EFFECTS OF *PUCCINIA RECONDITA* F. SP. *TRITICI* ON CERTAIN NUTRITIVE VALUES AND FORAGE YIELDS OF WINTER WHEAT

By

ERVIN WILLIAMS, JR.

Bachelor of Science
Kansas State University
Manhattan, Kansas
1951

Master of Science
Kansas State University
Manhattan, Kansas
1960

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1973

EFFECTS OF PUCCINIA RECONDITA F. SP. TRITICI
ON CERTAIN NUTRITIVE VALUES AND FORAGE
YIELDS OF WINTER WHEAT

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to his major adviser, Dr. Harry C. Young, Jr., for his guidance and assistance throughout this study and preparation of the manuscript. Appreciation is also expressed to the other committee members, Drs. G. L. Barnes, L. A. Brinkerhoff, E. L. Smith, and J. E. Thomas, for critical review and suggestions concerning the manuscript.

The author is grateful to Dr. R. R. Johnson and Mr. P. E. Weems for their assistance in the protein and carbohydrates analyses.

The author also acknowledges the aid of Dr. R. D. Morrison for assistance in conducting the statistical analyses.

The author wishes to thank the Department of Botany and Plant Pathology of Oklahoma State University for providing the facilities needed for this study.

The author is indebted to fellow botany graduate students and technicians who assisted in the harvesting of forage from field plots.

Finally, to his wife, Johna, and to his three children, Gerald, Marilyn, and Carolyn, the author wishes to express his sincere gratitude for their encouragement, patience, and sacrifice which were invaluable in the completion of the research reported herein.

TABLE OF CONTENTS

LIST OF TABLES

Table		Page

LIST OF FIGURES

CHAPTER I

INTRODUCTION

In mid December of 1971, nearly 40% of the fall-seeded wheat was being pastured by livestock through the fall and winter months in the three state region of Kansas, Oklahoma, and Texas. In western Oklahoma, 65% of the winter wheat acreage was being pastured (28).

Grazing of winter wheat can produce 2.27 kilograms of beef per day per hectare under average conditions (26). Winter wheat forage is a high-quality feed and protein content is high throughout the winter and early spring (12). This fact has brought about an increased interest in growing winter wheat primarily for livestock pasture. To increase the grazing potential, growers are planting earlier in the fall, which materially increases the chance of infection by the leaf rust fungus, Puccinia recondita Rob ex. Desm. f. sp. tritici. Earlier rust infections will, in turn, greatly increase the possibility of epiphytotic development, both in the fall and the spring seasons (41).

Since the time that leaf rust was recognized as a destructive disease of wheat, nearly all research on the effects of the disease has considered only the grain crop (21). Only recently have studies been conducted on the effect leaf rust may have on the grazing potential of wheat. The studies discussed herein deal with certain effects of leaf rust on young immature wheat plants grown under controlled conditions in a growth chamber, and from 1.22 X 3.05 meter field plots where simulated grazing was achieved by periodic foliage clippings made in the early fall and winter months of the 1971-1972 season.

CHAPTER II

LITERATURE REVIEW

Leaf rust has long been recognized as a common and widely distributed disease of wheat as pointed out by Carleton (9) early in the culture of winter wheat in the United States. The disease was then commonly called "orange leaf rust" of wheat. Carleton observed in 1898 that under certain conditions and in certain localities, considerable injury may follow if leaf rust develops abundantly much in advance of harvest.

Melchers (27) in 1917 reported that leaf rust in some fields in Kansas was very abundant, and that grain yield of a pure line winter wheat called P706 was reduced by 38%. Melchers stated that careful observations of such fields showed that no other factors could have been responsible for the poor quality and reduced grain yield. The foliage of the above mentioned variety was estimated to have 100% leaf rust infection.

Mains (25) in 1930 evaluated the effects of leaf rust on several wheat cultivars by controlling the disease using sulfur dust. He found that grain yield reduction, depending on the cultivar and the time of established infection, could vary from 24 to 97%. He also found that the straw weight of leaf rust infected plants was decreased from 11 to 70%.

Caldwell et al. (8) in 1931 made a study of the effects of a severe leaf rust epiphytotic on the yield and physical characters and chemical composition of the grain and plants of seven cultivars of winter wheat each of which produced a somewhat different response to the leaf rust disease. The host cultivars varied from highly susceptible to highly resistant. Control of the disease on susceptible cultivars was accomplished by frequent dustings with sulfur. Depending on the leaf rust severity and the disease reaction type, grain yields were reduced from 0 to 24%. Straw yields were reduced as much as 14% with severe leaf rust infections. In the same paper, they reported that the percentage of protein in the grain of susceptible cultivars of both hard and soft winter wheats was significantly reduced by severe leaf rust infections. However, a trend to the reverse was noted for the combined culms and leaves which actually contained higher percentages of total nitrogen. Sucrose concentrations of the mature grain were consistently reduced by leaf rust. Both the culms and leaves of rusted plants contained less sucrose and reducing sugars than did the culms and leaves from non-rusted plants.

Johnston and Miller (23) reported that leaf rust could reduce the average grain yields of susceptible varieties from 42 to 93%. They also reported that the yields of straw were significantly reduced by leaf rust infections, and that heavy rust infections on susceptible varieties resulted in a rapid and severe deterioration of the roots. This was indicated by root discoloration, a decrease in the number of fibrous roots, and a marked loss in total root weight. Their studies indicated that leaf rust infections increased the water requirement of the susceptible varieties from 31 to 104% based on total dry matter and on the length of the rust infection period.

Weiss (43) also reported that both leaf rust and stem rust lowered the water economy of the host, when either the dry matter of the entire plant tops or the grain was considered. Weiss reported that the actual quantity of water transpired was significantly related to rust infection when it was correlated with the dry matter produced.

Chester (10) reported a leaf rust epiphytotic in Oklahoma in 1938. After questionnaires were sent to leading farmers requesting that wheat grain yields be compared with the leaf rust free year of 1937 it was determined that the loss to the leaf rust disease was 34% in 1938. Again, only the grain was considered in determining the loss.

After Caldwell et al. (8) had found that leaf rust infected wheat plants actually contained higher percentages of nitrogen than rust free plants, D'Oliveria (13) of Portugal, in 1939, grew wheat and barley plants in water or nitrogen free media with and without rust infection. Analysis of the seedlings showed that those plants which were rust infected contained more nitrogen than the normal plants, and the longer the plants had been rusted the greater was this difference. From this D'Oliveria proposed that rusts were able to fix atmospheric nitrogen. There was no record that this theory of rust fixing nitrogen has been pursued any further.

Shaw (36) reported that C^{14}-labelled substances accumulate near the stem rust infection sites on susceptible wheat leaves. Johnson et al. (20) reported similar results with leaf rust on wheat by the accumulation of radioactive phosphorus in the region near the rust pustules. The P^{32}-labelled phosphorus was applied at the leaf tips and accumulated near the rust infection sites at the base of the leaves. The rust parasite apparently has a definite affect on the mobilization of certain metabolites within the host.

Johnson et al. (19) reported that they had found no pronounced change in total buffer-soluble protein content on leaf rust infected wheat compared with the healthy controls. In this comparison of rusted and non-rusted wheat seedlings of the cultivar Wichita, micro-Kjeldahl and Folin assays were used to determine the total protein at intervals of 0, 2, 4, 7, and 10 days after inoculation. At no time was there any appreciable difference in total protein between the healthy and rusted seedlings although the values for the rust infected tissues generally were slightly lower. They cautioned that the intensity of rust infection may be involved in these protein differences, since the total protein content of infected leaves is an average of protein contents from infection sites of active metabolic activity and interpustular areas. They were working with heavy concentrations of rust inoculum.

Hendrix and Fuchs (16) working with stripe rust in the Pacific Northwest wheat growing area of the United States reported in 1970 conspicuous centers of infection in early fall seeded wheat. They called these centers of infection "hotspots." The importance of the fall stripe rust infection was established by comparing growth and yield of plants growing within 24 of these so-called hotspots with disease free plants growing immediately outside these areas. "Hotspot" plants produced 18.6 to 24.0% fewer tillers, 19.6 to 25.4% less straw, and 18.3 to 30.8% less grain than the corresponding healthy plants.

Hendrix and Martin (17) reported on several observations of the affect of stripe rust on wheat plants grown in mist culture. Roots from plants infected with stripe rust had a reduction in stele cylinder diameter of 39 to 57% compared to non-rusted plant roots. The number of pericycle cells was reduced 34 to 47% while the number of root phloem cells was reduced 45 to 60%. In most cases, the size of the above described reductions was associated with the severity of rust infection. They also reported that the mitotic index in root cells of stripe rust infected plants was lower than in root cells of nonaffected plants. Also, the balance of amino acids was changed in the roots of stripe rust infected plants. Concentrations of aspartic acid, threonine, leucine, cystine, lysine, and arginine were at the highest proportionate levels among plants inoculated at early stages of growth. The proportion of these same amino acids receded when the plants were inoculated in the later stages of development. Concentrations of glutamic acid, glycine, and ornithine surpassed the controls in all inoculated treatments and proline, methionine, isoleucine, tyrosine, and histidine were always found to be at lower levels than in the noninfected controls.

An extensive search for chemicals effective in the control of cereal rusts has been conducted since the early 1920's when sulfur dust was reported to be a good protectant fungicide for leaf and stem rust disease (5). Since that time, an abundance of literature has accumulated on the use of various inorganic and organic chemicals for rust control (33). A complete review of that literature would be inappropriate here, but one of the most recent reports concerning chemicals for wheat leaf rust control involves 4-n-butyl-1,2,4-triazole (42). This reportedly systemic chemical appears to be limited exclusively to the control of wheat leaf rust. It persists at effective levels in the plant for practically the entire growing period of winter wheat.

LeGrand and McMurphy (24) reported that forage production from small grain pastures during the winter months has become very important and is an essential source of nutrition for the livestock industry in Oklahoma. Favorable cattle prices have, in many cases, made this forage production of greater value than the harvested grain.

Shipley and Regier (37), in an experiment in a Texas high plains area, found the average daily gain of cattle from November through May was 0.82 kilograms on irrigated winter wheat pasture. The stocking rate was 3.7 animals weighing 448.16 kilograms per hectare at the time the cattle were placed on wheat pasture in the fall. The average grain yield in this experiment, when grazing was terminated March 20, was 3,314.12 kilograms per hectare compared to 1,465.47 kilograms per hectare when grazing was extended to May 1. The average grain yield for nongrazed wheat was 4,167.86 kilograms per hectare.

CHAPTER III

MATERIALS AND METHODS

Growth Chamber Experiments

A uniformly mixed soil of six parts of clay loam, one part fine sand, and one part peat moss was obtained by using a Lindig soil shredder followed by an additional screening through a 3.17 mm mesh screen. Three liters of this soil were firmly packed into 3.78 liter capacity glazed stone jars. A 1.27 cm diameter drain hole located at the base and to the side of each jar was covered inside with a portion of paper towel to prevent the soil from escaping.

In two of the trials, 125 "Arasan" (50% Thiram)-treated seeds of the winter wheat (Triticum aestivum L. em. Thell.) cultivar 'Triumph 64' were planted in each of ten jars. The seeds were uniformly spread on top of the soil surface of each jar and firmly covered with an additional 2.54 cm of soil.

Water was slowly added to each jar until it started to drain at the base of the jars. When drainage stopped, each jar was weighed on a 20 kg capacity balance and the weights recorded. The first time water was added, and every third time thereafter, "Hyponex" fertilizer (7-6-19, N-P-K formulation) was added at the rate of 2 grams per liter of water.

The Triumph 64 cultivar was used because this and similar type cultivars dominate the wheat acreage in Oklahoma (29). Also, this cultivar, although showing some degree of tolerance to leaf rust in the field, carries no known genes for resistance to leaf rust in the seedling or early stages of growth (44).

The wheat seedlings were thinned to 100 per jar six days after planting except in the third trial where only 20 seeds were planted and then were thinned to 10 seedlings per jar. These experiments were performed in a Sherer-Gillett Model CEL 25-7 growth chamber which is capable of holding 10 jars in a randomized block design with five rows of two jars. The plants in one jar of each row selected at random, were inoculated with rust spores, and plants in the other jar of the row were not inoculated.

