Length of infection	Coefficient of infection: Total	Coefficient of infection: Average
March 3	4	12	10.5	10.0	9.5	30.0	2.50
5	6	18	20.5	16.5	14.0	51.0	2.83
12	4	12	18.5	18.0	20.0	56.5	4.71
26	4	12	15.5	15.5	13.5	44.5	3.71
April 2	13	39	40.5	39.0	39.0	118.5	3.04
11	28	84	98.5	101.0	103.0	302.5	3.60
25	9	27	35.0	37.0	36.5	108.5	4.02
May 4	20	60	27.5	37.0	38.5	103.0	1.72
16	8	24	29.0	28.0	27.0	84.0	3.50
June 1	22	66	73.5	70.0	67.5	211.0	3.20
11	5	15	20.0	22.0	23.0	65.0	4.33
19	23	69	98.0	91.5	90.0	279.5	4.05
July 17	22	66	76.0	78.5	72.5	227.0	3.44
Total	168	504	563.0	564.0	554.0	1681.0	44.65
Average			3.35	3.36	3.30		3.34

Table 14. Response of Hairy Peruvian and Hardistan alfalfa to inoculations with *Uromyces striatus* in the greenhouse. Manhattan, Kans., 1940.

Date of readings	Number of plants	Number of readings	Coefficient of severity	Type of infection	Length of infection	Coefficient of infection Total	Coefficient of infection Average
			Hairy Peruvian				
March 3	4	12	13.0	11.5	11.5	36.0	3.00
12	4	12	16.5	15.0	17.5	49.0	4.08
April 2	2	6	8.5	6.0	10.0	24.5	4.08
May 16	8	24	21.0	24.0	23.5	68.5	2.85
June 11	5	15	17.0	20.5	22.5	60.0	4.00
19	2	6	9.5	9.5	10.0	29.0	4.83
Total	25	75	85.5	86.5	95.0	267.0	22.84
Average			3.42	3.46	3.80		3.57
			Hardistan				
May 4	5	15	18.5	18.5	19.5	56.5	3.77
July 17	5	15	14.0	15.5	11.5	41.0	2.73
Total	10	30	32.5	34.0	31.0	97.5	6.50
Average			3.25	3.40	3.10		3.25

Table 15. Summary showing relative susceptibility of varieties and species of alfalfa to Uromyces striatus under greenhouse conditions. Manhattan, Kans., 1940.

Species, variety or selection	Severity rank	Total plants	Total readings	Coefficient of infection	
				Total	Average
Medicago ruthenica	1	10	30	0.0	0.00
Semipalatinsk	2	24	72	58.5	0.81
Ladak	3	63	189	307.0	1.63
Medicago falcata	4	25	75	128.5	1.71
Kansas Common	5	25	75	181.5	2.42
Kansas Common 1-2018	6	74	222	547.5	2.48
Kansas Common 1-205	7	44	132	343.0	2.60
Grimm	8	77	231	210.0	2.75
Hardistan	9	10	30	97.5	3.25
Turkestan 86696	10	158	474	1552.5	3.28
Turkestan 19304	11	168	504	1681.0	3.34
Hairy Peruvian	12	25	75	267.0	3.57
Total		703	2109		

Selections for Rust Resistance in Ladak Alfalfa. The control of diseases caused by rust fungi by the discovery of resistant strains in varieties, hybrids, or species from various sources or by selection in well-adapted commercial varieties of crop plants has received much attention in recent years. However, very little work or investigations have been done as far as alfalfa rust is concerned. During the summer of 1911 plots were examined at the Iowa experimental plots (19) for the purpose of noting to what extent rust prevailed among them. It was noted that one plot of an unnamed selection with nearly white flowers was badly rusted, while an adjoining plot of a recent introduction from Germany was sparsely affected. No rust was found on Melilotus alba and M. officinalis growing nearby. In another instance, it was noted in 1922 that several plants in a heavily infected field of alfalfa growing on the Soils and Crop Experiment Farm at Lafayette, Indiana, showed high resistance (16). These were transplanted to the greenhouse where their rust resistance was proved by inoculation. Mains (10) stated, in 1928, that "Individuals resistant to the alfalfa rust, Uromyces medicaginis Pass., have been found in strains of such varieties as Grimm, Cossack, Valo, Argentine, Dakota, and New Zealand." These three reports constitute the whole of the literature on resistance to alfalfa rust and they, in the true sense, carry little practical significance.

