THE EFFECTS OF INFECTION BY BOTH *PUCCINIA RECONDITA* F. SP. *TRITICI* AND *HELMINTHOSPORIUM SATIVUM* ON WHEAT

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CHAPTER I

INTRODUCTION

Puccinia recondita Rob. ex. Desm. f. sp. tritici Eriks. is the causal agent of leaf rust disease of wheat, Triticum sp. L. The name leaf rust is appropriate because of the common occurrence of the disease on wheat leaves, although it also occurs on leaf sheaths, and occasionally on the culms just below the heads. The disease appears as orange pustules in the urediospore stage and black pustules in the teliospore stage.

Leaf rust has been recognized for many years as a destructive disease of wheat in the central plains area of the United States. Johnston and Miller (28) reported that leaf rust could reduce the average grain yield of susceptible varieties from 42 to 90 percent. Salmon and Laude (41) reported that leaf rust, combined with stem rust, Septoria disease, heat and other detrimental influences, all share in the responsibility for losses in grain production.

Helminthosporium sativum Pam. King & Bak. was discovered by Pammel, King and Bakke (36) as the causal agent of a root rot disease of wheat. This fungus is also known to cause black point, spot blotch, leaf spot, seedling blight, kernel smudge, black joint, spike blight, and seed blight

depending on the location of the infection and disease development on or in the various parts of wheat, barley, or rye plants (1, 22, 31, 52). The distinguishing symptom which occurs in underground parts is a light-brown to black discoloration of the crown and roots.

The disease may kill plants at any time from germination until maturity. They may or may not be stunted. Symptoms usually are not apparent on adult plants unless their bases are examined (15).

In this study, leaf rust and root rot diseases were combined in order to determine whether a possible interaction occurred which could affect yield or plant part development. This study included plants in both seedling and adult stages.

CHAPTER II

LITERATURE REVIEW

Wheat leaf rust occurs wherever wheat is grown; it is the most common and widely distributed of all cereal rusts. It appears equally well adapted in the coldest (Canada and Siberia) and warmest (the Congo, Ethiopia, and India) climates in which wheat is grown (10).

Chester (10) estimated that the yield loss due to leaf rust for the United States in 1938 approximated 100,000,000 bushels and that production in Oklahoma was reduced about 34 percent. Johston and Miller (28) reported that leaf rust could reduce the average grain yield of susceptible varieties from 42 to 90 percent. Caldwell et al. (7) found that lower yields were due primarily to fewer kernels per head, and to a loss of kernel weight. Mains (32) showed that reduction in kernel size was a major factor in the yield reduction caused by leaf rust. He also reported that the yield loss of the cultivar named "Fulcaster" was dependent upon the duration of heavy infection as follows: from tillering to maturity, 97.4 percent; from shooting to maturity, 91.3 percent; from heads just showing in the boot to maturity, 54.3 percent; and from blossoming to maturity, 24.7 percent. Burleigh et al. (5) indicated that rust severity

at early dough accounted for 64 percent of the variation in crop loss.

Leaf rust not only reduces yields of wheat crops but also reduces grain quality. Caldwell (6) reported that the percentage of protein in grain of susceptible varieties of both hard and soft winter wheat was very significantly reduced by severe leaf-rust infection. Also, sucrose of the mature grain, although a minor constituent, was consistently reduced, but the percentage of phosphorus and total ash of the grain were not appreciably affected.

Johnson and Cunningham (26) studied peroxidase activity in primary leaves of healthy and inoculated near-isogenic lines differing in susceptibility to isolate UN1-68B of P. recondita. Peroxidase activity was similar in healthy leaves of both lines, and increased with leaf age. In inoculated Thatcher, which develops a high infection type, "4", peroxidase activities at 2-9 days were 20-48 percent higher than in healthy tissue. In a line, Lr 10 (TC), which developed a low infection type, "X," in this experiment, peroxidase had increased up to 109 percent over the healthy leaf after nine days. Johnson et al. (27) also studied the changes in nucleotide composition of RNA in infected wheat leaves. They showed that in primary leaves of M-1 wheat, six days after inoculation, the total nucleotide of salt-extractable RNA averaged 43 percent higher than in healthy leaves. As infection progressed, total RNA nucleotide content dropped below that in healthy leaves. Individual RNA

nucleotide responded differently to rust development.

Young and Prescott (51) studied race populations of P. recondita collected from a susceptible cultivars at 20 sites in Oklahoma. From 647 isolates, eight races (based on Unified Numeration (UN) differential cultivars), or 17 races (based on North American Wheat Leaf Rust Research Workers Committee (NA65) differential cultivars) were identified. Telial samples from the same uredial population were used to produce aecia on Thalictrum speciosissimum or T. dasycarpum. Identification of races from subsequent uredia produced 24 races based on the UN differentials, and 25 races based on the NA 65 differentials. Thus, in the original uredial population in the field, most of the genes conditioning avirulence must have been heterozygous since the uredia which developed from aeciospores either were F1's or were the result of selfing.

Statler and Watkins (42) evaluated virulence of 120 collections on near-isogenic differential lines (LR lines) and the cultivar Agent. Twenty-three virulence combinations were found. Most isolates were virulent on lines having genes Lr3 and Lr10. Genes Lr1, Lr2A, Lr2D, Lr3, Lr10 and Lr6 were ineffective against fewer of the isolates collected in 1972 than of those collected in 1971. The line having Lr18 and Agent (Lr24) were susceptible to more of the isolates collected in 1972 than in 1971. Statler and Nolte (43) later evaluated virulence of 45 collections and determined that near-isogenic lines having Lr2A and Lr2D were

susceptible to a higher percentage of isolates in 1975 and 1976 than in 1973 or 1974. Isogenic line having Lr16, Lr17, and Lr18 were susceptible to a lower percentage of isolates in 1975 and 1976 than in 1973 or 1974.

Boskovic et al. (3) compared pathogenicity of P. recondita on nine near-isogenic lines of T. aestivum by assaying samples from 24 European and Mediterranean countries and 32 states of the U.S. in 1972. These lines carried Lr1, Lr2A, Lr2D, Lr3A, Lr10, Lr16, Lr17, Lr18, or Lr3B. Virulence frequencies to all the lines were very high in the European and Mediterranean samples, whereas virulence frequencies were high on only two of the lines in samples from the U.S. and Canada. Sixty-three percent of the 545 isolates in the European-Mediterranean sample had combined virulence to all eight of the lines, but none of the isolates from the U.S. or Canada had virulence to more than seven of the lines.