Prior to the actual experiments on the effect of rust on seedling plant development, a uniformity trial was conducted within the growth chamber to be used. The wheat seedlings were grown in the same manner as in the rust studies which were to follow. At the end of 30 days the plants were harvested and the forage weights were recorded. Statistical analysis of the data showed a coefficient of variation for the fresh and dry forage weights of 5.40% and 2.45%, respectively. These were considered to be acceptable coefficients.

The bench in the growth chamber was adjusted to provide a light intensity of 2,152 luxes at the top of the jars. This light was composed of six F48T12/CW/VHO 110-watt fluorescent bulbs, and twelve 25-watt incandescent bulbs. A photoperiod of 12 hours of light and 12 hours darkness was provided. The temperature was maintained at 26 ± 2 C during the light period and 16 ± 2 C during the dark period. Humidity control was not provided; however, hygrometer measurements indicated the relative humidity to be near 50% during the light period and near 80% during the dark period.

The first inoculation of the wheat seedlings was made when the plants were seven days old. At this time, each jar containing plants to be inoculated was removed from the growth chamber and placed in a moist chamber large enough to hold five such jars. These moist chambers were made of 66 X 51 X 30 cm galvanized metal boxes with a grate of 1.27 cm wooden slats to hold the base of jars, above a thin layer of water placed in the bottom of the chambers to maintain high humidity. The sides and bottom of the moist chambers were thoroughly washed and left wet before the jars of wheat seedlings were placed in the chambers. The plants in five jars in one moist chamber were inoculated with rust spores and the plants in the five jars in another moist chamber remained uninoculated. The plants to be inoculated were sprayed with a fine mist of water using a hand-operated 473 ml polyethylene trigger sprayer. Approximately 30 wheat seedlings, heavily infected with physiologic race UN-2A (31) of the leaf rust fungus, and grown in a 10.16 cm clay pot were brushed over the wheat seedlings in the jars, causing unredio-spores to adhere to the leaves. These inoculated plants were sprayed again with water until the leaves were thoroughly covered with small water droplets. After the second spraying the top of the moist chamber box was covered with a 4.74 mm thick sheet of glass.

The uninoculated plants also were placed in a moist chamber and handled in exactly the same manner as the inoculated plants except that they were not brushed with the rust-infected wheat seedlings nor were they sprayed with water since, by chance, some rust spores may have fallen on the leaves. All plants were kept in their respective moist chamber for eight hours; sufficient time to insure infection with leaf rust at approximately 20 C.

Similarly, two additional inoculations were made on the same plants, one when the second leaf was fully developed, and the other when the third leaf was fully developed. A leaf rust infection severity of near 100% (modified Cobb scale (11)) was obtained following the third inoculation.

The culture of the leaf rust fungus used, physiologic race UN-2A, was isolated from a field collection made near Alva, Oklahoma, in December, 1970, by Dr. H. C. Young, Jr., Plant Pathologist, Oklahoma State University, Stillwater, Oklahoma. Currently, the UN-2A race group predominates among the isolates of *Puccinia recondita* f. sp. *tritici* in Oklahoma (45).

The first forage yields were obtained by cutting 42 day-old wheat plants with scissors at a level of 2.54 cm above the soil surface. Cuttings from each jar were placed in small paper bags, and weighed while still fresh on a Mettler P-1210N balance. After weighing, the samples were placed in a drying oven designed for such plant material for 96 hours at 62 C. These oven dry samples were again weighed and the results recorded.

After forage cuttings were made, both rusted plants and the non-rusted plants were allowed to grow for 14 days at which time another forage cutting was made, weighed, dried, and weighed again in the manner described above.

When forage yields were completed, root development data were obtained by separately placing the contents of each jar, soil and plants, on a 3.17 mm mesh screen and running a fine stream of water over the root mass until the soil was thoroughly washed through the screen and only the wheat plant roots remained. A root volume for the total of all plants in each jar was obtained by placing the roots from each jar in a 1,000 ml graduated cylinder and measuring the displacement of water. The roots in each jar were then weighed fresh, dried in the previously described drying oven, and weighed again as was done with the forage cuttings.

Protein determinations from 1 gm oven dried forage samples were made by using the standard Kjeldahl method (2). The percentage of total nitrogen for each distillate sample was determined by titration using 0.1253N solution of sulfuric acid and an indicator dye mixture consisting of a 5% solution of boric acid, methyl red, and methylene blue. The protein value for each sample was obtained by multiplying the percent total nitrogen by the factor 6.25. This factor is based on the fact that nitrogen occurs in different proteins at a fairly constant 16%.

For the determination of soluble carbohydrates in rusted and non-rusted forage another trial was made in the growth chamber following the same procedures and design used for the forage yield trials. This time, when the forage samples were clipped, they were immediately frozen by sealing each sample in a plastic bag and completely submerging it under crushed dry ice. These frozen samples were analyzed by a method developed by Dubois et al. (14) and modified by Johnson et al. (18) which involved the following steps:

1. The fresh frozen samples were pulverized by grinding each sample in a small Wiley mill. A sufficient amount of dry ice was added during the grinding process to prevent thawing.

2. Two grams of the frozen samples were rapidly transferred to a 200 ml volumetric flask.

3. The flasks containing the samples plus the addition of 150 ml distilled water were placed on a shaker (220 oscillations per minute) and shaken for 30 minutes.

4. After the shaking, the small plant particles were allowed to settle out. Then, the samples were each diluted with 50 ml of distilled water so that final volume of supernatant contained between 2 and 30 micrograms of soluble carbohydrates per ml. A 2 ml aliquot of supernatant from each sample was placed into a reaction tube.

5. To each tube, 1 ml of phenol reagent (50 mg phenol per ml in water) and 5 ml of concentrated sulfuric acid were added. The contents were mixed and let stand for 10 minutes, mixed again, and let stand for 20 minutes.

6. A standard curve was prepared by using spectrophotometer readings of 0.5, 1.0, 1.5, and 2.0 ml of a standard solution using an equal mixture of glucose and xylose. This curve gave values of 20, 40, 60, and 80 μg.

7. Optical density readings were taken at 490 mμ on a spectrophotometer against a prepared reagent blank using 2 ml of distilled water instead of sample.

8. The milligrams of soluble carbohydrates per gram of sample was calculated in the following manner:

$$\text{mg CHO per gm} = \frac{\mu\text{g per tube} \times A}{\text{sample wt} \times \text{D.M.} \times 4\ B}$$

A = Total volume of second dilution used for color deviation.

B = Aliquot volume of original extraction used for the second dilution.

D.M. = Dry matter content expressed as decimals rather than percent.

Sample wt is in grams.

To determine sample moisture percentage, a separate 2 gm sample was placed in an oven and dried for 48 hours. The milligrams of extracted carbohydrates per gram were then converted to percent carbohydrates on a moisture free bases.

Field Experiments

To evaluate the effect of leaf rust on forage production in the field a series of several different cultivars plus a pair of near isogenic lines of winter wheat were planted at the following Oklahoma locations: Goodwell, Lahoma, Stillwater, and Woodward. A randomized complete block design with four replications was followed. The plot size was 1.22 X 3.05 meters and each plot contained four rows spaced 30.48 cm apart.

The cultivars grown at some or all of these locations which processed some degree of either specific or nonspecific resistance or tolerance to leaf rust were:

'Agent' (AG) (6), an Oklahoma release (38), that has a gene conditioning resistance to all known physiologic races of leaf rust except for at least one culture recently found to be virulent at that locus (7).

'Caprock' (CRC), a Texas release (4), which has a gene or genes for resistance in the field to all of the predominant races of leaf rust found in the North American hard red winter wheat area.

5*SUT/AG (30), an experimental strain (OK696731), selected at the Oklahoma Agricultural Experimental Station from a cross of 'Scout' (SUT), a Nebraska release (22), and Agent made at the Colorado Agricultural Experiment Station. This strain carries the leaf rust resistance of the Agent cultivar and possibly other genes conditioning resistance.

The cultivars grown and classified as susceptible to leaf rust in the early stages of growth were:

'Comanche' (CMN), a Kansas release of 1942, was considered to have many superior agronomic characteristics (32). The Comanche cultivar does not exhibit any resistance to leaf rust in the seedling stage; however, it does exhibit what has been called adult plant resistance in the field (46).

'Danne,' a recent Oklahoma release (39), and is a "Triumph-type" wheat with similar agronomic characteristics. Danne expresses a leaf rust infection type analogous to that on Triumph 64.

'Nicoma,' another recent Oklahoma release, considered superior to the "Triumph-type" wheats in both yield potential and baking qualities (40), but of similar maturity. Nicoma expresses no resistance to leaf rust in the seedling stage, but like Comanche exhibits some degree of adult plant resistance in the field.

Atkins and Mangelsdorf (3), in 1942, suggested the use of near isogenic lines to compare the effects of the awns on grain production in wheat. An isogenic line can be defined as, "Two or more lines differing from each other genetically at one locus only" (1).

In this study, a pair of near isogenic lines was used in the field plots to help in the evaluation of the effect of leaf rust on forage production. This near isogenic pair consisted of a TF/5*CMN leaf rust resistant line (TF/CMN(R)) and a TF/5*CMN leaf rust susceptible line (TF/CMN(S)). These lines were developed by Dr. H. C. Young, Jr., at the Oklahoma Agricultural Experiment Station and involved a primary cross between the cultivars 'Transfer' (TF) and Comanche, described earlier. The Transfer cultivar was the result of the work by E. R. Sears (34) who was able to effect a translocation of a chromosome segment from Aegilops umbellulata containing a gene or genes for resistance to leaf rust to a spring wheat cultivar named 'Chinese.' Although at least one leaf rust culture occurring in nature has been reported to be virulent on Transfer (35), that culture has not yet been found in Oklahoma, and the resistant member of the near isogenic pair remained free of rust in these studies.

The four row plots at all locations were planted with a John Deere model 71 planter equipped with a cone type seed hopper for the purpose of evenly distributing 7.5 gm of wheat seeds over the 3.05 meter row. This seeding rate was equivalent to a rate of 80 kg per hectare. The planter was mounted on an Allis-Chalmers model G tractor.

Planting dates at each location were as follows for 1971: Goodwell, August 7; Woodward, August 16; Lahoma, August 30; and Stillwater, September 1. These dates are all a few days earlier than the planting date normally used for wheat that is intended for fall pasture. The planting was accomplished this early to ensure optimum time for leaf rust development.

At Goodwell, a preplant application of fertilizer was applied at the rate of 134 kg of nitrogen and 67 kg of P_2O_5 per hectare. In February an additional 79 kg of nitrogen was top-dressed on the plots. At Stillwater, only a preplant fertilizer application was made at the rate of 134 kg of nitrogen plus 67 kg of P_2O_5 per hectare. No fertilizer was applied on the plots at Lahoma or Woodward.

At Goodwell, one preplant flood irrigation and four additional irrigations of 9 cm each were applied giving a total of 45 cm of irrigation water for the growing season. Sprinkler irrigation was used at Stillwater primarily to increase relative humidity within the plots to enhance the spread of leaf rust. These periodic irrigations supplied approximately 12 cm of water from September through November of 1971. No irrigation water was applied at either Lahoma or Woodward.

Leaf rust inoculations were made only at Stillwater. A border of eight or more rows of the leaf rust susceptible cultivar Triumph 64 was planted around the plot area. These border rows on the south side of the plots were planted one month in advance of the date the plots were planted. Within these early planted border rows, a series of 10.16 cm clay pots containing sporulating leaf rust infected wheat seedlings were placed at random. These plants had been inoculated with a composite of physiologic races indigenous to Oklahoma. However, the Agent virulent race was not included in the composite. The rust infection which did ultimately occur on the Agent cultivar in the plots was the result of naturally occurring inoculum.

After the rust infected seedlings had been placed in the field, the overhead irrigation sprinklers were operated for one hour periods just before sundown twice a week. This promoted heavy morning dew in the plots and provided an ideal environment for rust spore germination.

Duplicate plots of each cultivar were planted within each replication of the field design. One of these plots remained unsprayed, and in the other the two center rows were sprayed with the experimental systemic chemical 4-n-butyl-1,2,4-triazole, later referred to in this paper as triazole, developed and produced by the Rohm and Haas Company (42).