Although foregoing experiments demonstrated considerable difference among strains of alfalfa in their reaction to rust, casual observations in the field already had indicated the existence of such differences. Notes taken in 1938 by D. B. Creager, then connected with the Kansas Agricultural Experiment Station, suggested that plants and varieties varied in susceptibility.

During the summer of 1939 he also selected two plants of Ladak from an old and established experimental field at McLouth, Kansas. Both were taken from one of the variety test plot series which had been planted for seven years. Other varieties had almost disappeared due to wilt and even some of the Ladak plants had been killed. These were selected for three main purposes: (1) They seemed resistant to leaf and stem diseases, especially blotch and blackstem, which were present on many near-by plants, while very few lesions were present on the two plants selected, (2) they apparently were resistant to wilt because of survival for so many years in the presence of the disease, and (3) they were fine-stemmed, leafy, abundant flowered, and upright in habit of growth.

Several cuttings were made from these plants in the greenhouse with the idea of testing them for rust resistance. During the winter these plants were run with other groups of plants in the inoculation chamber until all cuttings had been tested three times. The results indicated that both original plants were fairly resistant, but their reactions to rust were not greatly different from that of ordinary Ladak plants. Although the resistance of Ladak and the two selections definitely is an improvement over the susceptibility of Turkestan and Kansas Common types, the discovery of a more resistant strain in Ladak made it seem advisable to discard the earlier selections.

During the fall of 1939 extensive rust notes were taken on replicated rows of six varieties of alfalfa grown in nursery blocks. Rust infection was very heavy on susceptible strains of Turkestan, while Ladak showed only light infection. Nearly all plants had some infection, but one plant was found in one replication that showed no infection. Rust readings were made at three different dates, but at no time was any infection found on this selection. This plant was rather spreading in its growth habit, and had many small stems and leaves. No rust pustules ever were found on any part of the plant. Because of its promising possibilities this plant was carefully transplanted to a pot and brought to the greenhouse for further and more severe tests.

It was sometime, as already described, before a successful method of inoculation could be discovered. In the meantime, this plant was cared for in one of the sand beds with many other plants. Finally, after the desired method for inoculation had been worked out, the plant was tested with other potted plants. On none of the first four tests were there any signs or symptoms that infection had taken place. These early tests suggested that this plant might be immune. This seemed very logical in view of the fact that other plants placed directly beside it in the moist chamber were heavily infected at the end of two weeks.

It was decided to give the plant the most severe test possible. A heavy suspension of spores was prepared and atomized on the lone plant now placed in one of the small metal cylinder moist chambers. It remained in the moist chamber for 48 hours after which the glass cover was removed, but the plant was left in the moist chamber and sand kept well watered. On the fourteenth day after inoculation very faint flecks began to appear. The rust failed to continue development, however, and no indications of pustules ever were observed. The final stage might be described as small, minute, necrotic areas, which definitely showed that the organism failed to reach fruition within the tissue of the host. This, however, was proof that infection did take place but that the plant carried a very high form of morphological or physiological resistance.

With such a lone promising plant, the next problem was to propagate more, or in some way obtain more plants from the original selection in order to make more tests and not at the time risk losing the plant in some way.

Through very fortunate circumstances, the writer was able to obtain 57 vigorously growing cuttings from the one parent plant. These were given tests during the fall of 1940 and found to carry the same resistance as the parent stock, as would naturally be expected.

The only hopes of alfalfa rust control lies in resistant varieties, since such a forage crop cannot be sprayed, dusted, or treated properly with fungicides on a commercial scale. Therefore, this plant, in the opinion of the writer, may have great possibilities in the future, especially if it proves to be resistant to other alfalfa diseases, such as wilt and blackstem. The variety, as a whole, carries some resistance to both of these diseases as well as a high resistance to rust, but the problem is in the degree of resistance carried by certain plant selections. Recent tests by C. O. Grandfield, of the Division of Forage Crops and Diseases, have shown that this rust-resistant selection also is highly resistant to blackstem. Part of the cuttings made from this selection will be retained by the writer for further study. The remainder will be released to the alfalfa improvement project of the Kansas Agricultural Experiment Station for other disease tests and possible increase.

Field Experiments

Material and Methods. A nursery consisting of five 60-foot blocks of 4-foot rows was available for alfalfa rust work in the field. Six varieties, Grimm, Ladak, two selections of Kansas Common, 1-205 and 1-2018, Turkestan 86696, and Turkestan 19304 were planted in this nursery. These were replicated in different manners; some were randomized, two blocks were made up of two row replicates grown side by side and the remainder were planted in 12-row series of each variety. Plants were spaced eight inches apart, six plants per 4-foot row. This allowed ample room for cultivation and permitted a study of each plant individually as well as a comparison of rows for varietal differences.