Wiese and Ravenscroft (48) studied environmental effects on inoculum quality of rust urediospores. They took newly harvested, mature, and dormant urediospores; subjected them to specific light, temperature, and relative humidity (RH) treatments for 24 and 48 hours, then tested for germinability and infectivity. They found germination and infectivity were reduced or eliminated by increased temperature, especially above 25°C and both germinability and especially infectivity were further reduced by exposure to 100 percent RH. Supplemental light reduced or eliminated the detrimental effect of high RH, but otherwise caused no measureable

effect. Reductions in the infections per leaf and in infection efficiency always were preceded by losses in germinability. Sood (41) also exposed spores of P. recondita to different environmental treatments for periods up to 48 hours and then tested them for germinability. Germination was observed under standard conditions in which non-treated spores routinely germinated at levels greater than 84 percent. Light, temperature, RH and the duration of those parameters all influenced germination levels, but had only minor effects on germination rate. Germination was markedly reduced or did not occur after spores were exposed to temperatures above 25°C especially in dark, water-saturated atmospheres.

Eyal and Peterson (19) reported that light intensities of 1600, 2600, and 3600 ft-c at 24°C affected total spore production but not sporulation rate or pustule maturation time. Chang and Calpouzos (9) reported germination of urediospores of P. recondita inhabited by blue, red and far red light. Inhibition of 97 percent or greater occurred at wavelengths of 400, 419, 651, 710, 720 and 750 nm. Moderate inhibition occurred at 390, 425, and 603 nm. Little or no inhibition occurred at 452, 493, and 552 nm. A response to far-red radiation is unusual in fungi. Eversmeyer and Burleigh (18) found that urediospores of P. recondita remained viable but ungerminated on dry (that is without surface moisture) wheat foilage for 45 days at 5-8°C, nine days at 18-39°C and 12 days in a variable temperature regime of 10-24-27°C. He believed uredospore longevity on wheat forage

plays a significant role in initiating leaf rust epiphytotics, since urediospore deposition and conditions that permit infection do not always coincide.

Takahashi et al. (41) reported that the majority of the wheat leaf rust isolates readily formed teliospores on the first leaf of young wheat seedlings. Teliospores were normally formed 18-24 days after inoculation of some varieties. The degree of teliospore formation was not related to the physiologic race of the fungus, but did vary with different leaf rust isolates and wheat varieties. Jackson and Young (24) reported that teliospores were occasionally found in abundance in highly susceptible varieties. Teliospore formation on leaves of seedling plants appeared to be correlated with senesence as telia are present on leaves of adult maturing plants. However, moisture stress and other factors, such as high levels of infection, which were detrimental to plant growth appeared to limit teliospore formation. These factors apparently brought about more rapid deterioration of the leaf and thereby reduced the length of time available for teliospore formation.

Raju et al. (39) studied the combined effect of two viral diseases and leaf rust on wheat. He reported that leaf rust was more severe in field grown wheat plants also infected with wheat streak mosaic virus (WSMV). Greenhouse experiments with six of seven cultivars or lines of wheat confirmed this. The presence of wheat streak mosaic changed the rust infection type of the wheat lines 7*Wichita/Malakof,

6*Wichita/Loros, and 6*Wichita/Webster, which normally are resistant to race 15. The susceptibility cultivar Bison, Wichita and Triumph (susceptible to races 9 and 15) showed more profuse rust development in the presence of streak mosaic. Combined streak mosaic and rust infection in greenhouse experiments reduced dry weight of plants as compared with dry weights of plants infected with each disease alone. Virus infection significantly changed rust infection type, and, when established in the host for ten days, significantly increased rust severity. Combined streak mosaic and rust infection of field grown plants significantly reduced yield when compared with those infected only with rust, but not when compared with those infected only with the virus.

James and Shih (25) in 1973, studied relationships between incidence and severity of powdery mildew and leaf rust on winter wheat. They recorded the disease incidence and severity from powdery mildew (Erysiphe graminis f. sp. tritici) and leaf rust (P. recondita f. sp. tritici) on winter wheat during three surveys in Ontario in 1960 and 1970. An exponential equation was used to describe the relationship between the incidence (percentage of leaves infected) and severity (percentage of leaf area affected) for the two diseases on particular leaves. A linear regression was found to be adequate to estimate severity for incidence values of 65 percent or below. The relationship between incidence and severity for the two diseases was consistent over a large geographical area, but differed for the two years.

Nelson et al. (35) worked on yield reduction caused by S. nodorum and other pathogens. He evaluated disease severity on various yield components. A septoria disease index (SDI) was derived from these data. The SDI correlated with yield, 1,000 kernel weight, test weight and plant height. Multiple regression equations were utilized to predict yield from disease severity caused by S. nodorum, E. graminis f. sp. tritici, P. recondita and several agronomic factors. When 1,000 kernel weight was included in an equation of SDI and E. graminis, 81 percent of the yield variation could be explained.

Root rot of cereals were prevalent in the United States many years ago, but they did not attract attention until about 1900. H. L. Bolly in North Dakota emphasized their importance. He proved that the root rot of wheat was caused by several fungi that accumulated in the soil, particularly if crop rotation was not practiced (14). Root rot was reported from Madison County, Idaho, in 1921, and investigations indicated that it was confined to dry land farms. The fields attacked were spotted with stunted and lighter colored plants. There was evidence that there had been considerable tillering earlier in the season, most of which had been killed. Counts made in the most severely infected spots, showed only one plant in 50 survived. Heads of the surviving plants were stunted and contained only shriveled grain (38). Later, it was reported that the disease had spread to the southeastern states (4, 17). The disease is

caused by Cochliobolus sativus (Helminthosporium sativum). Cultural studies of monoascosporic isolates showed that the fungus is heterothallic in the sense that isolates are hermaphroditic, self-sterile, intra-group sterile and inter-group fertile. Two compatibility groups in C. sativus are randomly distributed in nature (45). Christiansen and Schneider (15) calculated that the frequency of mutation based on spore numbers in colonies of H. sativum on nutrient agar, ranged from 1/2,400 to 1/20,000 depending on conditions, and on living plants the rate was approximately 1/2,900. Mutation of H. sativum has been studied at the University of Minnesota continuously for more than 30 years, and many thousands of mutants have been isolated. The number and kinds of mutants may differ widely or only slightly from their parents. It is harder to observe mutations in pathogenicity than for cultural characters. When it does occur, there usually is partial or complete loss in pathogenicity and only occasionally is there a gain.

In 1949, Pon (37) isolated an exceptionally virulent race of H. sativum from a leaf of Kindred barley. On potato dextrose agar it produced pinkish mycelium with light-colored spores. The conidia resemble those of dark-spored races of H. sativum in size, shape, and number of septa. This new race also attacked many standard varieties of spring wheats, and thus was definitely more virulent than the common dark-spored races.