The rate of application was 1.014 kg of active material per hectare. The sprays were applied with a hand operated 7.56 liter knapsack sprayer at the time the third leaf had begun to expand.

One of the 2 center rows of each 4 row plot was clipped. The center 2.43 meter portion of the row was used for forage yield data. Hopefully, this removed any border effect. Clippings were made whenever the majority of the plots showed sufficient plant growth to produce ample forage, generally when the plants were from 15 to 20 cm in height. Forage clippings taken in February, however, were shorter due to the slow plant growth rate at low temperatures. At the Goodwell, Lahoma, and Stillwater locations, three cuttings were made between late September and middle February. However, only two cuttings were made at Woodward because inadequate moisture inhibited growth.

Hand operated grass shears were used for clipping. A uniform clipping height was achieved by placing a 2.54 cm wooden board along the side of the row of wheat plants to be clipped. This board was used as a guide by sliding the grass shears along the top side.

After clipping, the fresh forage from each plot was placed in a plastic bag and sealed until the sample could be weighed and recorded. The contents were then transferred to paper bags and placed in the drying oven for 96 hours at 62 C. Oven dry weights were obtained immediately upon removal from the drier. All weighing was done on a Mettler P-1210N balance.

The field plot data were converted to kilograms per hectare by the following equation:

$$\text{kg/hectare} = \frac{\text{Plot wt.} \times 43{,}560 \times 2.47}{8 \times 1{,}000}$$

43,560 = square feet per acre

2.47 = number of acres per hectare

8 = square feet within plot actually harvested

1,000 = gm/kg

Time and expense limited the number of samples that could be analyzed for protein and soluble carbohydrates. As a consequence, only the forage samples for the first 2 cuttings of the cultivars Danne, Caprock, Agent, TF/CMN(S), and TF/CMN(R) from the Stillwater field plots were analyzed. The methods used for these analyses were the same as those described for growth chamber experiments.

CHAPTER IV

RESULTS

Leaf Rust Effects on Immature Wheat Plants Grown in the Growth Chamber Experiments

The initial trial showed the non-rusted plants produced 42% more oven dry forage than rusted plants (Table I). Statistical analysis showed an F value of 157.31 which exceeded the 1% level of probability. This work was repeated with similar results (Table I). Figure 1 shows the appearance of the 42 day-old wheat plants before the first clipping.

The amount of new growth after the first cutting of forage was indicated by the results of the second cutting (Table II). Regrowth of the rusted plants was much slower than of the non-rusted plants and after 14 days had produced an average of 68% less regrowth than the non-rusted plants.

The severe reduction in recovery of the inoculated plants prompted a study of the roots. There have been reports that leaf rust disease of wheat can reduce root development (23), and the roots of rusted plants grown in the stone jars in this study appeared to be greatly retarded (Figure 2). Examination of individual rusted plants, indicated a definite reduction in growth (Figure 3). Root development was measured immediately after the second cuttings had been made. Oven dry root weight and root volume measurements are given in Table III. Since the

TABLE I

EFFECT OF LEAF RUST ON WINTER WHEAT FORAGE PRODUCTION,
GROWTH CHAMBER EXPERIMENTS

	Weights (g/jar)[a]			
	Fresh		Oven Dry	
Test	Rusted	Non-Rusted	Rusted	Non-Rusted
1	36.2	71.6	6.1	10.4
2	28.9	77.3	5.5	10.6

LSD, 0.001, for fresh forage = 15.9

LSD, 0.001, for dry forage = 2.4

[a]Means of 5 replications of 100 plants per jar.

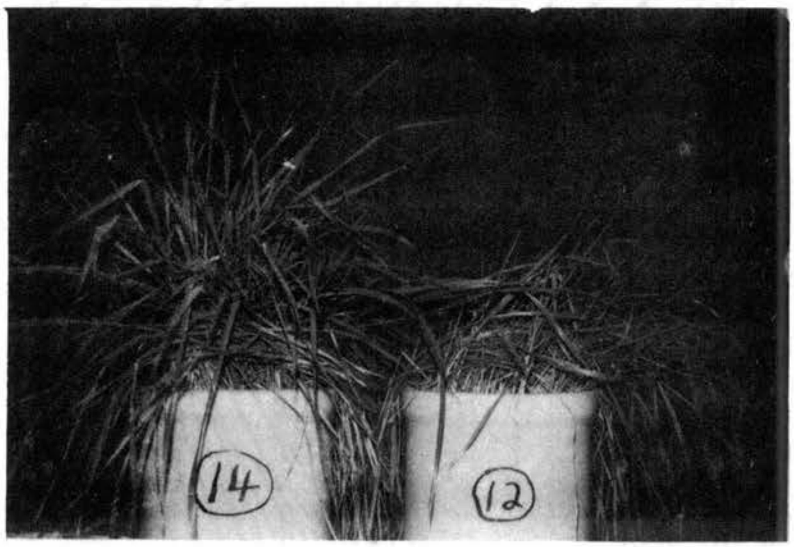

Figure 1. Six Week Old Winter Wheat Plants, Cultivar Triumph 64 Grown in a Controlled Environment. Leaf Rust Free Plants are in the Left Jar. The Plants in the Jar on the Right Had Been Infected With Leaf Rust Since Seven Days After Planting.

TABLE II

WINTER WHEAT REGROWTH 14 DAYS AFTER THE FIRST FOLIAGE CLIPPING, GROWTH CHAMBER EXPERIMENTS

	Weights (g/jar)[a]			
	Fresh		Oven Dry	
Test	Rusted	Non-Rusted	Rusted	Non-Rusted
1	2.5	9.7	0.7	2.1
2	1.4	7.1	0.2	0.8

LSD, 0.001, for fresh forage = 3.7

LSD, 0.01, for dry forage = 0.9

[a]Means of 5 replications of plants remaining of an original 100 plants per jar.

Figure 2. Differences Occurring in Root Development of 56 Day-Old Winter Wheat Plants, Cultivar Triumph 64 Grown in Stone Jars in a Growth Chamber as a Result of Heavy Leaf Rust Infection.

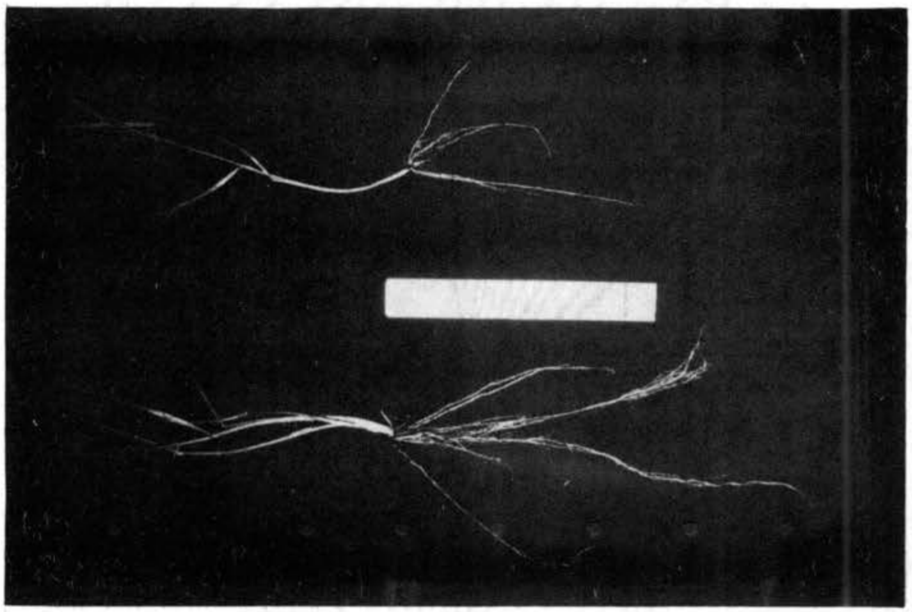

Figure 3. Two Individual 42 Day-Old Cultivar Triumph 64 Wheat Plants. Top Plant was Severely Infected With Leaf Rust. Lower Plant was Disease Free. The White Marker Between the Two Plants is 15 cm Long.

TABLE III

EFFECT OF LEAF RUST ON ROOT GROWTH OF WINTER WHEAT, GROWTH CHAMBER EXPERIMENTS

	Root Production			
	Oven Dry Weights (g/jar)[a]		Volume (ml/jar)[a]	
Test	Rusted	Non-Rusted	Rusted	Non-Rusted
1	2.0	8.7	35	104
2	5.4	10.7	30	70

LSD, 0.001 for dry roots = 4.0

LSD, 0.001 for root volume =34.9

[a]Means of 5 replications of plants remaining of an original 100 plants per jar.

soil was washed away from the roots with water, the actual amount of water still adhering to the roots could not be equated from jar to jar and therefore fresh weights were quite variable and unreliable indications of growth. The differences in root growth for the rusted and the non-rusted plants were highly significant for both oven dry weights and root volume. The roots of the rusted plants were obviously discolored and appeared to be deteriorating.

There was no significant difference in the total amount of water added to each jar during the growing period of the above described trials (Table IV). However, when the amount of water required for each gram of dry matter was determined, it was found that the heavily rusted plants used 33% more water to produce the same amount of dry matter as the healthy plants. These results were similar to those found by other leaf rust workers (23, 43).

A reduction in plant survival following the initial forage clipping of the severely rusted plants was noted. Therefore, a third growth chamber test was conducted using only 10 plants in each jar to avoid the "crowded" condition of the plants in the jars in the earlier experiments where 100 plants were used. This thinly spaced planting reduced the competition between plants and, as a result, tillering occurred. In addition to increased vigor, the non-rusted plants in this trial had an average of two tillers per plant seven days after the first cutting (Figure 4). No tillering occurred on the rusted plants. Reduced tillering has also been reported to be a result of infections with the stripe rust disease of wheat (16).

Two weeks after the first clipping in this thinly spaced test,

TABLE IV

EFFECT OF LEAF RUST ON WATER ECONOMY OF FORAGE PRODUCTION FROM IMMATURE WINTER WHEAT PLANTS, GROWTH CHAMBER EXPERIMENTS

	Weights (g/jar)[a]			
	Total Water Added		Water Required /g Dry Matter	
Test	Rusted	Non-rusted	Rusted	Non-rusted
1	5,036	5,834	830.41	561.07
2	5,255	6,474	955.59	619.30
LSD, 0.01	Not Significantly Different		38.52	

[a]Means of 5 replications of 100 plants per jar.

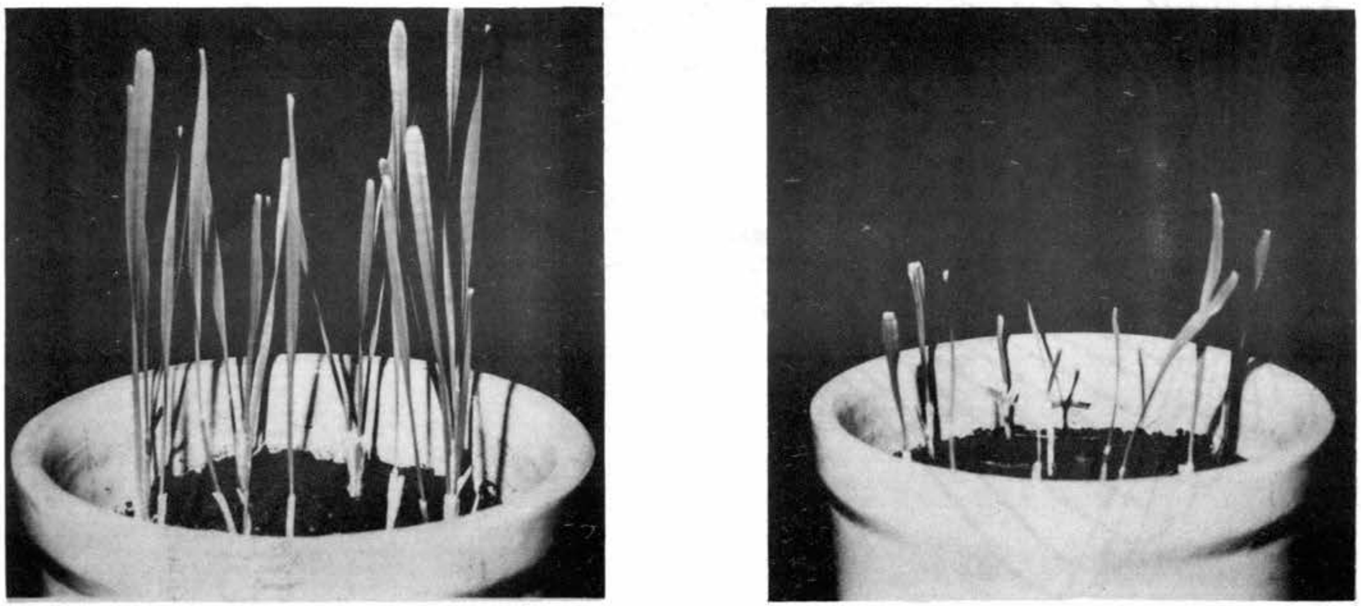

Figure 4. Foliar Regrowth and Tillering Seven Days Following Initial Clippings of Ten Disease Free, 42 Day-Old, Cultivar Triumph 64 Wheat Plants (Left) Compared With Ten Rusted Plants (Right).

only 64% of the previously rusted plants were still alive; whereas, 96% of those plants that had never been infected were still alive.