Besides this nursery, the Agronomy farm plots were available for observation at all times. Various general notes were taken during the fall of 1939 and valuable observations made at frequent intervals. Interesting strains from India, Turkey, Russia, Canada, and other foreign countries as well as many introduced strains from other states were grown in the plots at the Agronomy farm.

Alfalfa rust made its first appearance of the year at Manhattan on July 17. It was not until after the first week in September, however, that infection seemed sufficiently heavy and well-distributed to justify notes on varietal susceptibility. Frequent waterings at the Rust Nursery during late July and August and considerable late summer rain provided ideal conditions for good rust infection in the alfalfa plots.

Notes on severity of infection were taken at three different dates on the six varieties in one of the blocks to determine their field reaction to rust. These six varieties were placed in randomized 2-row series replicated in the block, ten rows or 60 plants of each variety. Each plant was examined carefully for rust appearing on leaves, petioles and stems. Each row was then given a numerical value from 0-9, the same system of numbering that is used by the United States Department of Agriculture in reporting damage done by the leaf-spot diseases of alfalfa. The lowest number, in this case, represents the more desirable plants, those that are most resistant to rust. Thus <u>0</u> denotes no rust at all, <u>2</u> noticeable, <u>4</u> to <u>5</u> medium attack, <u>7</u> severe, and <u>8</u> to <u>9</u> very severe, with marked defoliation. Readings were taken on three different dates, beginning September 8, 1939, and repeated at two-week intervals, on September 22 and October 6, respectively.

<u>Field Readings in 1939</u>. Tables 16 and 17 show the results of readings taken on the six varieties mentioned heretofore. Ladak, a variety already known to be fairly resistant to leaf and stem diseases, had a low average reading of 2.6. Rust was noticeable but caused no real damage to the plant. Two selections of Kansas Common, 1-205 and 1-2018, exhibited considerable resistance as shown by average readings of 2.9 and 3.1, respectively. Grimm ranked next with a reading of 3.6 indicating only a moderate degree of resistance. By far the most susceptible were the two Turkestan varieties, 66696 and 19304. These two strains, which are known to be more susceptible to leaf and stem diseases, were very definitely damaged by the rust. Pustules were large and very numerous on the leaves and stem pustules were undoubtedly causing severe injury to the plant. Badly infected leaves were drying up and falling to the ground. By the time of the last readings on October 6 the Turkestan rows generally could be distinguished from a distance due to their greater loss of leaves. Turkestan also showed very plainly another fact that was later substantiated with potted plants in the greenhouse studies, i.e., that the new growth was larger on the more susceptible plants.

Table 18. Response of six different varieties of *Medicago sativa* to a natural infection of alfalfa rust, *Uromyces striatus* in the rust nursery, Manhattan, Kansas, 1939.

Rows	Variety	Severity of infection, 0 to 9				
		Sept. 8	Sept. 22	Oct. 6	Total	Average
1-2	Grimm	3	3	2-3	8.5	2.8
3-4	Ladak	2	2-3	2	6.5	2.2
5-6	Kansas Common 1-205	4-5	3	3	10.5	3.5
7-8	Kansas Common 1-2018	5	3-4	2-3	11.0	3.7
9-10	Turkestan 86696	8	8-9	7	23.5	7.8
11-12	Turkestan 19304	9	9	8	26.0	8.7
13-14	Grimm	6	6	5	17.0	5.7
15-16	Kansas Common 1-205	5	4	3	12.0	4.0
17-18	Ladak	2-3	3	2	7.5	2.5
19-20	Kansas Common 1-2018	2	2-3	2	6.5	2.2
21-22	Kansas Common 1-205	3	3	2	8.0	2.7
23-24	Turkestan 86698	9	8	5-6	22.5	7.5
25-26	Kansas Common 1-2018	2-3	3-4	2	8.0	2.7
27-28	Turkestan 19304	7	7-8	5	19.5	6.5
29-30	Grimm	5	4	3-4	12.5	4.2
31-32	Kansas Common 1-2018	3	5	3	11.0	3.7
33-34	Ladak	3-4	3	4-5	11.0	3.7
35-36	Turkestan 86698	6	6	5	17.0	5.7
37-38	Kansas Common 1-205	2-3	4	2-3	9.0	3.0
39-40	Turkestan 19304	7	7	6	20.0	6.7
41-42	Grimm	2	3-4	3	8.5	2.8
43-44	Turkestan 86696	6-7	5	7	18.5	6.2
45-46	Ladak	2	3	2	7.0	2.3
47-48	Turkestan 19304	6	5-6	5	16.5	5.5
49-50	Kansas Common 1-2018	2	2	3	7.0	2.3
51-52	Turkestan 86698	4	4-5	3-4	12.0	4.0
53-54	Grimm	2	2-3	3	7.5	2.5
55-56	Turkestan 19304	5	5	4-5	14.5	4.8
57-58	Ladak	2	2-3	3	7.5	2.5
59-60	Kansas Common 1-205	2-3	2	2	6.5	2.2

Table 17. Summary of field reactions of alfalfa varieties to rust. September-October, 1939.