Tinline and Dickson (46) studied the inheritance of

several characters in H. sativum. Segregation ratios could not be determined accurately for pathogenicity, growth rate, and conidial production since segregation was largely indistinguishable from normal variation. Some isolates, derived from randonly selected ascospore, differed significantly in pathogenicity from one to another and from the common parental isolates. The distribution curves of data indicated recombination and segregation of the factors. When four pairs of isolates (derived from the eight ascospores of an ascus) were compared with the parental isolates, the data indicated that pathogenicity was controlled by multiple factors. Tinline (45) reported that conidial color and pathogenicity appeared to be inherited independently. Wood (50) also reported that H. sativum is comprised of many races that differ greatly in their parasitic ability; not only on wheat, rye, oats, and corn, but also on varieties of barley. Pathogenicity tests with many isolates indicated that races virulent on Kindred barley were prevalent and widely distributed. During the years 1950-1953, numerous varieties and thousands of hybrid wheat or barley lines were subjected to artificial epidemics of H. sativum created by using a mixture of biotype and races. Most of the material tested was susceptible. Wood (50) isolated H. sativum, from many sources if wheat that differed in ability to cause seedling blight and root rot of cereals. Of 103 isolates tested, 28 percent were virulent on barley, wheat, and oats; 19 percent on barley and wheat, 1 percent on wheat, oats; 15

percent on wheat only; 5 percent on barley only; and 1 percent on oats only; 31 percent were non-pathogenic or caused no apparent damage on any of the three hosts. There was no association between the source of an isolate and its virulence. Progenies from a single conidium differed strikingly in pathogenicity. Seedling blight was most severe on barley at soil temperature of 10 and 34°C which were unfavorable to growth of the host. Apparently, the effect of soil temperature was primarily on the host, rather than on the pathogen.

Mathre (33) tested spores of H. sativum and reported that they germinated well at 30-39°C. Viability of the spores remained high (above 80 percent) for over two years when stored with a moisture content of 11 percent at 4°C. The constituents of spores are quite similar with lipids composing about 10 percent of the dry weight and carbohydrates 5.5-8.3 percent.

Dosdall (16) in 1923, reported the optimum temperature for growth of H. sativum in the laboratory to be 24 to 28°C with minima and maxima of 0 to 20 and 35 to 39°C, respectively. She also concluded that H. sativum induces severe disease injury in situations unfavorable for development of the host.

McKinney (34) also, in 1923, tested the influence of soil temperature and moisture on infection of wheat seedling by H. sativum. His results indicated that optimum foilage development of wheat occurred between 20 and 24°C while 16 to 20°C favored root development. The optimum temperature

for growth of the parasite was 24 to 28°C. Disease development, however, was favored by a temperature suitable to neither the host nor the parasite, but at 28 to 32°C.

While McKinney found that relatively high moisture favored the Helminthosporium disease of wheat, and that the optimum temperature for disease development remained constant at all soil moisture levels, numerous workers have stressed the importance of predisposing factors on wheat plants exposed to H. sativum. Fenster (20) reported that, in western Nebraska, the incidence of H. sativum can be checked or reduced through later seeding dates. He also suggested that if adequate moisture becomes available during fall, early planting may result in lush growth, which in turn could produce severe water stress on the plants if moisture later became limited. He found that root rot caused by H. sativum thrived at the expense of plants weakened due to water stress. Chinn and Ledingham (12) showed that viability of spores in a dry soil did not decline over a nine-month period whereas survival of spores in saturated soil dropped markedly in the same period. Intermediate moisture levels had intermediate effects on spore survival.

Ledingham (30) in 1970, found that conidia of H. sativum cultures on sterile wheat straw were nearly 100 percent germinable after 52 months when maintained at relative humidity at 50 percent or lower. At higher relative humidity, spore longevity was much reduced. Increased temperature decreased spore longevity.

Chinn et al. (13) in 1960, studied the relationship of the spores of H. sativum in soils and the occurrence of common root rot of wheat in the greenhouse and field in 1959 and 1960. Results were similar in both years. In 47 fields studied in 1960, the number of viable spores of H. sativum ranged from 28 to 253 per gram of soil. Disease ratings ranged from 1 to 33 for seedlings grown in a greenhouse in soils from the fields, and 2 to 36 for seedlings and 7 to 56 for mature plants grown in the field, H. sativum was isolated more frequently from mature field plants than from seedlings grown in either greenhouse or field. Disease ratings of seedlings in the greenhouse and field tended to vary with the logarithm of the population of spores in the soil. No such relationship was found for mature plants. However, fairly good correlations were found between spore population and the frequency with which H. sativum was isolated from all of these sources. Spore populations of H. sativum were negatively related to the frequency of isolation of Fusarium spp. from mature field plants, but were not related to the incidence of Fusarium in greenhouse seedlings.

Campbell (8) in 1965, studied the influence of six soil fungi on the pathogenicity of H. sativum. Phoma humicola, Epicoccum purpurascens and Trichoderma viride strongly inhibited the pathogenic activity of H. sativum, while Actinomucor ripens, Sclerotinia trifoliorum, and Myrothecium verrucaria were only slightly inhibitory. The pathogenicity of H. sativum was increasingly depressed by each of six

fungi as soil temperature was increased from 15 to 26°C. The pH shifts which these fungi induced in soil were not sufficient to be considered as a factor in depressing pathogenicity. In a plate test on nonenriched soil-extract medium, S. trifoliorum and A. ripens did not appreciably affect H. sativum. The other four fungi all caused distortion and breakdown of the spores, while M. verrucaria and E. purpurascens were found as internal parasites in spores of H. sativum. P. humicola and E. purpurascens were found as internal parasites of the mycelium. These fungi also produced substances in the medium which adversely affected the germination and growth of germ tubes of spores of H. sativum. A. ripens and S. trifoliorum were the least effective. T. viride and P. humicola were intermediate in their action and E. purpurascens and M. verrucaria were very severly limiting. Two antagonistic mechanisms, antibiosis and direct parasitism, were demonstrated, both of which were responsible for disorganization of the mycelium and were able to depress the pathogenicity of H. sativum appreciably.

Hugelet and Kiesling (23) in 1973, reported that H. sativum alone or in combination with Alternaria alternata caused decreases in kernel weight of durum wheat but the decrease was more pronounced when the ratio of the pathogens in combination favored H. sativum. On plants inoculated first with one organism and then 24 hours later with the other, the fungus initially introduced rapidly colonized the wheat heads and restricted the isolation frequency of the

second organism. The growth rate of each organism was reduced more than 40 percent when grown in the presence of the other organism presumably due to competition for nutrients and not to the production of diffusible inhibitors.