Protein analysis of oven dry forage samples from the growth chamber tests 1 and 2 indicated an average of 20.70 and 21.48%, respectively, for the rusted plants. In the same two tests, the protein of samples from non-rusted plants averaged 19.93 and 20.55%, respectively. Although a higher percent of protein tended to occur in the rusted forage samples, the differences observed were not significant.

In a growth chamber test designed solely for obtaining plant material for analysis of soluble carbohydrates, differences observed between the leaf rust infected forage and disease free forage were found to be highly significant. Forage from healthy plants produced nearly twice as much soluble carbohydrates as the forage from leaf rust infected plants. Mean values for soluble carbohydrates on a moisture free bases was 12.13% for the non-rusted forage, and 6.45% for the rusted forage, a difference of 5.68%. The LSD for such a difference at the 1% level of probability was 5.52%.

Effects of Leaf Rust on Forage Production in Field Grown Winter Wheat Experiments

Readings of leaf rust severity were recorded just prior to each forage clipping. These readings were based on the modified Cobb scale (11). Statistical analyses of these readings were not made, but obvious differences in severity readings between the resistant and susceptible cultivars are evident (Table V). Table V also contains the readings of both the chemically sprayed and unsprayed plots. Severity readings

TABLE V

PERCENT LEAF RUST SEVERITY IN FIELD PLOT EXPERIMENTS

	Goodwell			Lahoma			Stillwater		
Clipping	1	2	3	1	2	3	1	2	3
Date	9/27	10/27	2/22	10/26	12/13	2/21	10/15	11/19	2/8
Cultivar	Percent Severity[a]								
Agent									
Sprayed	0	0	0	0	0	0	0.25	0	0
Unsprayed	0	0	0	0	0	0	0.25	1.25	0
Caprock									
Sprayed	Not Entered			0	0	0	10	0	0
Unsprayed	Not Entered			0	0.50	0	10	1.0	0
Comanche									
Sprayed	0	0	0	0	0.25	0	15	0	0
Unsprayed	0	1.0	0	0	25	0	20	30	0
Danne									
Sprayed	0	0	0	0	1.5	0	50	0.25	0
Unsprayed	0	1.0	0	0	40	0	30	60	0
Nicoma									
Sprayed	Not Entered			Not Entered			7.5	0	0
Unsprayed	Not Entered			Not Entered			10	10	0
5*Sut/Ag									
Sprayed	Not Entered			Not Entered			0	0	0
Unsprayed	Not Entered			Not Entered			0.50	0	0
TF/5*CMN(R)									
Sprayed	0	0	0	0	0	0	0	0	0
Unsprayed	0	0	0	0	0	0	0	0	0
TF/5*CMN(S)									
Sprayed	0	0	0	0	0	0	13.7	0.50	0
Unsprayed	0	1.0	0	0	30	0	20	60	0

[a]Means of four replications of readings made on the second youngest fully expanded leaf.

for Woodward were not recorded since only a trace of leaf rust was observed and that only before the first clipping.

The earliest and most severe leaf rust infection occurred at Stillwater. This, of course, was due partially to the rust inoculation and the periodic sprinkler irrigations. A naturally occurring late fall infection high enough to attribute some significant evaluation of the effects of leaf rust did occur at Lahoma. The leaf rust infection at Goodwell was too minute for any valid assessment.

At Stillwater, where leaf rust infection developed in the early fall, all cultivars containing a gene or genes conditioning leaf rust resistance were those that produced the highest levels of total oven dry forage when only the unsprayed samples are considered (Table VI). At Lahoma, however, where leaf rust infection did not occur until late fall, the leaf rust susceptible cultivar Danne produced more total oven dry forage than two of the cultivars classified as leaf rust resistant (Table VII). Oven dry forage weights were used for rankings because the fresh weights were influenced by the percentage of moisture in the sample which was quite variable between clippings (Table VIII). Yields for all the leaf rust resistant cultivars were pooled and compared with the pooled yield of all the leaf rust susceptible cultivars. (The sprayed and unsprayed comparisons are considered later.) Tables IX and X contain the data for the field plots at Stillwater and Lahoma, respectively. At Stillwater, where leaf rust infection occurred early in the fall, the greater forage production of the pooled resistant cultivars was significantly higher than the pooled susceptible cultivars at the 1% level of probability. This difference was significant both for the fresh and oven dry weights. At Lahoma, the only clipping when the

TABLE VI

FORAGE PRODUCTION OF LEAF RUST RESISTANT AND SUSCEPTIBLE CULTIVARS OF WINTER WHEAT WHEN LEAF RUST INFECTION OCCURRED EARLY IN THE FALL, STILLWATER, OKLAHOMA 1971-1972

	(Kg/hectare)[a]							
	Clipping I		Clipping 2		Clipping 3		Total	
Cultivar[b]	Fresh	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry
TF/5*CMN(R)	3,354.55	553.43	7,445.11	1,202.35	1,579.94	644.55	12,379.60	2,400.33
Caprock	2,869.71	473.07	4,611.04	806.95	1,770.24	704.06	9,250.99	1,984.08
5*Sut/Ag	2,043.60	350.35	5,448.59	953.54	1,330.79	530.23	8,822.98	1,834.12
Agent	2,256.09	362.12	5,370.58	905.46	1,161.33	486.19	8,788.00	1,753.77
TF/5*CMN(S)	2,579.21	384.64	3,067.70	588.40	709.44	304.95	6,356.35	1,277.99
Danne	1,738.30	300.25	3,047.91	565.20	835.86	349.34	5,622.07	1,214.79
Comanche	657.33	107.26	2,956.79	563.52	923.96	429.03	4,538.08	1,099.81
Nicoma	1,134.09	191.98	2,296.78	434.74	580.67	253.52	4,011.54	880.24
LSD 0.05	1,603.20	266.20	1,975.33	329.92	732.10	278.62	1,178.73	222.99
LSD 0.01							1,556.67	294.48
CV (%)	68	67	34	33	43	40	63	57

[a]Means of 4 replications.

[b]Cultivars arranged in descending order of total production of dry forage.

TABLE VII

FORAGE PRODUCTION OF RESISTANT AND SUSCEPTIBLE CULTIVARS OF WINTER WHEAT WHEN ONLY A LATE FALL LEAF RUST INFECTION OCCURRED, LAHOMA, OKLAHOMA 1971-1972

	(Kg/hectare)[a]							
	Clipping 1		Clipping 2		Clipping 3		Total	
Cultivar[b]	Fresh	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry
Agent	4,027.35	669.09	4,038.44	772.99	1,025.16	291.85	9,090.95	1,733.33
Danne	4,033.06	682.21	3,300.42	738.36	582.34	195.01	7,915.82	1,615.57
Caprock	3,246.96	529.89	3,338.07	824.43	636.14	207.12	7,221.17	1,561.44
TF/5*CMN(R)	3,550.57	556.12	3,322.28	733.65	744.75	236.03	7,617.60	1,525.80
TF/5*CMN(S)	3,562.01	524.18	3,054.64	708.10	639.84	219.89	7,256.49	1,452.16
Comanche	2,760.10	447.18	2,979.99	687.59	693.63	232.00	6,433.72	1,366.77
LSD 0.05	629.08	106.30	529.29	105.89	199.30	78.65	504.27	112.38
LSD 0.01							668.31	148.95
CV (%)	15	15	15	13	21	21	22	18

[a]Means of 4 replications.

[b]Cultivars arranged in descending order of total production of dry forage.

TABLE VIII

MOISTURE PERCENTAGE OF FRESH FORAGE SAMPLES AT THE THREE CLIPPINGS, STILLWATER AND LAHOMA

Cultivar	Clipping 1	Clipping 2	Clipping 3
	STILLWATER		
TF/5*CMN(R)	84%	84%	59%
Caprock	84	83	60
5*Sut/Ag	83	83	60
Agent	84	83	58
TF/5*CMN(S)	85	81	58
Danne	83	82	58
Commanche	83	81	54
Nicoma	83	83	62
	LAHOMA		
Agent	83%	82%	72%
Danne	83	78	66
Caprock	84	75	67
TF/5*CMN(R)	84	78	68
TF/5*CMN(S)	85	77	66
Comanche	83	77	61

TABLE IX

A COMPARISON OF POOLED FORAGE YIELDS OF RESISTANT VERSUS SUSCEPTIBLE CULTIVARS, STILLWATER, OKLAHOMA 1971-1972

(Kg/hectare)[a]

Host Response	Clipping 1		Clipping 2		Clipping 3		Total	
	Fresh	Oven Dry	Fresn	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry
Resistance	2,630.99	434.74	5,718.83	967.07	1,460.57	591.51	9,810.39	1,993.32
Susceptible	1,527.23	246.03	2,842.29	537.96	762.48	334.61	5,132.01	1,178.60
LSD 0.01	994.40	169.17	2,537.32	209.44	532.44	209.96	1,325.01	263.94

[a]Pooled means of the 4 resistant (TF/5*CMN(R), CRC, AG, 5*SUT/AG) and 4 susceptible (TF/5*CMN(S), CMN, DANNE, NICOMA) cultivars in the experiment.

TABLE X

A COMPARISON OF POOLED FORAGE YIELDS OF RESISTANT VERSUS SUSCEPTIBLE CULTIVARS, LAHOMA, OKLAHOMA 1971-1972

(Kg/hectare)[a]

Host Response	Clipping 1		Clipping 2		Clipping 3		Total	
	Fresh	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry
Resistance	3,608.29	585.03	3,566.26	777.02	802.02	245.00	7,976.57	1,606.86
Susceptible	3,451.17	581.19	3,111.68	711.34	638.60	215.00	7,202.01	1,478.17
LSD 0.05	NS	NS	294.96	61.06	NS	NS	NS	NS

[a]Pooled means of the 3 resistant (TF/5*CMN(R), CRC, AG) and 3 susceptible (TF/5*CMN(S), CMN, DANNE) cultivars in the experiment.

pooled leaf rust resistant yields were significantly higher than the pooled susceptible cultivars was at the second cutting, which was at the time of the highest level of leaf rust severity for this test location. Although there was a significantly higher forage production of pooled resistant cultivars over the pooled susceptible cultivars (Table IX) at the time of the first clipping, an even greater difference occurred at the second clipping. Bar graph diagram (Figure 5) illustrates that the greatest increase in forage production of leaf rust resistant cultivars over leaf rust susceptible cultivars at Stillwater was at the time of the second clipping. This was the period when leaf rust severity had developed to the highest level (Table V).

The third clipping was made February 8, 1972, after leaf rust development on actively growing wheat leaves had been stopped due to hard freezes during January. Even so, the resistant cultivars still produced a significantly larger amount of forage (Table IX) than the susceptible cultivars indicating, as in the growth chamber studies, the lasting effects of the rust on plant growth.

At Lahoma, where leaf rust developed much later than at Stillwater, susceptible cultivars produced equal or larger amounts of forage at the first clipping (Figure 6). At the second clipping, however, the resistant cultivars produced forage yields significantly greater than the susceptible cultivars (Table X). Again, this second clipping corresponds with the time when leaf rust severity readings were the highest (Table V).