Variety	Total rows	Total readings	Total severity	Average severity	Severity rank
Grimm	10	5	54.0	3.6	4
Ladak	10	5	39.5	2.6	1
Kansas Common 1-205	10	5	46.0	3.1	3
Kansas Common 1-2018	10	5	43.5	2.9	2
Turkestan 86696	10	3	93.5	6.2	5
Turkestan 19304	10	5	96.5	6.4	6

Note: Rows replicated, readings based on observations on two rows.

Ladak and Kansas Common 1-2018 seemed to show definite resistance. In the case of Kansas Common, many of the leaflets exhibited a chlorotic killing of the tissue around the small pustules with a necrosis following, and later, a dropping out of the diseased tissue giving the leaflet a sort of shot-hole effect. The remainder of the leaflet remained green and apparently continued to function normally.

There seemed to be large differences in susceptibility even within a variety. Very resistant and very susceptible plants were marked and left standing. These were later transplanted to pots and moved to the greenhouse for winter inoculation work.

Not only were rust readings made on plants in the Rust Nursery, but varieties and selections grown in an adjacent wilt nursery were available for study. In one section of the wilt nursery eleven different varieties of alfalfa were planted in three-row replicates. These plants were to serve as transplants to be grown in pots in the greenhouse but they also showed excellent varietal differences to rust. Rust notes were taken on two different dates, September 8 and September 22, 1939. These readings are found in Table 18. Additional plant and rust notes were taken at the time and these are combined with the table. These same varieties were transplanted and tested in the greenhouse during the winter.

Rust notes were also taken on a group of 49 wilt-resistant strains of alfalfa at the Agronomy farm during the month of September 1939. These selections were made from several different varieties and were at the time being grown for seed increase. In some instances there were several plants under one selection number, other times, only one. Many of these strains were of foreign origin, having been developed either in other states or in foreign countries, such as Russia, Turkey, Canada and India. It was interesting to observe that there was a great range in the susceptibility to the rust organism, even within the same selection. For example, a range of 0.0 to 7.5 occurred for Kansas Common Selection 1-102 and 1.0 to 7.0 for Kansas Common Selection 1-108. In a similar manner, Selection 2-102 of the variety Kaw varied from 1.5 to 8.5. Such variations can be noted for most varieties of alfalfa. Whether it is actually due to a difference in the genetic make-up of the plants is still a problem to be solved. It is true, however, that most alfalfa plants are not homozygous for many characters, and probably not for rust resistance.

All of the plants that were examined in this plot had gone to seed and some of the seed had already been stripped. However, some plants showed a definite loss of leaves, while others showed very little defoliation from

Table 13. Notes on susceptibility of species and varieties of alfalfa to rust in field plots at the Agronomy farm. Manhattan, Kans., September, 1939.

Rows	Variety	Severity reading Sept. 8	Severity reading Sept. 22	Additional notes
1-3	Kansas Common	3-4	3-4	Pustules mostly large and distinct. Secondary infection by latter reading.
4-6	Kansas 308	6-7	6	Pustules more numerous, leaves falling.
7-9	Turkestan 86696	8	8-9	Very susceptible, definite loss of leaves due to rust.
10-12	Ladak	3	3-4	Lesions mostly smaller, no marked loss of leaves.
13-15	Turkestan 19304	5	7	Definitely susceptible.
16-18	Grimm	4-5	4	Rust pustules numerous, average size.
19-21	Kansas Common 1-3018	2-3	2	Definite ringing effect of chlorotic nature around pustules, killing plant tissue and preventing spread of rust. Pustules smaller and restricted. No secondary infections.
22-24	Semipalatinsk F.C. 22613	2-3	2-3	More prostrate form, 2-3, as a whole, but a few individual plants affected much worse, others hardly at all. Pustules mostly very small.
25-27	Hairy Peruvian	2-3	2	Very erect, upright plants. Large pustules but not numerous.
28-30	Medicago ruthenica	0	0	Prostrate, growing flat on the ground. No rust present.
31-33	Medicago falcata	2-3	2	In general, not so susceptible as other strains.

rust. The only rust found in any quantity in the Agronomy nursery during the fall of 1939 was on these old plants. The large uniform agronomic nursery had been cut, the plants had grown up and were blooming again, but very little rust was present. However, some rust was found in an adjacent plot of seedlings that had been transplanted from the greenhouse early in the spring. Rust of those plants was not nearly so heavy as that on the seedlings grown in the regular rust nursery, however. The latter plants had been watered frequently and showed good leaf growth and excellent rust infection. This proved that abundant moisture is necessary for good rust infection in alfalfa.