CHAPTER III

MATERIALS AND METHODS

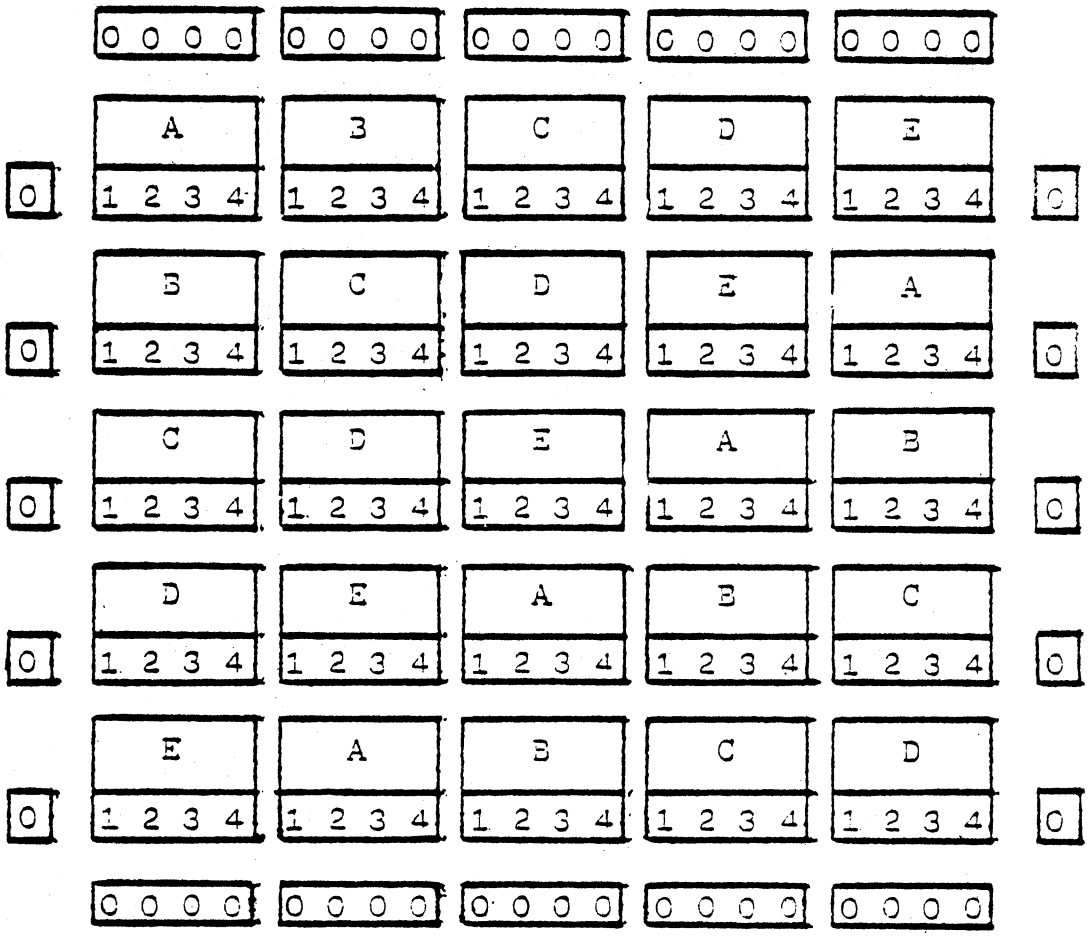
The winter wheat cultivar Triumph 64 was used for the experiments under controlled conditions in the greenhouse. Pure cultures of H. sativum and P. recondita f. sp. tritici were used as test organisms (hereafter, P. recondita f. sp. tritici will be referred to as P. recondita for brevity). Two experiments at two stages of plant growth were conducted as described below:

Experiment I. H. sativum and P. recondita affects on seedling wheat development.

The test, conducted in the greenhouse was arranged in a five-row X 5-column latin square with four plant units per square to provide 20 observations for each treatment (Figure 1). Each plant unit consisted of five seeds of Triumph 64 sown in an 11 cm. clay pot containing a 1:1:1 mixture of sand, peat moss, and silty clay loam soil. The soil mixture was steam sterilized twice for three hours at 200°C and 1,055 g/sq. cm. The two sterilization periods were separated by a 48 hour interval.

Five treatments which served as experimental units were:

1. Seed untreated, no inoculation.



Legend:

A Control.
B Control with alcohol.
C Infested with H. sativum.
D Infected with P. recondita.
E Infested with H. sativum.
and infected with P. recondita.
O Border row plant.

Figure 1. The Experimental Design of Experiment I Arranged in a Five-Row X Five-Column Latin Square with Four Plant Units Per Square

2. Seed treated with alcohol, no inoculation.
3. Seed untreated and sown in soil infested with *H. sativum*.
4. Seed untreated and inoculated with *P. recondita*.
5. Seed untreated and sown in soil infested with *H. sativum* and inoculated with *P. recondita*.

Treatments were randomized in both blocks and columns and the entire design bordered with untreated plant units.

The seedling tests were conducted in greenhouses at the Oklahoma State University Agricultural Experiment Station during the spring and summer. A pure culture of *H. sativum* (designated isolate 47) from Custer County, Oklahoma, was obtained from Dr. L. L. Singleton, Department of Plant Pathology, Oklahoma State University, Stillwater. Isolate 47 was used throughout all experiments and hereafter will be referred to as *H. sativum*.

Conidial inoculum of *H. sativum* was obtained by culturing the fungus on wet wheat kernels (two parts wheat kernels to one part water by volume) in 250 ml. Erlenmeyer flasks. The wheat kernels were autoclaved for 90 minutes at 1,055 g/cm^2, cooled, inoculated with *H. sativum* and incubated for 25°C for 14 days. Conidial suspensions were obtained by repeatedly flooding the cultures with sterile distilled water, agitating them and then filtering them through cheesecloth. Clean conidial suspensions were then obtained by decanting the upper liquid layer when the spores had settled to the bottoms of the flasks. The number of conidia/ml of

of water was determined by pipetting 0.1 ml of the suspension into a nematode counting dish and counting them under a stereoscopic microscope.

To a known weight of sterile soil mixture specified earlier, known volumes of H. sativum conidial suspensions were added to produce a population of 250 conidia/g of soil on a dry weight basis. The inoculated soil was mixed thoroughly in an Electric Bucket Mixer (No. 7658711, McMaster-Carr Supply Company, Chicago, Il. 60680, U.S.A.) for 30 minutes to obtain uniform distribution of conidia in the soil. Water was added while mixing to raise its content in the soil to field capacity. Conidia-infested soil was put in 11 cm. clay pots, and five seeds of Triumph 64 were sown in each pot. Also, seeds were sown in non-infested soil for subsequent leaf rust treatment and for disease-free control plants.