Only at Stillwater, where leaf rust infections occurred early in the fall, was any significance found between the forage yields of triazole treated and untreated plots (Table XI and Figure 7). In spite

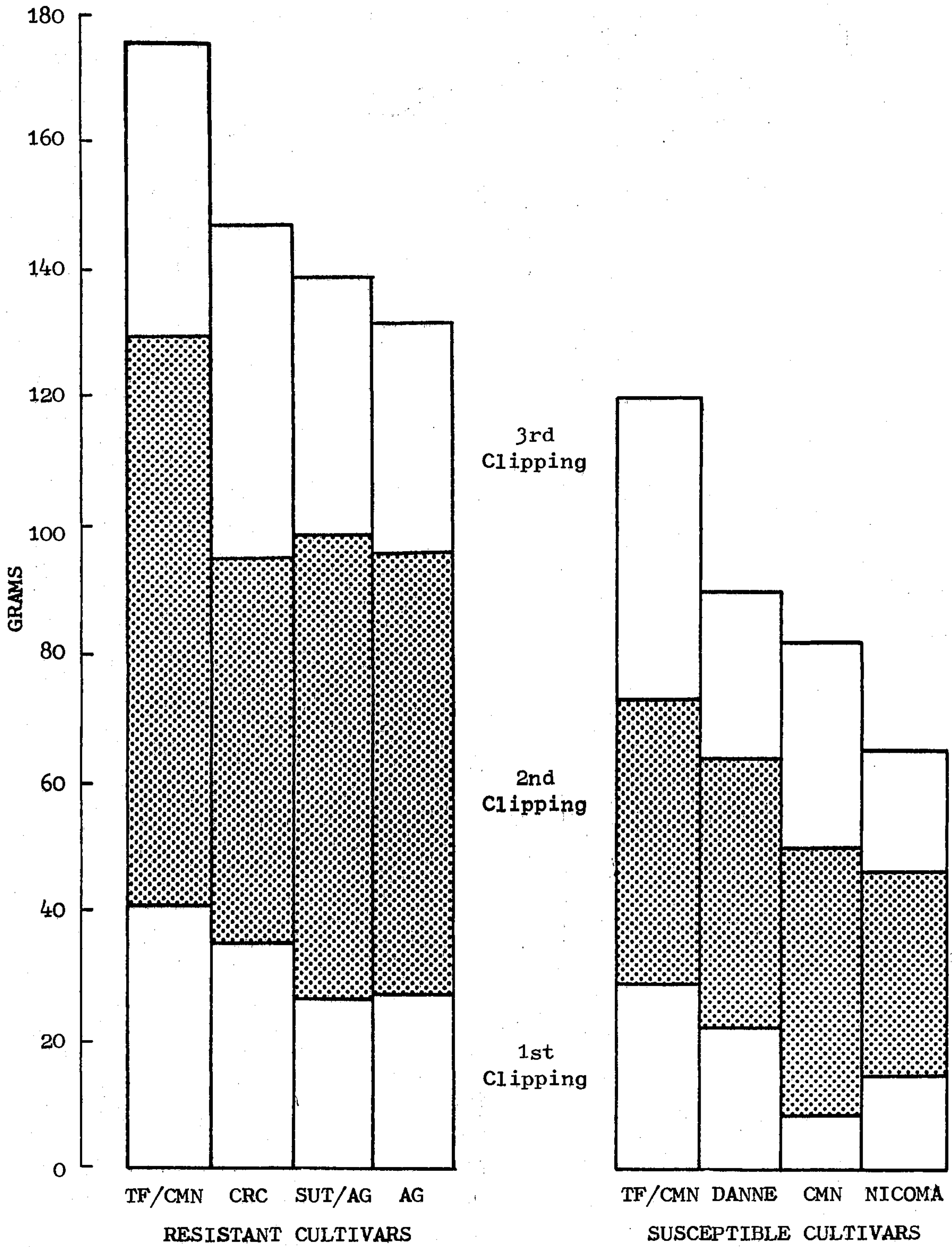

Figure 5. A Comparison of Oven Dry Forage Yields of Leaf Rust Resistant and Susceptible Cultivars for Three Clippings. Stillwater, Oklahoma 1971-1972

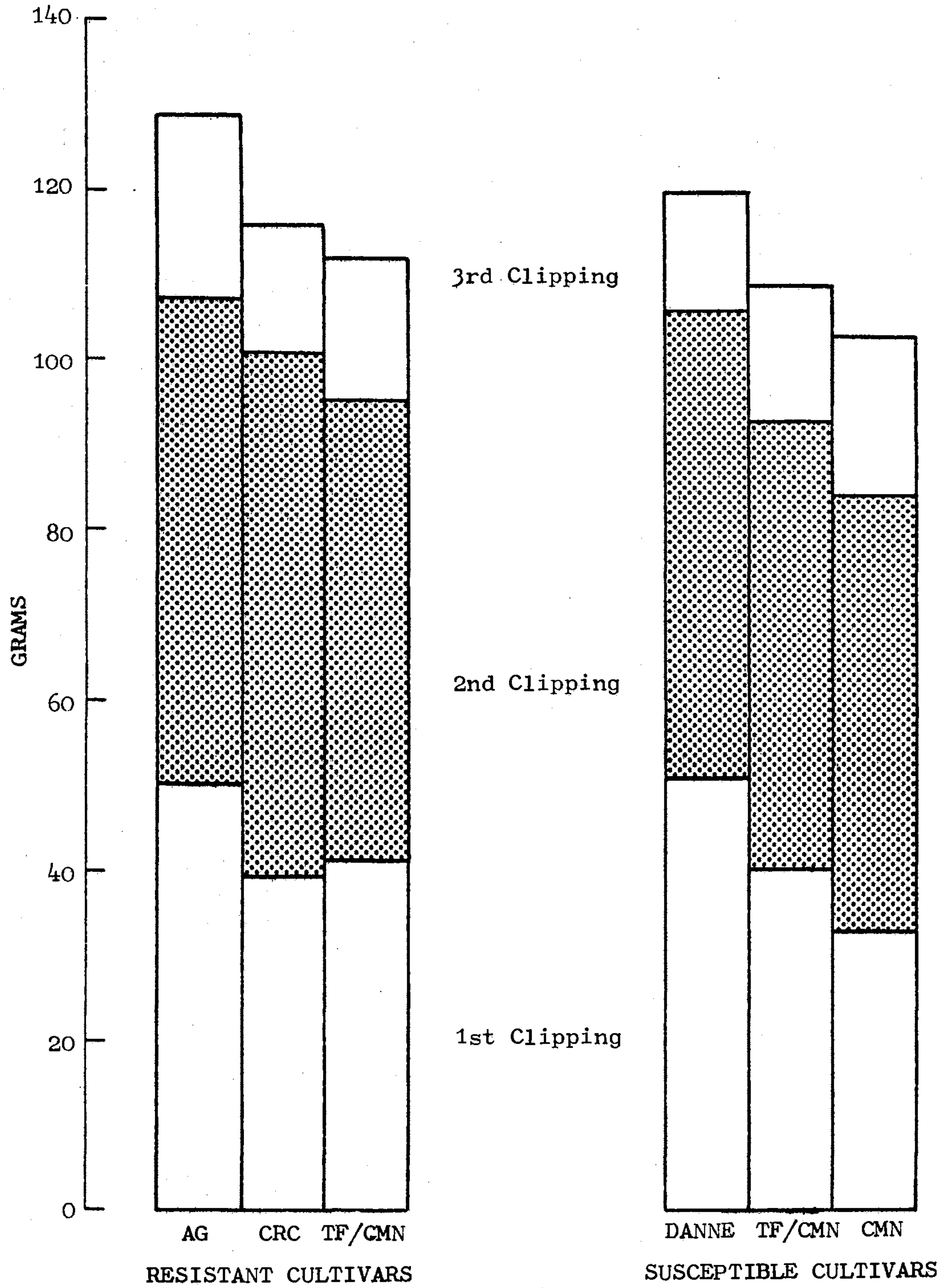

Figure 6. A Comparison of Oven Dry Forage Yields of Leaf Rust Resistant and Susceptible Cultivars for Three Clippings. Lahoma, Oklahoma 1971-1972

TABLE XI

COMPARISON OF WINTER WHEAT FORAGE PRODUCTION OF FOUR SUSCEPTIBLE CULTIVARS SPRAYED WITH TRIAZOLE AND UNSPRAYED, STILLWATER, OKLAHOMA 1971-1972

		(Kg/hectare)[a]							
		Clipping 1		Clipping 2		Clipping 3		Total	
Cultivar[b]	Treat-ment	Fresh	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry
TF/5*CMN(S)	Sprayed	2,734.54	440.79	4,997.70	825.44	1,636.42	615.97	9,368.66	1,882.20
TF/5*CMN(S)	Unsprayed	2,579.21	384.64	3,067.70	588.40	709.44	304.95	6,356.35	1,515.03
Danne	Sprayed	2,037.54	367.83	3,900.92	705.74	1,231.27	532.92	7,169.73	1,606.49
Danne	Unsprayed	1,738.30	300.25	3,047.91	565.20	835.86	349.34	5,622.07	1,214.79
Nicoma	Sprayed	1,426.95	248.47	3,927.82	710.45	1,013.73	423.64	6,368.50	1,382.56
Nicoma	Unsprayed	1,134.09	191.98	2,296.78	434.74	580.67	253.52	4,011.54	880.24
Comanche	Sprayed	734.32	128.77	3,227.12	579.65	1,052.39	420.95	5,013.83	1,129.37
Comanche	Unsprayed	657.33	107.26	2,956.79	563.52	923.96	429.03	4,538.08	1,099.81
LSD 0.05[c]		NS	NS	1,411.80	248.90	347.57	131.63	976.30	188.75
CV (%)		69	68	40	39	35	32	67	60

[a]Means of four replications.

[b]Cultivars arranged in descending order by total production of dry forage of the sprayed treatments.

[c]For comparison of sprayed versus unsprayed plots only. Interactions between cultivars and spray treatments did not form a part of this analysis.

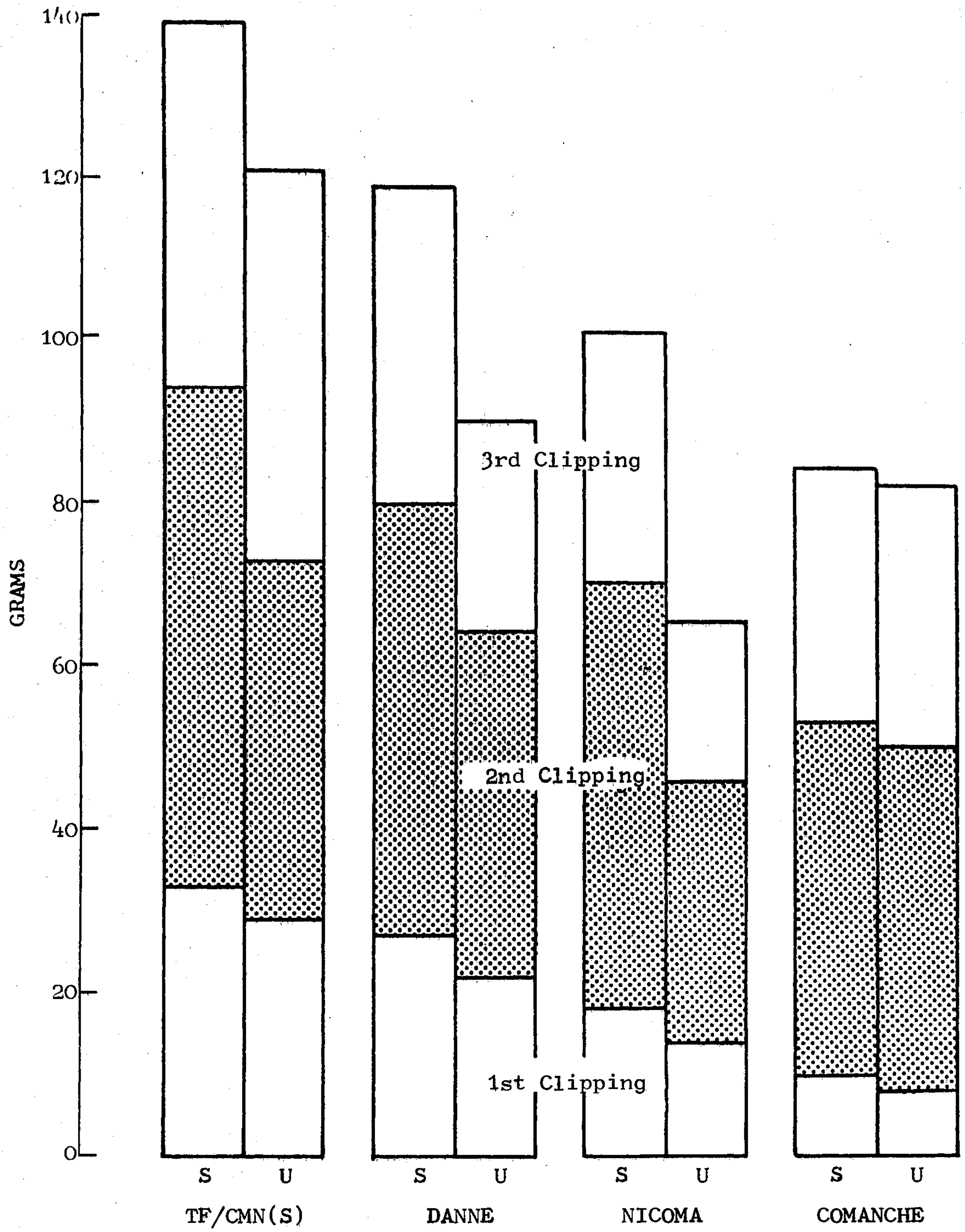

Figure 7. A Comparison of Oven Dry Forage Yields of Triazole Sprayed (S) and Unsprayed (U) Plots of Four Leaf Rust Susceptible Cultivars. Stillwater, Oklahoma 1971-1972