The field data that have been presented seem to agree with readings made on the varieties, selections and species in the laboratory and greenhouse. However, it was desired to bring all readings together so results could be prepared directly. Table 19 was prepared with this object in view. Here the rankings of the various strains under each of the three conditions are shown. The latter figure in the table is considered to represent the final ranking of each strain and to indicate its relative susceptibility. This table, therefore, shows the ranking of each strain for each test and its average severity rank for the three conditions.

Such variables as the use of a different number of varieties and unequal numbers of plants are known by the writer to exist in these data. Another differing factor in all cases is the prevailing environmental condition. However, after placing all the rankings of the basis of 12 varieties, the greatest number used in any one experiment, a final average ranking was obtained that corresponded very closely to all observations made by the writer in this study. Therefore, even with such variables and imperfec-

Table 19. Summary table showing relative susceptibility of alfalfa varieties and species to rust as determined by experiments conducted in the laboratory, greenhouse and field. Manhattan, Kans., 1939-1940.

Species variety, or selection	Severity rank in green-house	Severity rank in field Test I	Severity rank in field Test II	Severity rank in laboratory Test I	Severity rank in laboratory Test II	Total tests	Rank in each test on basis of twelve varieties tested: Green house	Field I	Field II	Laboratory I	Laboratory II	Total	Average rank	Final severity rank
Medicago ruthenica	1		1		1-2	3	1.0		1.09		1.8	3.89	1.30	1
Semipalatinsk	2		5		3	3	2.0		5.45		3.6	11.05	3.68	3
Ladak	3	1	6	1	5	5	3.0	2.0	6.54	2.0	6.0	19.54	3.91	4
Medicago falcata	4		2-3		1-2	3	4.0		2.73		1.8	8.53	2.84	2
Kansas Common	5		7		4	3	5.0		7.63		4.8	17.43	5.81	6
Kansas Common 1-2018	6	2	2-3			3	6.0	4.0	2.73			12.73	4.24	5
Kansas Common 1-205	7	3		3-4		3	7.0	8.0		7.0		20.00	6.67	7
Grimm	8	4	8	3-4	8	5	8.0	8.0	8.72	7.0	9.6	41.32	8.26	10
Hardistan	9					1	9.0					9.00	9.00	11
Turkestan 88698	10	5	11	5-6	9	5	10.0	10.0	11.99	11.0	10.8	53.79	10.76	12
Turkestan 19304	11	6	10	5-6	10	5	11.0	12.0	10.90	11.0	12.0	56.90	11.38	13
Hairy Peruvian	12		4		6	3	12.0		4.36		7.2	23.56	7.85	9
Kansas 308			9	2	7	3			9.81	4.0	8.4	22.21	7.40	8
Total	12	6	11	6	10									

tions, such a numerical ranking seems to be rather accurate and certainly a very worth while contribution to the summary of this study.

On the basis of the data shown in Table 19, Medicago ruthenica was the most resistant of all plants tested. The next in rank were M. falcata and Semipalatinsk, both of which showed considerable resistance. Ladak ranked fourth but it might be classed as first among the commercial varieties. Kansas Common types showed only moderate resistance in all cases, hence they all had intermediate rankings. Although Hairy Peruvian proved to be the most susceptible in greenhouse tests, its final ranking was below Grimm and Turkestan. This was due to its freedom from rust in the field. Kardistan and Turkestan were by far the most susceptible of any plants tested under either of the three conditions.

Field Readings in 1940. During the fall of 1940 very little alfalfa rust was present due mainly to the lack of rainfall during the months of August and September. It was first seen at Manhattan on July 26, which is about the usual date of its first appearance in the central plains area, but failed to develop as it did the previous year. However, a light but uniform infection of rust did develop a few days before the first killing frost. Thus, rust infection was very light and of little importance in the vicinity of Manhattan in the fall of 1940. This was not true for the entire state, however, since on November 5, J. C. Miller, Extension Plant Pathologist for Kansas, reported numerous outbreaks of rust on fall-planted alfalfa, but failed to estimate the damage.