Cultures of P. recondita race 6B were collected from susceptible cultivars with a cyclone-separator-collector, and then sealed in small glass tubes and then stored in liquid nitrogen. Prior to inoculation, the tubes were removed from the liquid nitrogen and the spores heat shocked by putting the tubes in water at 47°C for five minutes. One mg. of spores were suspended in a 0.5 ml. of a light paraffin base oil (Soltrol 170, Phillips Petroleum Company) and then sprayed on 15-day old wheat seedlings with a Venturi Micro-sprayer at approximately 352 g/cm^2. Inoculated plants then were sprayed with Tween 20 (polysorbate 20) in

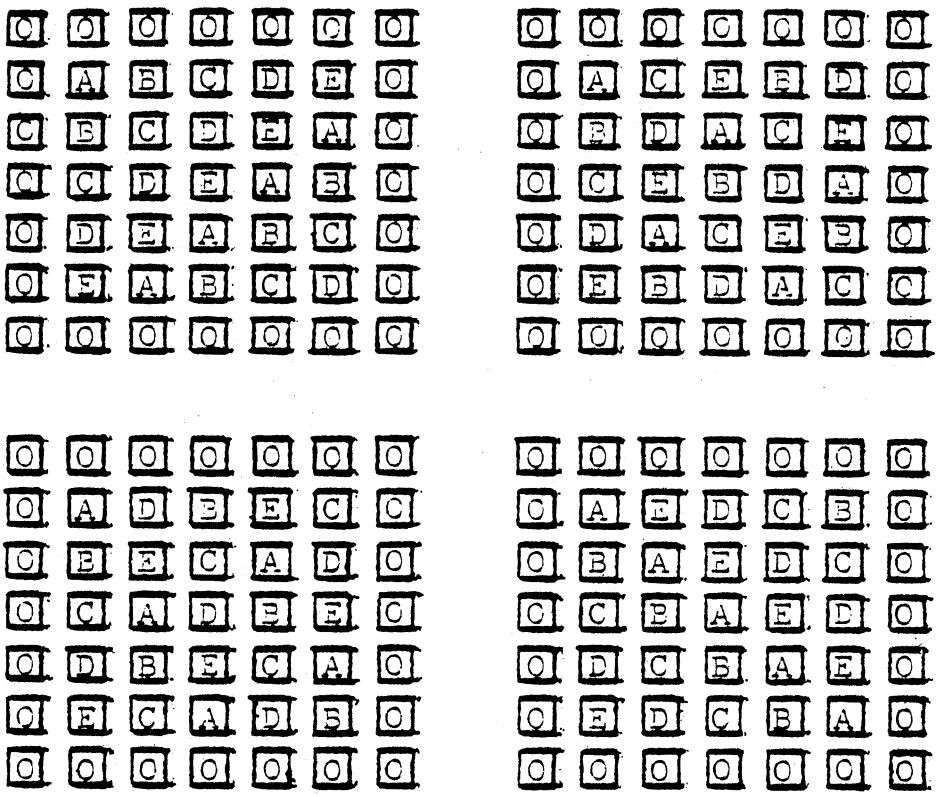
water (1 drop/200 ml) and kept in a moist chamber at 20°C for 12 hours.

Infected leaves of two replicates of this experiment were harvested after 45 and 60 days, respectively, dried overnight in a hot air oven at 60°C then weighed. Then, all stems were cut to the soil level. The pots, soil, and roots were soaked in water for one hour or until the roots and crown portions could be removed from the pots. Each root mass was washed gently in water over a fine mesh screen to remove soil particles, then pressed between paper towels to remove exogenous water. The volume of each root mass was measured in ml by water displacement. After obtaining the root volumes, each root mass was dried overnight at 60°C in a hot air oven and weighed.

All data were summarized and analyzed by using analysis of variance, including all possible means of each variable, interactions between the treatments, and the correlation of variables.

Experiment II. H. sativum and P. recondita effects on adult plants.

Experiment II was conducted in the greenhouse, in a four orthogonal Latin square design with 25 plants in each group, and 20 observations per treatment. Within each group, one seed of Triumph 64 was sown in each of 25 sterilized 15 cm pots. Randomization of the design is shown in Figure 2. Five treatments of inoculation were applied as follows:



Legend:

A Control.
B Control with alcohol.
C Infested with H. sativum.
D Infected with P. recondita.
E Infested with H. sativum and infected with P. recondita.
O Border plant row.

Figure 2. The Experimental Design of Experiment II Arranged in a Four Orthogonal Latin Square

1. Seed untreated, no inoculation
2. Seed treated with alcohol, no inoculation.
3. Seed untreated and sown in soil infested with *H. sativum*.
4. Seed untreated and inoculated with *P. recondita*.
5. Seed untreated and sown in soil infested with *H. sativum* and inoculated with *P. recondita*.

Soil mixtures, maintenance of cultures and methods of inoculations were similar to those of Experiment I. The only difference between the two experiments was that the pots were infected with *H. sativum* and seedlings were inoculated only once with *P. recondita*, in Experiment I, but the mature plant pots were infested three times with *H. sativum* at 0, 90 and 120 days of age and plants were inoculated with *P. recondita* race 6B at 15, 110, 140 days of age.

The inoculations with the organisms were made alternately. Thirty days after the first inoculation with leaf rust, the first three leaves of all plants were excised, dried at 60°C in a hot air oven overnight, then weighed. Immediately after removing the leaves, the plants were moved to a cold frame to vernalize for 40 days. After vernalization they were returned to the greenhouse.

The second and third inoculations with *H. sativum* were made 90 and 120 days after the first by pouring conidial suspensions on the soil surface close to the plants and then covering the surface with sterilized soil. A concentration of 250 conidia per gram of dried soil was used.

When the plant tillers had elongated, the second inoculation was made with P. recondita. The third inoculation was made one month later. All plants were allowed to mature in the greenhouse.

Thirty days after the third inoculation with P. recondita, the leaf rust disease incidence was evaluated on individual leaves. The evaluation was made on a scale of 0-9; from no infection to heavy infection. Before harvesting the number of tillers and heads per plant were recorded. The height of each tiller was also measured.

At the end of the experiment the seed yield of each plant was harvested separately, counted, and weighed. All plants were cut at the soil level, and the pots were soaked in water for 24 hours to facilitate removal of the root mass. The roots from each pot were rinsed gently in tap water over a fine-mesh screen. The roots of each plant were pressed between paper towels to remove any excess water, and their volumes measured by water displacement. Finally, the roots were dried at $60^{o}C$ in a hot air oven overnight, and weighed.

Analysis of variance was used to analyze the results. Computation and summarization were based on means of five plants in the seedling stage tests and on single plants in the mature plant test. All statistical analyses were conducted at the Oklahoma State University Computer Center with assistance by faculty of the Department of Statistics.

CHAPTER IV

RESULTS

Experiment I: Effect of H. sativum and P. recondita Infection on Seedling Wheat

Seven to 14 days after inoculation with H. sativum and P. recondita, the plants showed symptoms of both leaf rust and root rot. Symptoms produced by P. recondita were discoloration and uredinial pustules scattered over the leaf surfaces (Figure 3). Symptoms produced by H. sativum were manifested as black necrosis at the base of the stem (Figures 4 and 5).