of large differences in forage yields, significance at the 5% level of probability was attained only at the last two clippings. This was undoubtedly a result of a rather large coefficient of variance, the reasons for which will be discussed later in this paper. The positive effect of the chemical control is best seen when the forage yields of the four unsprayed leaf rust susceptible cultivars were pooled and compared with the pooled yields of the same cultivars that were sprayed (Table XII). The problem of the high coefficient of variability is still evident, however. There was very little variation in percent of moisture of the fresh forage between the sprayed and unsprayed treatments (Table XIII).

A more accurate evaluation of the effect of leaf rust on wheat forage production can perhaps be found by a separate analysis of only the resistant and susceptible near isogenic lines. Unfortunately, with just one comparison and with the high coefficient of variance of forage yields occurring among the plots at Stillwater, even large differences in production were not significant at the first and third clippings (Table XIV). However, at the second clipping when leaf rust severity was the highest, and the coefficient of variance was the lowest, significant differences in forage production were found at the 5% level of probability. Total production from the second and third clippings also yielded significant differences (Table XV).

When the triazole sprayed and unsprayed plots of the susceptible near isogenic line were compared nearly the same results were obtained. Differences in forage yield that were statistically significant were found only for fresh weight at the second clipping and for the combined yields of the second and third clippings (Tables XVI and XVII). It was

TABLE XII

A COMPARISON OF POOLED FORAGE YIELDS OF FOUR LEAF RUST SUSCEPTIBLE CULTIVARS SPRAYED WITH TRIAZOLE AND UNSPRAYED, STILLWATER, OKLAHOMA 1971-1972

	(Kg/hectare)[a]							
	Clipping 1		Clipping 2		Clipping 3		Total	
Treatment	Fresh	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry
Sprayed	1,733.34	296.46	4,013.39	705.32	1,233.45	498.37	6,980.18	1,500.15
Unsprayed	1,527.23	246.03	2,842.29	597.22	762.48	334.61	5,132.01	1,177.46
LSD 0.05	NS	NS	815.10	143.70	200.66	76.00	488.15	94.38

[a]Pooled means of the chemically sprayed and unsprayed cultivars listed in Table XI.

TABLE XIII

VARIATION IN PERCENT MOISTURE BETWEEN FRESH FORAGE OF SPRAYED AND UNSPRAYED PLOTS OF FOUR SUSCEPTIBLE CULTIVARS, STILLWATER, OKLAHOMA 1971-1972

Cultivar	Treatment	Clipping 1	Clipping 2	Clipping 3
TF/5*CMN(S)	Sprayed	84%	84%	62%
TF/5*CMN(S)	Unsprayed	85	81	58
Danne	Sprayed	82	82	57
Danne	Unsprayed	83	82	58
Nicoma	Sprayed	83	82	58
Nicoma	Unsprayed	83	83	62
Comanche	Sprayed	83	82	60
Comanche	Unsprayed	84	81	54

TABLE XIV

COMPARISON OF FORAGE PRODUCTION OF LEAF RUST RESISTANT AND SUSCEPTIBLE NEAR ISOGENIC WINTER WHEAT LINES, STILLWATER, OKLAHOMA 1971-1972

Near Isogenic Line	(Kg/hectare)[a] Clipping 1 Fresh	Clipping 1 Oven Dry	Clipping 2 Fresh	Clipping 2 Oven Dry	Clipping 3 Fresh	Clipping 3 Oven Dry
TF/5*CMN(R)	3,354.55	553.43	7,445.11	1,202.35	1,579.94	644.55
TF/5*CMN(S)	2,579.21	384.64	3,067.70	588.40	709.44	304.95
LSD 0.05	NS	NS	3,577.05	528.02	NS	NS
CV (%)	62	63	30	26	41	40

[a]Means of 4 replications.

TABLE XV

COMPARISON OF TOTAL FORAGE PRODUCTION OF LEAF RUST RESISTANT AND SUSCEPTIBLE NEAR ISOGENIC WINTER WHEAT LINES, STILLWATER, OKLAHOMA 1971-1972

	Clippings			
	1, 2, and 3		2 and 3	
	Fresh	Oven Dry	Fresh	Oven Dry
TF/5*CMN(R)	12,379.60	2,400.33	9,025.05	1,846.90
TF/5*CMN(S)	6,356.35	1,515.03	3,777.14	893.35
LSD 0.05	NS	NS	4,428.40	872.18
CV (%)	67	64	44	40

TABLE XVI

COMPARISON OF THE FORAGE YIELDS OF TRIAZOLE SPRAYED AND UNSPRAYED PLOTS OF SUSCEPTIBLE NEAR ISOGENIC WINTER WHEAT LINE, STILLWATER, OKLAHOMA 1971-1972

	(Kg/hectare)[a]					
	Clipping 1		Clipping 2		Clipping 3	
Treatment	Fresh	Oven Dry	Fresh	Oven Dry	Fresh	Oven Dry
Sprayed	2,734.54	440.79	4,997.70	825.44	1,636.42	615.97
Unsprayed	2,579.21	384.64	3,067.70	588.40	709.44	304.95
LSD 0.05	NS	NS	1,786.19	NS	NS	NS
CV (%)	68	64	20	22	41	39

[a]Means of 4 replications.

TABLE XVII

COMPARISON OF TOTAL PRODUCTION OF TRIAZOLE SPRAYED AND UNSPRAYED PLOTS OF THE SUSCEPTIBLE NEAR ISOGENIC WINTER WHEAT LINE, STILLWATER, OKLAHOMA 1971-1972

	Clippings			
	1, 2, and 3		2 and 3	
	Fresh	Oven Dry	Fresh	Oven Dry
Sprayed	9,368.66	1,882.20	6,634.12	1,441.14
Unsprayed	6,356.35	1,515.03	3,777.14	893.35
LSD 0.05	NS	NS	539.92	130.18
CV (%)	45	44	18	20

noted that the differences in forage production between the fungicide treated and untreated plots of the leaf rust susceptible near isogenic lines were not as great as that between the resistant and susceptible near isogenic lines. This may be attributed partly to the fact that at the time of the first clipping fungicidal control of the rust was incomplete (Table V). In turn, this does indicate that the leaf rust fungus did have an effect on the early development of the plants.

Since the near isogenic lines were planted at four locations during the 1971-1972 season, and leaf rust was severe at only one location and essentially nonexistent at two locations, the situation became almost ideal for comparing the two near isogenic lines for forage production with and without leaf rust infection. Figure 8 is a graphic illustration of the comparison of the fresh forage weights for the second clipping at all four locations. It was at the time of this clipping when forage production and leaf rust severity were at their peak.

The only time that a significant difference between the forage production of the resistant and susceptible near isogenic lines occurred was at Stillwater where leaf infection developed to the greatest severity. Forage production for each line at the other locations was nearly the same, which indicates that forage production capacity of these two lines in the absence of leaf rust was essentially the same. It should be pointed out also that fresh forage samples from leaf rust infected plots at Stillwater were 3% lower in moisture than the uninfected samples (Table XVIII). These results from field plots support the earlier findings from growth chamber studies that leaf rust reduces the water economy of wheat plants.

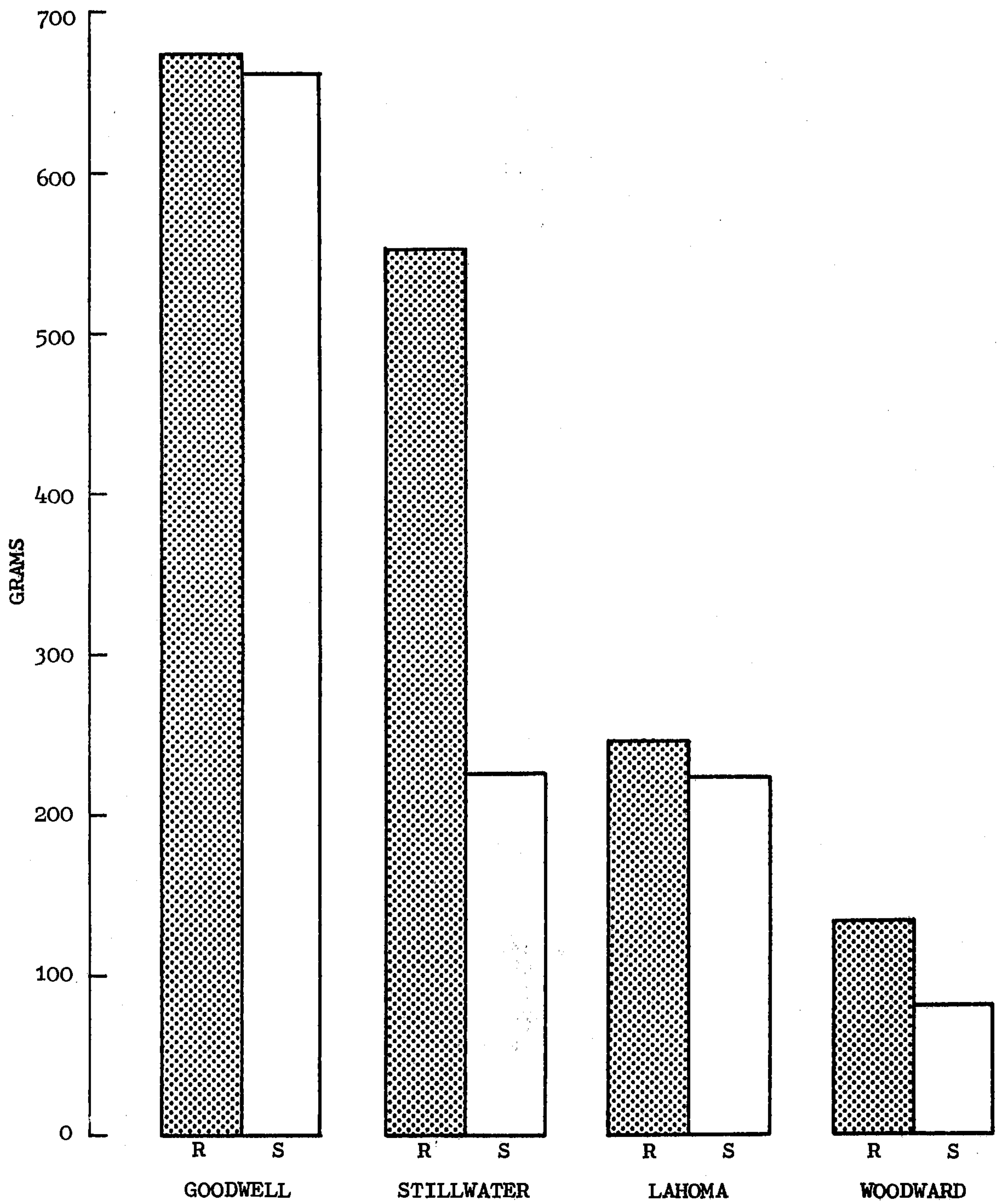

Figure 8. A Comparison of Second Clipping Fresh Forage Yields of Leaf Rust Resistant (R) and Susceptible (S) Near Isogenic Lines at Four Locations in Oklahoma 1971

TABLE XVIII

COMPARISON OF THE PERCENT OF MOISTURE IN FORAGE SAMPLES FROM LEAF RUST RESISTANT AND SUSCEPTIBLE NEAR ISOGENIC LINES IN THE SECOND CLIPPING AT FOUR LOCATIONS, 1971

	Percent of Moisture			
Line	Goodwell	Lahoma	Stillwater	Woodward
TF/5*CMN(R)	84%	78%	84%	60%
TF/5*CMN(S)				
Sprayed	84	77	84	59
Unsprayed	84	77	81	59

The near isogenic lines at the four locations also was ideal for an inquiry into the effect of the triazole fungicide with and without leaf rust infection. It can be readily seen in Figure 9 that no effects were measured from the fungicide treatment when leaf rust infections were either absent or at a low severity. Again, significant differences in forage yields between the fungicide treated and untreated plots of the susceptible near isogenic lines only occurred at Stillwater for the fresh weights of the second clipping.