Through correspondence with Dr. K. Starr Chester it was found that the same conditions prevailed in Oklahoma. He stated that he had not seen any fields suffering from rust in 1940 beyond a negligible extent and that no samples of rusted alfalfa had been sent in for control recommendations. He remarked, concerning the latter, that this was most unusual.

Due to this lightness of infection, no reliable rust notes could be taken in the field during the fall of 1940. However, the writer was very fortunate in having W. T. Emery, United States Department of Agriculture Entomologist, call his attention to the rust infection in the pea aphid nursery grown at the Bureau of Entomology and Plant Quarantine at Manhattan.

During the winter, rust had interfered greatly with pea aphid work in the greenhouse. After infection had started, it increased at a rapid rate due to the ideal conditions prevailing for rust infection. Rust was found to be more severe on the cuttings near a mist or spray used to keep the plants tender and succulent for pea aphid work. The disease seemed to dry up the leaf and stem tissues of the plants until the pea aphid would not feed.

Mr. Emery transplanted cuttings from plants known to be resistant to the pea aphid to the nursery during the first week in May. Unknowingly, he thereby carried the rust to the plots. The cuttings were watered daily by means of an overhead sprinkler until they were well started, after which water was applied once a week throughout the month of July. The main purposes in watering was to keep the plants growing and to protect them from the potato leaf hopper. This proved to create ideal conditions for producing a rust epiphytotic, as evidenced by the almost perfect infection obtained over the plot. If the conditions had been more dry and less humid, rust would have undoubtedly caused less damage. Leaf-fall of the seed crop was estimated on September 21 to be from 15 to 90 percent, depending on the variety. Damage to the seed crop in the plots ranged from 5 to 50 percent, again depending on the variety. Even the new growth appearing around the crowns of the seed plants was showing considerable rust infection at this time. The following notes were taken on different varieties in the pea aphid plots (readings based on the same 0-9 system used in the varietal studies in the Rust Nursery):

1. Cossack No. 6S37-S38-15, selected in 1936 for aphid resistance and selfed since 1937. Rust reading was 2.5. The plant seemingly showed considerable resistance, especially until very late in the season when rust was prevalent on the leaves. There was very little stem infection and the plants produced a good crop of seed.
2. Cossack, Plant Sel. 6S37-14, 7.5, a much more susceptible line. Many leaves lost due to rust, leaf pustules much more numerous where leaves are present. Stem pustules abundant. Seed crop definitely damaged, pods dried and shrivelled, turned brown before time to mature.
3. Cossack, Sel. 6S37-12 and S38-7, 6.5, fewer stem pustules than preceding plant but leaf lesions very prevalent.
4. Cossack, Sel. CS37-12, 4.5, fewer pustules on both stems and leaves.
5. Cossack, Sel. 6S37-22, 6.5, leaf rust very severe, some stem pustules. No seed produced.
6. Turkestan, Sel. T16S37-16, 8, very susceptible. Reaction the same as that of Turkestan in rust plots and greenhouse. Both stem and leaf infection, not so heavy leaf fall, however. Very little seed set.
7. Turkestan, Jones 16, 9, completely susceptible, most seriously rusted of all of the plants examined. Many stems devoid of

leaves, fall due to rust. Very little seed set.

8. Turkestan 86696, Sel. BIS38-11, 9, better seed set than the preceding selection, but much of it ad not matured normally due to rust.

9. Turkestan 86696, Sel. BIS38-12, 7.5, much like preceding, except less stem infection and better seed set.

10. Kansas Common, Sel. M24S38-1, 4.5, heavy leaf infection, none on stems. Good seed set.

11. Kansas 308, selected in 1938, Agronomy No. 5-20-2, 1.0, highly resistant to rust. Showed no infection until very late in the season when little damage could be done. These plants had been selfed from one plant at the Agronomy nursery that showed resistance to pea aphids.

12. Kansas 308, Sel. 29S38-5, 4.5, susceptible to leaf infection but no stem pustules. Not so good as preceding plant. Good seed set.

13. Ladak, Sel. D11S38-3, 5.5, rather heavy rust infection for variety, both stem and leaf. Not much seed set.

14. Ladak, Sel. D14S38-2, 6, rusted heavily, especially leaves. Seemingly a very susceptible line for Ladak.

An interesting hypothesis was reached after a careful study of these plants. From observation, it seemed that those plants having heavy stem infection had a much smaller seed set than those having very few or no stem pustules. In most cases, large, normal seeds were produced on plants free from stem pustules. It seems possible that the large lesions on the stems interfere with the normal translocation of the storage products to the seed. They undoubtedly lead to a greater loss of water by the plant.