Dry leaf weight was reduced significantly when the plants were infected with P. recondita, but not when infected with H. sativum alone (Table I) or the combination of both organisms. Dry root weights of plants infected with H. sativum and P. recondita alone differed significantly from uninoculated plants, however, only the root volume of plants infected with P. recondita was significantly less then the uninoculated control.

In the first seedling test, dry leaf weight (2.23 g), root volume (4.72 ml), and dry root weight (0.60 g) of

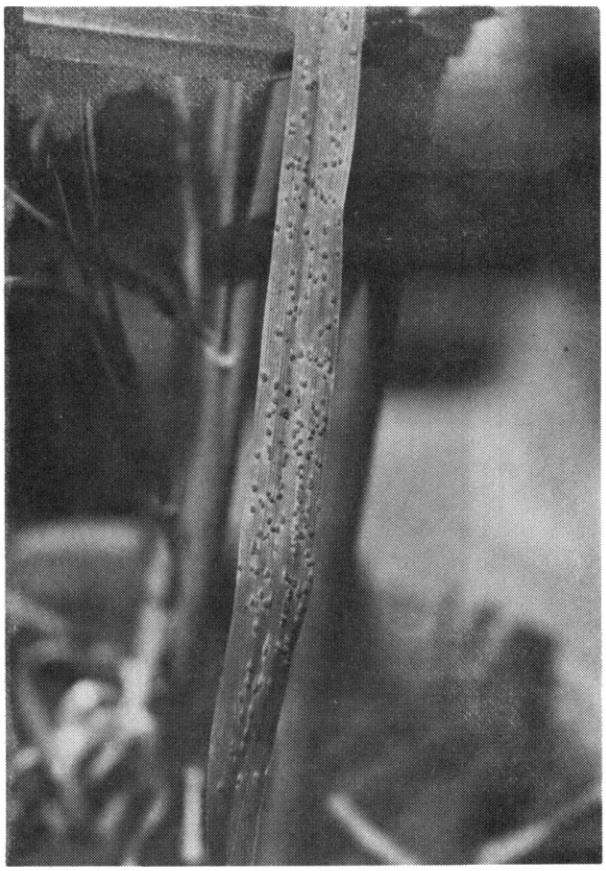


Figure 3. Wheat Cultivar Triumph 64 Infected with P. recondita

Figure 4. "Wheat Cultivar Triumph 64 Infected with H. sativum

Figure 5. Wheat Cultivar Triumph 64 Infected with H. sativum and P. recondita on the Basal Stem, Leaves and Leaf Sheaths

TABLE I

1/
MEAN DRY LEAF WEIGHT, ROOT VOLUME, AND DRY ROOT WEIGHT OF 45 DAY OLD TRIUMPH 64 WHEAT SEEDLINGS INOCULATED WITH H. SATIVUM AT 0 DAYS AND WITH P. RECONDITA F. SP. TRITICI AT 15 DAYS AFTER PLANTING

Treatments	Dry leaf Weights (g)	Root Volumes (ml)	Dry Root Weights (g)
Control	1.98	4.53	0.64
Control with alcohol	2.08	4.79	0.64
Infected with H. sativum	1.83	3.49	0.45
Infected with P. recondita	1.45	2.90	0.43
Infected with H. sativum and P. recondita	2.23	4.72	0.60
LSD .05	0.42	1.18	0.15
CV (Percent)	18.74	39.75	36.27

1/ Mean of 100 plants.

plants inoculated with both H. sativum and P. recondita did not differ significantly from those of the control which were 1.98 g, 4.53 ml, and 0.64 g, respectively.

Thes results of the second seedling test were similiar to the first one. For example, plants infected with P. recondita showed a significant reduction in dry leaf weight, root volume, and dry root weight (Table II), but plants infected with H. sativum showed no significant reduction in either dry root weight or root volume when compared to the control. Plants infected with both H. sativum and P. recondita were not significantly different from the control in either dry leaf weight or root volume but were significantly higher than the control plants in dry root weight (Table II).

In all cases the value for all three parameters with the plants inoculated with both fungi were higher than for those plants inoculated only with P. recondita. With one exception (root volume in the second test) the plants infected by H. sativum had lower values for these parameters than the plants inoculated with both fungi. Since H. sativum is known to cause root rot, and P. recondita has been shown to restrict root development and reduce foliar growth (48, 50), it is difficult to understand how the effect of the two organisms together acts to negate the damage caused by each one alone, or perhaps even bring about some stimulation in growth.

TABLE II

1/
MEAN DRY LEAF WEIGHT, ROOT VOLUME, AND DRY ROOT WEIGHT OF 60 DAY OLD TRIUMPH 64 WHEAT SEEDLINGS INOCULATED WITH H. SATIVUM AT 0 DAYS AND WITH P. RECONDITA F. SP. TRITICI AT 15 DAYS AFTER PLANTING

Treatments	Dry leaf Weights (g)	Root Volumes (ml)	Dry Root Weights (g)
Control	2.17	10.21	1.38
Control with alcohol	2.45	13.68	1.67
Infected with H. sativum	2.36	11.32	1.60
Infected with P. recondita	1.74	9.91	1.25
Infected with H. sativum and P. recondita	2.44	10.43	1.75
LSD .05	0.22	2.03	0.36
CV (Percent)	36.58	23.33	41.83

1/ Mean of 100 plants.

Experiment II: Effect of H. sativum and P. recondita Infection on Mature Plants

An attempt was made to determine the effects of infection by H. sativum and P. recondita singly and in combination on dry leaf weight, number of tillers, mainstem height, average tiller height, number of heads, number of seeds, seed weight, root weight, and root volume.

In this experiment, as mentioned earlier, the plants were inoculated three times. The first inoculations were made while the plants were in the seedling stage. Results of dry leaf weight of plants infected with H. sativum and P. recondita singly and in combination are presented in Table III. There were no significant differences in the dry leaf weight.

The second and third inoculations were made after the plants had been vernalized. Infection with H. sativum and P. recondita alone and in combination significantly reduced the number of seed produced per plant, and the yield in terms of grain weight (Table IV, Figure 6). Plants inocukated with both H. sativum and P. recondita produced an average of 44.2 seeds which was significantly less than other plants inoculated with P. recondita alone, but not significantly less than other plants inoculated with H. sativum alone. The weight of seed of plants inoculated with both organisms was less than from plants inoculated with either

TABLE III

MEAN DRY LEAF WEIGHT OF 45 DAY OLD TRIUMPH 64 WHEAT SEEDLINGS INOCULATED WITH *H. SATIVUM* AT 0 DAYS AND WITH *P. RECONDITA* AT 15 DAYS AFTER PLANTING [1/]

Treatments	Dry Leaf Weight (g)
Control	0.42
Control with alcohol	0.40
Infected with *H. sativum*	0.39
Infected with *P. recondita*	0.39
Infected with *H. sativum* and *P. recondita*	0.42
LSD .05	0.04
CV (Percent)	14.92

1/ Mean of 20 plants.