The variation in forage production between locations could be due to many factors, but certainly the fertilizer and irrigation water applied at Goodwell and Stillwater were significant in this regard.

The percent of protein in the dry forage sample of the five cultivars selected for analysis ranged from 29 to 32% for the first clipping and from 27 to 31% for the second clipping. Differences in percent of protein among the cultivars and between the fungicide treated and untreated plots were not statistically significant.

The percent of soluble carbohydrates ranged from 8 to 12% for the first clipping and from 20 to 30% for the second clipping. Fresh frozen samples were used for this analysis, but the percent of soluble carbohydrates were calculated on an oven dry weight bases. Since analyses were made from a composite of the forage samples from all four replications, no statistical analysis of the data could be made. However, no differences in the levels of soluble carbohydrates could be associated with leaf rust infection.

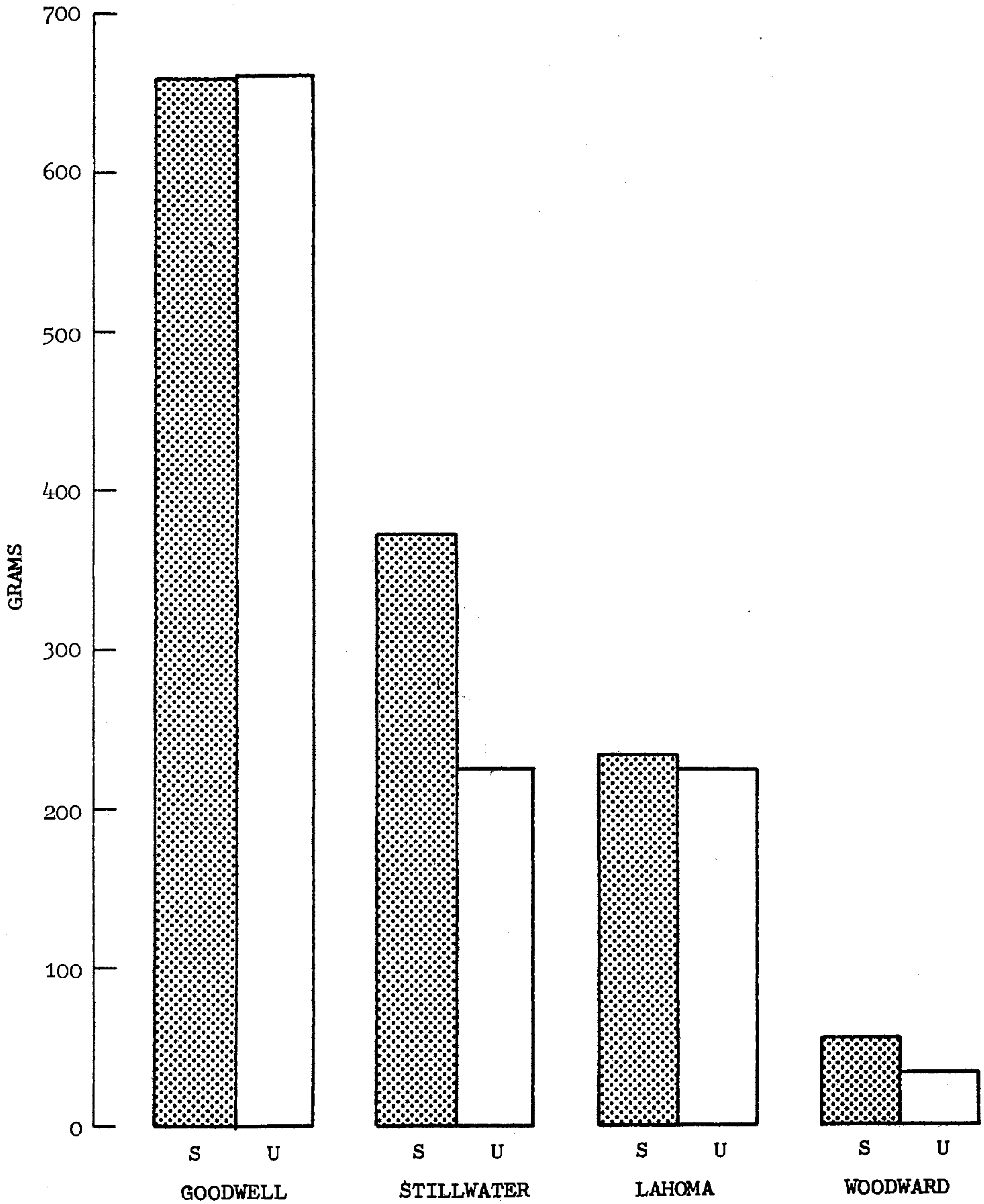

Figure 9. A Comparison of Fresh Forage Yields at the Second Clipping of Triazole Sprayed (S) and Unsprayed (U) Plots of a Susceptible Line of a Near Isogenic Pair at Four Locations in Oklahoma 1971

CHAPTER V

DISCUSSION

There can be no doubt that leaf rust can adversely affect forage production of winter wheat. Experiments in the growth chamber showed that leaf rust reduced forage production by nearly 50 percent. Although the statistical significance was not as striking, the data from field experiments also indicated reductions in forage production due to leaf rust on the order of 50 percent when the disease developed early and to a high level of severity.

Properly planned experiments in growth chambers can be expected to yield low coefficients of variance, and so it was in these experiments when the coefficient of variance was seldom higher than 5%.

Field experiments, however, are very much influenced by the vicissitudes of the weather and other factors which often lead to coefficients of variance in the range of 15 to 25%. It was somewhat surprising, and disheartening, to find coefficients of variance in the field experiments reported here ranging to almost 70%. Yet it was evident that at all locations the stands and early season growth were lacking in uniformity from one row to another, one plot to another, and one replication to another. It would appear that the equipment used for planting grain yield measurements is not adequately precise for measurements made such a short time after planting. Uneven stands from one end of the row to the other at Goodwell, for example, was traced to the fact that the cones of the seed distribution system were not exactly level at the time the seed was dropped into them. Uneven stands and growth at Stillwater and Lahoma seemed to be due to the fact that the seed was not uniformly being covered with moist soil, since some seedlings emerged quickly and others days later or not at all. Such differences may be evened out or overshadowed by other factors by the time grain is harvested 9 or 10 months later, but they are still a major source of variation within a month or two after planting. This is substantiated by the fact that the level of coefficients of variability dropped at the time of the second forage clipping in these studies.

In addition, at Woodward particularly, it was observed that high soil temperatures above normal affected the seed germination of some of the cultivars differently. For example, the cultivar Comanche did not emerge until a rain occurred about 10 days after planting which reduced the soil temperature. The cultivar Danne, however, appeared to be unaffected and emerged almost immediately after planting. With the increased interest in early planting of winter wheat for pasture this variability in germination at high temperatures warrants further investigation. Although many other factors may be involved, these appear to be the main causes of high coefficients of variance for forage production in field plots.

In any study of diseased versus healthy plants or production from them, some method of disease control must be exercised. The less side effects the control measure has, the better. In the growth chamber experiments exclusion of leaf rust inoculum was used for the maintenance of non-rusted plants. Yield differences under these conditions between rusted and non-rusted plants of the same cultivar could be directly compared.

In the field, one method was the use of leaf rust resistant cultivars compared with susceptible cultivars. Comparing the yields of cultivars with different host responses to the leaf rust fungus assumptions were made that in the absence of disease these cultivars as a group are relatively equal in yield. In these experiments, resistant cultivars did yield more than susceptible cultivars in the presence of the disease, and in the absence of disease yields were not greatly different. In some cases, however, as with the cultivar Danne at Lahoma, the susceptible cultivars may yield quite well even when some disease is present. It serves to demonstrate that when leaf rust was not the major limiting factor, some of the well adapted susceptible cultivars were superior in forage production.

The effect of these cultivaral characteristics other than disease response can be at least partially eliminated by the use of near isogenic lines. The existence of near isogenic lines, one leaf rust resistant and one leaf rust susceptible, proved to be valuable for comparing the effects of leaf rust on forage production in these studies. The results obtained confirm that in the absence of the disease these lines were practically equal in yield, but when the disease was severe production of the resistant line was nearly double that of the susceptible line in forage production.

Another method used in the field was spraying with a fungicide. Usually, when fungicides are used in studies of this nature the side effects such as the control of other diseases or insects, the supplying of a minor element, or phytotoxicity can confound the results. The chemical used in these studies appears to be unique in that it controls only the leaf rust pathogen, Puccinia recondita f. sp. tritici (42), and does not interfere with normal plant metabolic activity. Again it was found that where the disease was not present the yields of sprayed and unsprayed plots of susceptible cultivars were essentially equal. At Stillwater, however, where the disease did develop, the sprayed plots of susceptible cultivars yielded more forage than the unsprayed plots. Inoculum was present at Stillwater at the time of wheat plant emergence in the test plots and the triazole fungicide was not applied until October 2, which was when the third leaf of the wheat plants was fully developed. Although this is the growth stage recommended by the Rohm and Haas Company for application, in these particular plots, this stage was too late to accomplish much disease control for the growing period which produced the forage of the first clipping. By the time of the second clipping, however, the fungicide treatments were providing nearly complete control and forage yields of the sprayed plots were up.

It can readily be observed that the second clipping was the time of highest fall and winter forage production. Also, at Lahoma and Stillwater, this was the time when the leaf rust fungus was most active. Second clipping forage production from resistant cultivars, resistant near isogene, and from fungicide sprayed plots definitely shows that when leaf rust was present, forage production was reduced if the disease was not controlled.

The fall and winter season of 1971-1972 was generally mild. However, on January 3, 1972, the night time temperature dropped to a -18 C. During the remainder of January most of the night time temperatures, especially during the time of the day when moisture might be present for spore germination, were too low for infection, growth, and development of leaf rust within the field plots. Leaf rust was almost totally inactive at the time of the third clipping for all locations. Nevertheless, there was ample evidence that forage yields at the third clipping were still being influenced by the disease that had been present earlier. An example can be seen in the third clipping at Stillwater where leaf rust was controlled with the fungicide. The third clipping production for the unsprayed cultivars Danne and Nicoma was only half of the amount produced by these same cultivars in plots that had been sprayed. Since at the time of the third clipping, leaf rust was almost gone, it would appear that this reduction in forage production was the result of reduced plant recovery following the second clipping.

Regrowth in the growth chamber tests was shown to be extensively retarded when the wheat plants had previously been infected with leaf rust. This fact was also associated with extensive reduction in root growth and would certainly appear to at least partially explain the residual damage to the third forage clipping production in the field.

An interesting observation concerned the cultivars Nicoma and Comanche. Leaf rust severity readings on Nicoma never exceeded 10%. Yet when leaf rust on this cultivar was controlled by the fungicide, forage production was significantly increased. On the other hand, forage production for the cultivar Comanche remained unaffected by the fungicide treatment even though leaf rust severity readings for the unsprayed plot were as high as 30%. Such comparisons indicate that differences in forage yield may well occur among cultivars that exhibit a compatible response between the wheat plant and the leaf rust fungus.