Observations pointed toward the fact that a heaf leaf infection did not cause nearly so much injury to the seed set as a heavy stem infection. Plants of the Kansas 308 variety showed this very plainly. All were relatively free from stem pustules but some showed an abundance of leaf pustules. In all cases, however, the seed set appeared normal. The variety, Turkestan 86696, is very susceptible to both leaf and stem infections. With the exception of Selection B1S33-12, they all produced only small amounts of normal, plump seed. This selection was much like the proceding, as far as leaf pustules were concerned, but had an exceedingly small number of stem pustules, hence, a much better seed set. From the evidence provided by these and other plants, the writer is inclined to believe that heavy infections of rust on the stems of alfalfa cause much greater reductions in the seed crop than equally heavy infections on the leaves only.

Spraying Alfalfa for Rust Control. Two blocks of 12-row replicates at the field nursery were utilized in a spray test in order to study the effect of sulphur upon the rust organism. Five varieties, Turkestan 86696, Turkestan 19304, Kansas Common 1-205, Kansas Common 1-2018, and Ladak were planted in 12-row replicates but there were only six rows of the variety Grimm.

One-half of the rows of each variety were sprayed with a 98.5 percent wettable sulphur at the rate of four pounds to 100 gallons of water. The other six rows were left as checks. Two sprayings were made at weekly intervals during the second and third weeks in August 1939, and the plants were cut just a month later. No definite results could be seen due to the spray. Leaves on most rows of the tested plants were rusted as badly as the checks. At least, there was little significant difference. After cutting, the plants were sprayed again while the growth was still very young and in a week the process was repeated. An early frost on September 30 killed the plants so no results were obtained. It was hoped that tests could be run again during the summer and fall of 1940 but not enough rust appeared to warrant the work. Evidently spraying must be started earlier, with or before the first signs of rust infection, in order to get the best fungicidal results, if any, from the sulphur.

Even though sprays or dusts would control rust, it is very questionable as to their practicability, due to the expense of the fungicide and the labor involved. They, however, might be applied handily to small plots when there is danger of losing an important seed crop.

DISCUSSION

After noting differences in varietal susceptibility to alfalfa rust under moderate infections in the field, it was assumed that under favorable, controlled conditions in the greenhouse severe infections might be produced that would lead to a much more complete knowledge of the reaction of commonly used alfalfa varieties to rust.

Very little work had been done on any phase of this problem up to the time of these studies, especially in culturing this Uromyces in the greenhouse. Therefore, it was necessary first to explore all the possibilities in an effort to discover a simple method of obtaining rust infection in the greenhouse comparable to that in the field. After much experimenting it was proved that greenhouse inoculations conducted in the right manner would yield even better results than field tests. It was found that fresh urediospores must be used in order to obtain the best infection. This was exemplified by germination experiments in the laboratory which proved that unless the urediospores were stored under the most ideal conditions there was a rapid decline in their germinating power. Another retarding factor was the dearth of spores often produced by alfalfa plants, as contrasted to wheat rust, for example. The mode or method of collecting the rust inoculum from small leaflets also was a perplexing problem. Finally, however, it was discovered that excellent infection could be obtained on a large number of plants by placing them in a moist chamber add applying spores by means of a small fly sprayer. The spores were placed in suspension after scraping them from alfàlfa leaflets with a small, flattered needle. This method of inoculation was very satisfactory because of its relative ease and simplicity.

The greenhouse, it seems, is very important in the study of varistal differences to rust infection. A great number of plants can be studied and since conditions for obtaining good infections can be produced, reliable notes on varietal reactions to rust may be taken.

A summary of all the greenhouse tests showed the loid species, *Medicago ruthenica*, to be the most resistant of any plants tested. It was most impressive to note the wide range in susceptibility among species and varieties of alfalfa, e.g., from a rust reading of 0.00 for *M. ruthenica* to 3.56 for Hairy Peruvian. The results with *M. ruthenica* might indicate that low chromosome plants carry a much higher degree of resistance. Further breeding work may be able to clarify this point.

In general, there was a very close correlation between the laboratory and greenhouse tests and observations made in the field. This indicates that a testing program may be carried out under controlled conditions and give the same results in a much shorter time than if one depended only on field observations.