TABLE IV

MEAN GRAIN YIELD AND NUMBER OF SEEDS PER PLANT OF TRIUMPH 64 WHEAT AFTER INOCULATION WITH *H. SATIVUM* AND *P. RECONDITA* F. SP. *TRITICI* [1/]

Treatments	No. of Seeds/Plant	Weight of Seeds/Plant in g
Control	79.3	2.84
Control with alcohol	81.3	3.04
Infected with *H. sativum*	48.0	1.62
Infected with *P. recondita*	58.1	1.62
Infected with *H. sativum* and *P. recondita*	44.2	1.52
LSD .05	12.7	0.41
CV (Percent)	31.71	30.26

1/ Mean of 20 plants.

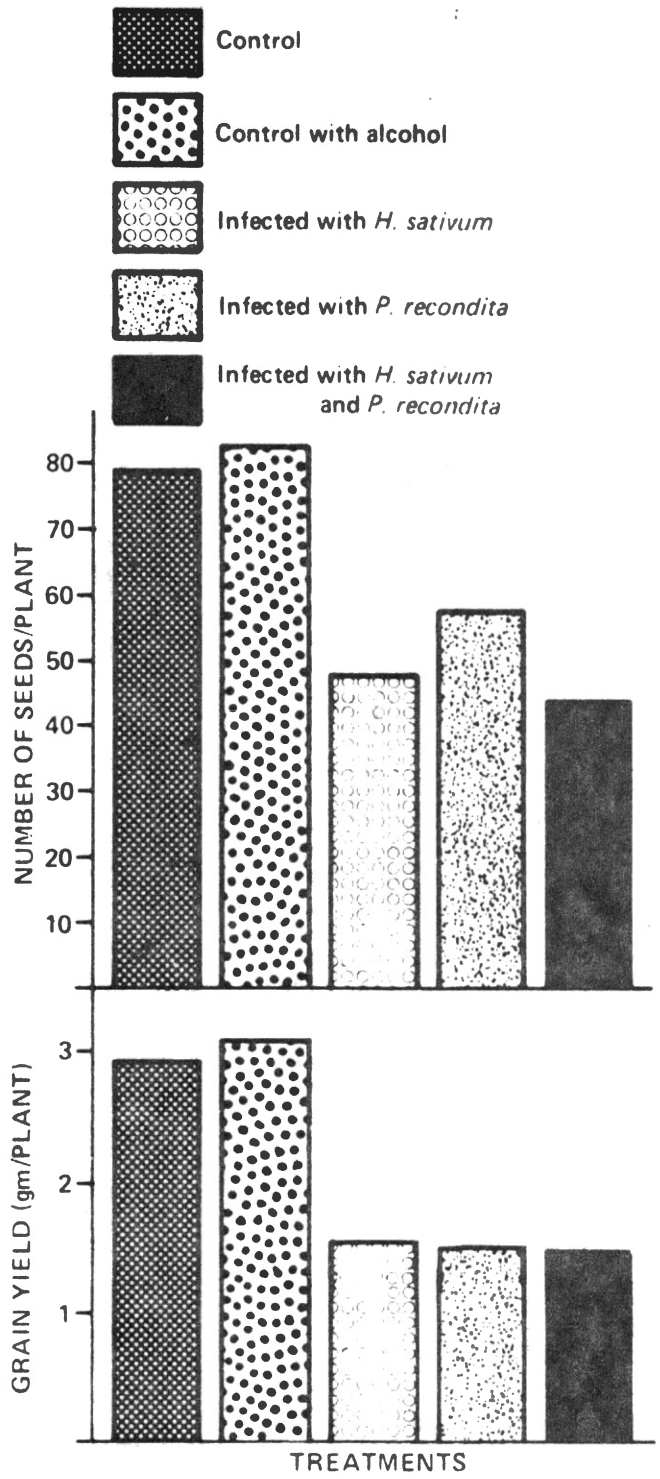


Figure 6. A Comparison of Grain Yield and Number of Seeds Per Plant of Triumph 64 Wheat After Inoculation with H. sativum and P. recondita

organism alone, but not significantly. Also there was no significant difference in yield between the two sets of control plants, i.e. plants derived from seed treated with alcohol and plants derived from untreated seed. The mean of grain yields obtained from the control treated with alcohol was 3.04 g while that from the untreated control was 2.84 (Table IV).

Root weights and root volumes of mature plants were measured after harvesting. The data in Table V and Figures 7 and 8 show a comparison of the root volumes of the various treatments. There were significant reductions in root weight and root volume for plants infected with H. sativum, P. recondita and the combination of both H. sativum and P. recondita. In this experiment the greatest reduction in root growth was caused by P. recondita although the difference between the values for H. sativum and for both fungi were not significant. There was no significant difference in root weight and root volume between the two controls.

The average number of tillers produced by plants infected with only H. sativum and with H. sativum in combination with P. recondita was significantly greater than that produced by plants in the two control groups and by plants inoculated with P. recondita alone (Table VI, Figure 9). The average number of heads produced by plants infected with H. sativum and with the H. sativum - P. recondita was less than the number produced by the control plants or plants infected only with P. recondita, but not significantly so. Neither number of tillers nor number of heads produced by plants

TABLE V

MEAN ROOT WEIGHT AND ROOT VOLUME OF MATURE PLANTS OF TRIUMPH 64 WHEAT AFTER INOCULATION WITH *H. SATIVUM* AND *P. RECONDITA* F. SP. *TRITICI* [1/]

Treatments	Root Weights (g/plant)	Root Volumes (ml/plant)
Control	0.63	4.20
Control with alcohol	0.76	4.73
Infected with *H. sativum*	0.52	3.19
Infected with *P. recondita*	0.43	3.06
Infected with *H. sativum* and *P. recondita*	0.48	3.40
LSD .05	0.11	0.72
CV (Percent)	31.03	30.49

1/ Mean of 20 plants.