Reduction of plant survival of previously rusted plants following clipping in the growth chamber tests reached serious proportions. None of the described soils were sterilized for these tests. When the crowns of some dead as well as healthy plants were examined, conidia of several soil borne fungi were found. Some of these fungi may have contributed to the demise of some of the plants, however, equal amounts of these fungi were found on the rusted and non-rusted plants. Supporting evidence is provided by Fenster, et al. (15) who have indicated that leaf rust weakens growing wheat, making it more vulnerable to attacks from soil pathogens.

The increased water requirement of leaf rust infected wheat has been known for a long time (23, 43). However, none of these studies have been directed toward the effect of leaf rust on water usage as it relates to forage production. Water requirements for leaf rust infected plants were definitely increased when actual production of dry matter was considered. This was supported by certain field data in this study that shows a lower percentage of moisture in rusted fresh forage samples. A lower percent of moisture in the rusted samples would indicate that water was being lost somehow as a result of the disease.

In the growth chamber tests there was a trend toward slightly higher protein in the samples from rust infected plants. The differences were not statistically different, however. Protein analysis of the field forage samples varied from 27 to 32%, but these differences were not associated with rust infection and were not significant statistically.

The soluble carbohydrates analyses of wheat forage could only be considered preliminary tests. For the method of analysis used, the percentage of soluble carbohydrates did not appear to be affected by the level of leaf rust severity established in the field. Analysis of one test grown in the growth chamber did show soluble carbohydrates to be considerably reduced in rusted forage samples. Unfortunately these studies on the effect of leaf rust on the nutrient level of wheat forage were incomplete and unconclusive. A need for more detailed research in this area is certainly indicated.

CHAPTER VI

SUMMARY

1. Severe infection of leaf rust reduced the oven dry weight production of forage from winter wheat as much as 47% during a six weeks growth chamber study.
2. A field comparison of leaf rust resistant versus susceptible cultivars showed oven dry forage production for resistant cultivars to be 41% greater when early fall rust severities were as high as 60% on the susceptible cultivars.
3. Rust control with an experimental fungicide, triazole, increased oven dry forage yields by 22%.
4. A field trial using a pair of near isogenic lines, which expressed either leaf rust resistance or susceptibility showed oven dry forage of the susceptible line was reduced by 48% when leaf rust severities on the susceptible line were 60% in mid-November.
5. Growth chamber studies indicated that a leaf rust severity of 100% reduced the growth of wheat plant roots by 50% within six weeks after planting.
6. Growth chamber experiments showed that regrowth after clipping was greatly retarded if the wheat had been previously infected with leaf rust.
7. Survival of 42 day-old wheat seedlings was 32% less in growth chamber experiments after early severe infections of leaf

rust. Tillering also appeared to be reduced by leaf rust infection.

8. Measurements of water requirements in growth chamber experiments showed 33% more water was needed per unit of oven dry forage for rusted plants than non-rusted plants.

9. No significant differences in protein content were found between rusted and non-rusted wheat forage.

10. A limited study indicated that the percent of soluble carbohydrates of wheat forage may be reduced by severe leaf rust infections.

LITERATURE CITED

1. Allard, R. W. 1966. Principles of plant breeding. John Wiley and Sons, Inc. New York. p. 468.

2. Association of Official Agricultural Chemists. 1960. Official methods of analysis. 9th ed. Washington, D. C. 832 p.

3. Atkins, I. M., and P. C. Mangelsdorf. 1942. The isolation of isogenic lines as a means of measuring the effects of awns and other characters in small grains. Jour. Amer. Soc. Agron. 34:667-668.

4. Atkins, I. M., K. B. Porter, and K. A. Lahr. 1969. Registration of Caprock wheat. Crop. Sci. 9:852.

5. Bailey, D. L., and F. J. Greaney. 1926. Preliminary experiments on the control of leaf and stem rusts of wheat by sulfur dust. Phytopathology 16:64 (Abstr.).

6. Briggle, L. W., J. W. Schmidt, E. G. Heyne, and H. C. Young, Jr. 1960. Rules for abbreviating wheat variety names. Agron. J. 52:613.

7. Browder, L. E. 1973. Probable genotype of some *Triticum aestivum* 'Agent' derivatives for reaction to *Puccinia recondita* f. sp. *tritici*. Crop Sci. 13:203-206.

8. Caldwell, R. M., H. R. Kraybill, J. T. Sullivan, and L. E. Compton. 1934. Effect of leaf rust (*Puccinia triticina*) on yield, physical characters, and composition of winter wheats. J. Agr. Res. 48:1049-1071.

9. Carleton, M. A. 1898. Cereal rusts of the United States. U.S. Dept. of Agr. Div. Veg. Phys. and Path. p. 73.

10. Chester, K. S. 1946. The cereal rusts. Chronica Botanica, Waltham, Mass. p. 31.

11. ________. 1947. Nature and prevention of plant diseases. Second edition. McGraw and Hill Book Co., Inc. p. 23.

12. Denman, C. E., and J. Arnold. 1970. Seasonal forage production for small grains species in Oklahoma. Okla. Agr. Exp. Sta. Bull. B-680, Stillwater, Okla. p. 21.

13. D'Oliveria, B. 1939. Can rusts fix nitrogen? Nature 144:480.

14. Dubois, M., K. A. Giles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

15. Fenster, C. R., M. G. Boosalis, and J. L. Weihing. 1972. Date of planting studies of winter wheat and winter barley in relation to root and crown rot, grain yields, and quality. Nebr. Agr. Exp. Sta. Res. Bull. No. 250, Univ. of Neb., Lincoln, Neb. p. 11.

16. Hendrix, J. W., and E. Fuchs. 1970. Influence of fall stripe rust infection on tillering and yield of wheat. Plant Disease Reptr. 54:347-349.

17. ________, and N. Martin. 1972. The interaction of rust and wheat roots in mist culture. Proceedings of the European and Mediterranean Cereal Rusts Conference. Praha, Czechoslovakia. pp. 137-140.

18. Johnson, R. R., T. L. Balwani, L. J. Johnson, K. E. McClure, and B. A. Dehority. 1966. Corn plant maturity. II. Effect on in vitro cellulose digestibility and soluble carbohydrate content. J. Anim. Sci. 25:617-623.

19. Johnson, L. B., B. L. Brannaman, and F. P. Zscheile, Jr. 1968. Protein and enzyme changes in wheat leaves following infection with Puccinia recondita. Phytopathology 58:578-583.

20. ________, J. F. Schafer, and A. C. Leopold. 1966. Nutrient mobilization in leaves by Puccinia recondita. Phytopathology 56:799-803.

21. Johnson, T., G. J. Green, and D. J. Samborski. 1967. The world situation of the cereal rusts. Ann. Rev. Phytopathol. 5:163-182.

22. Johnson, V. A., J. W. Schmidt, A. F. Drier, and P. J. Mattern. 1965. Registration of Scout wheat. Crop Sci. 5:485-486.

23. Johnston, C. O., and E. C. Miller. 1934. Relation of leaf rust infection to yield growth and water economy of two varieties of wheat. J. Agr. Res. 49:955-981.

24. Le Grand, F. E., and W. E. McMurphy. 1972. Forage production from small grain. Current Report, Okla. State Univ. Ext. Ser. Stillwater, Oklahoma, 4 p.

25. Mains, E. B. 1930. Effect of leaf rust (Puccinia triticina Erikss.) on yield of wheat. J. Agr. Res. 40:417-446.

26. McMurphy, W. E., and B. B. Tucker. 1970. Pasture research. Okla. Agr. Exp. Sta. Prog. Rep. P-637. pp. 25-26.

27. Melchers, L. E. 1917. Puccinia triticina Erikss. Leaf-rust of winter wheat causes damage in Kansas. Phytopathology 7:224.

28. Oklahoma Crop and Livestock Reporting Service. Dec. 1971. Wheat pasture. U.S.D.A. Stat. Rept. Ser. Oklahoma City, Okla. pp. 1-2.

29. Oklahoma Certified Seed News. Aug. 1972. Small grains crops performance. Okla. Crop Imp. Assoc. Stillwater, Okla. pp. 1-3.

30. Purdy, L. H., W. Q. Loegering, C. F. Konzak, C. J. Peterson, and R. E. Allan. 1968. A proposed standard method for illustrating pedigrees of small grain varieties. Crop Sci. 8:405-406.

31. Raymundo, S. A. 1972. Affects of media and media amendments on the in vitro culture of Puccinia recondita f. sp. tritici. Ph.D. Thesis, Okla. State Univ. Stillwater, Okla. p. 7.

32. Reitz, L. P., and C. O. Johnston. 1954. Varieties of hard red winter wheat in the United States. Circular No. 938, U.S.D.A. pp. 7-8.

33. Sarojak, D. J. 1971. The effect of three systemic chemicals on reaction type in winter wheat infected with Puccinia recondita Rob ex. Desm. f. sp. tritici. Master's Thesis. Okla. State Univ. Stillwater, Okla. pp. 7-12.

34. Sears, E. R. 1956. The transfer of leaf rust resistance from Aegilops umbellulata to wheat. Brookhaven Symp. in Biol. 9:1-22.

35. Shaner, G., J. J. Roberts, and R. E. Finney. 1972. A culture of Puccinia recondita virulent to the wheat cultivar Transfer. Plant Disease Reptr. 56:827-830.

36. Shaw, M. 1961. The physiology of host-parasite relations. IX Further observations on the accumulation of radioactive substances at rust infections. Can. J. Bot. 39:1393-1407.

37. Shipley, J., and C. Regier. 1972. Optimum forage production and the economic alternatives associated with grazing irrigated wheat. Texas A&M Univ. Bull. MP-1068. College Station, Texas.

38. Smith, E. L., A. M. Schlehuber, H. C. Young, Jr., and L. H. Edwards. 1968. Registration of Agent wheat. Crop Sci. 8:511-512.

39. ________, L. H. Edwards, H. Pass, D. C. Abbott, and H. C. Young, Jr. 1971. Registration of Danne wheat. Crop Sci. 11:139.

40. ________, L. H. Edwards, H. Pass, D. C. Abbott, H. C. Young, Jr. 1973. Registration of Nicoma wheat. Crop Sci. Vol 13. In print.

41. Van der Plank, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press. New York. pp. 275-284.

42. von Meyer, W. C., S. A. Greenfield, and M. C. Seidel. 1970. Wheat leaf rust: control by 4-n-Butyl-1,2,4-trizole, a systemic fungicide. Science. 169:997-998.

43. Weiss, F. 1924. The effect of rust infection upon the water requirement of wheat. J. Agr. Res. 27:107-118.

44. Young, H. C., Jr. 1970. Variation in virulence and its relation to the use of specific resistance for the control of wheat leaf rust. Plant Disease Problems. Indian Phytopathological Soc. Indian Agr. Research Institute, New Delhi. pp. 3-8.

45. ________. 1972. Unpublished leaf rust survey studies on file at the Okla. Agr. Exp. Sta.

46. ________. 1973. Personal Communication.

2

VITA

Ervin Williams, Jr.

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF PUCCINIA RECONDITA F. SP. TRITICI ON CERTAIN NUTRITIVE VALUES AND FORAGE YIELDS OF WINTER WHEAT

Major Field: Botany

Biographical:

Personal Data: Born in Longford, Kansas, March 22, 1926, the son of Ervin, Sr., and Cecil E. Williams.

Education: Attended Walker Rural Elementary School; graduated from Clay County Community High School, Clay Center, Kansas, 1944; received the Bachelor of Science degree from Kansas State University with a major in Agriculture, May, 1951; received the Master of Science degree from Kansas State University with a major in Botany and Plant Pathology, June, 1960; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in July, 1973.

Professional Experience: Farmed 1953-55; Plant Pathology Technician U.S.D.A., 1955-60; Assistant Professor, Department of Agricultural Services, New Mexico State University, 1960-69; Graduate Research Assistant, Department of Botany and Plant Pathology, Oklahoma State University, 1969-70; Instructor, Department of Botany and Plant Pathology, Oklahoma State University, 1970-71; Graduate Research Assistant, Department of Botany and Plant Pathology, Oklahoma State University, 1971-73.

Professional Organizations: American Phytopathological Society, Oklahoma Academy of Science, and Sigma Xi.