It was possible to select resistant forms from several different strains of alfalfa. Ladak and *Medicago falcata*, especially varied considerably. Plants with different degrees of resistance often were observed. This was not the case, however, with such varieties as Grimm and Turkestan, which were rather uniformly susceptible to rust.

Practical use was made of the differences in susceptibility by the selection of a very promising plant of Ladak alfalfa in field plots during the fall of 1939. It was potted and brought to the greenhouse where it was subjected to the most severe rust tests. All efforts to produce rust on the plant failed, hence, it obviously carries some high form of morphological or physiological resistance. Propagations were made from the parent stock and these also proved resistant, as would be expected. Recent tests made through the cooperation of the Agronomy Department have shown that this rust-resistant selection likewise is highly resistant to the blackstem disease caused by *Ascochyta imperfecta*. Hence, it seems highly probable that this selection carries strong resistance to several leaf and stem diseases of alfalfa. It, therefore, should be valuable parental stock for alfalfa breeders interested in disease resistance. If the resistance carried by this selection could be combined with the good agronomic qualities of Turkestan, a superior strain of alfalfa could be released to the farmers of the western Mississippi valley.

The role of the alternate host of this organism was also studied. The teliospores of Uromyces striatus refused to germinate under any laboratory condition. In the greenhouse a very few infections produced pycnia, but no aecial infection was obtained on the alternate host, Euphorbia cyparissias. Whether this was due to the methods used, or to some peculiarities in the rust or host is not known. It seems very likely, however, that neither the teliospore nor the alternate host playsan important part in the life cycle of alfalfa rust in the central part of the United States.

Considerable knowledge concerning the role and length of life of the urediospore was compiled by means of overwintering and germination studies, and also by correspondence with several investigators in mid-western states. This information, in addition to that noted in the previous paragraph, indicates that the urediospore, alone, is responsible for all rust outbreaks in this region. Furthermore, all indications point toward the fact that urediospores do not overwinter as far north as Kansas. Annual outbreaks, then, during August and September are no doubt due to the carrying of rust spores northward by prevailing southurly winds from areas in Louisiana, Texas, New Mexico and Arizona, where the organism can live over winter on green alfalfa plants.

Although alfalfa rust is not the most important disease of the crop today, it often causes much more damage than is realized. Any disease that attacks and injures such a crop as alfalfa, which not only has a great value as a forage crop but as a soil builder as well, deserves some study.

Rust epiphytotics often appear in the later part of the summer or early fall, just at the time the plant needs all the available food materials for seed formation. It has been noted that the greatest damage done by the disease in this area is manifested in a reduction in the seed crop. Heavy rust infection on the stems is considered to be mainly responsible for this reduced seed yield.

SUMMARY

1. A general study was made on alfalfa rust (Uromyces striatus Schroet.) under laboratory, field and greenhouse conditions. Facts concerning the life history of the organism were determined by experiments, and a testing program was developed by which resistant strains of alfalfa could be identified.

2. In laboratory experiments urediospores retained their viability for periods of eight months or more when stored under cool (30-40° F.), moderately moist conditions (relative humidity 70-80 percent). Urediospores overwintered outside failed to germinate the following spring.

3. Teliospores of this rust in laboratory mounts did not germinate under any conditions.

4. A method of inoculating excised alfalfa leaves in Petri dishes was developed in the laboratory. This method was found to be sufficiently accurate to determine varietal and species differences in susceptibility.

5. In greenhouse experiments a simple technique was developed by which rust infection comparable to that in the field was obtained. It consisted of spraying with a small fly sprayer the fresh rust inoculum on potted plants in a moist chamber. These plants remained 48 hours in the

moist chamber. Rust pustules appeared seven to ten days after inoculation and final rust readings were generally taken about three weeks later.

6. Marked differences in resistance to rust among varieties and species of Medicago were observed in the greenhouse. _M. ruthenica_ carried the highest form of resistance. Turkestan and Hairy Peruvian were the most susceptible.

7. Readings on varieties and species made in the greenhouse correlated very closely with those taken in the field and laboratory.

8. A plant selection of Ladak made in the field exhibited the most resistance of any strains or varieties of the _M. sativa_ group. This selection not only showed high resistance to rust in greenhouse tests, but also recently exhibited high resistance to blackstem disease of alfalfa caused by _Ascochyta imperfecta_ Peck. Cuttings of this selection have been released to the alfalfa improvement project of the Kansas Agricultural Experiment Station for other disease tests and possible increase.

9. Alfalfa rust generally appears late in the season here at Manhattan. Often, as in 1940, no damage is done, but occasionally, severe damage is caused to the seed crop. The latter is especially true when infections become heavy on the stem.

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