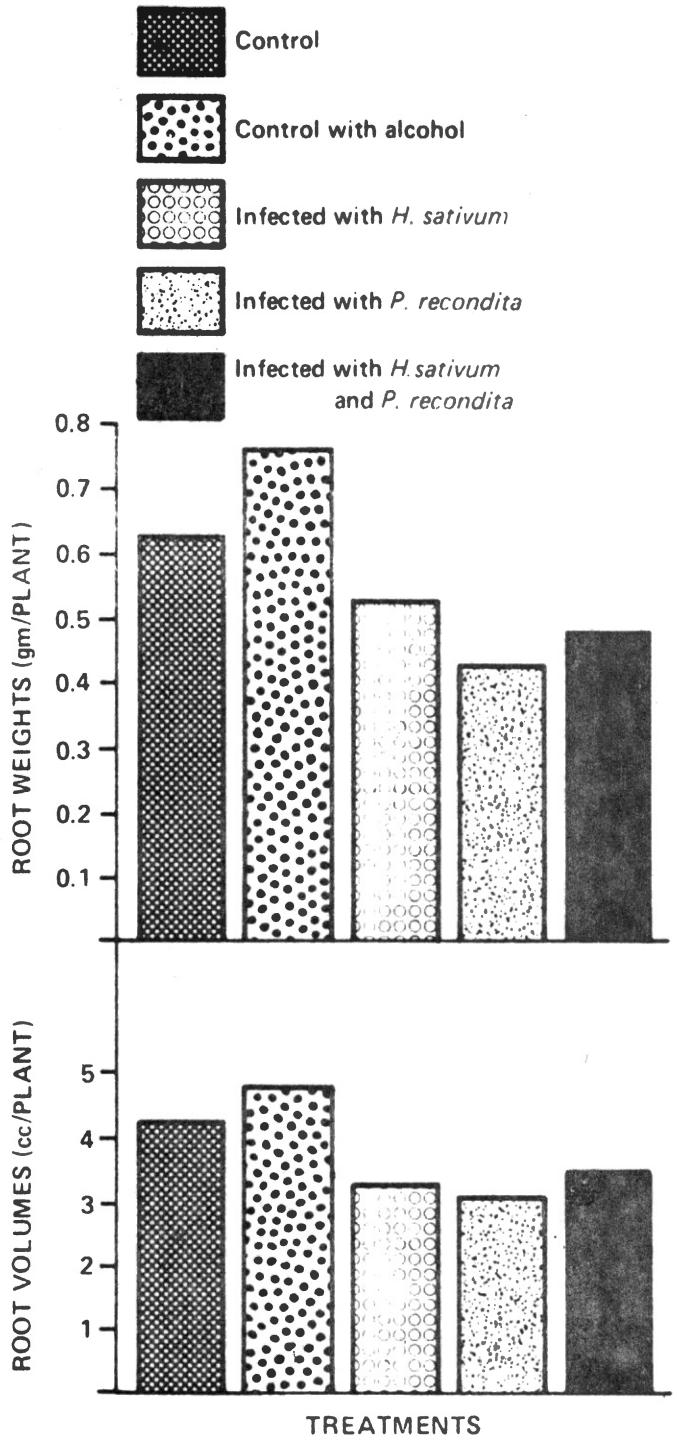


Figure 7. A Comparison of Root Weights and Root Volumes of Mature Plants of Triumph 64 Wheat After Inoculation with H. sativum and and P. recondita

R Inoculated with P. recondita f. sp. tritici
H Inoculated with H. sativum
H+R Inoculated with H. sativum and P. recondita f. sp. tritici
W Uninoculated (seed treated with alcohol
W/O Uninoculated (seed untreated)

Figure 8. A Comparison of the Root Volume of Plants of the Wheat Cultivar Triumph 64 Following Five Inoculation Treatments

TABLE VI

1/
MEAN NUMBER OF TILLERS AND HEADS PER PLANT OF TRIUMPH 64 WHEAT AFTER INOCULATION WITH H. SATIVUM AND P. RECONDITA

Treatments	No. of Tillers Per Plant	No. of Heads Per Plant
Control	4.45	3.05
Control with alcohol	4.40	3.45
Infected with H. sativum	5.40	2.75
Infected with P. recondita	4.05	3.00
Infected with H. sativum and P. recondita	6.05	2.65
LSD .05	0.72	0.58
CV (Percent)	37.97	30.51

1/ Mean of 20 plants.

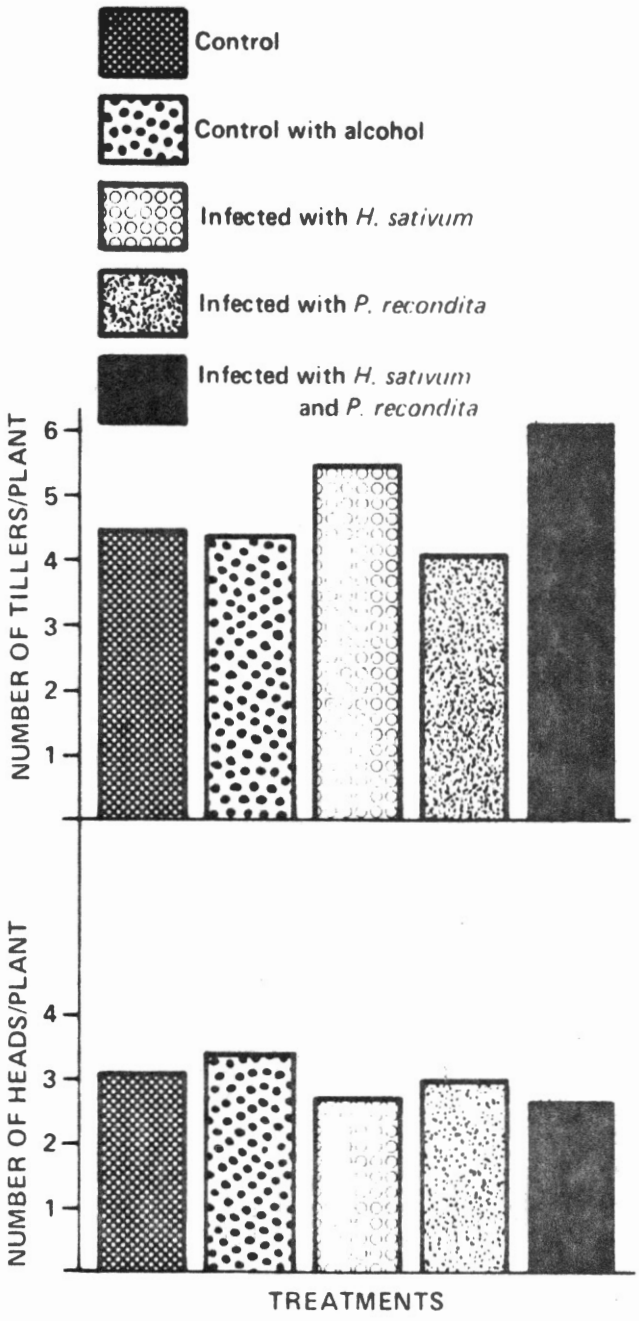


Figure 9. A Comparison of Numbers of Tillers and Heads Per Plant of Triumph 64 Wheat After Inoculation with H. sativum and P. recondita

infected with only P. recondita differed from the controls, and the controls did not differ from each other.

The heights of mainstems of plants infected with H. sativum and with the combination of H. sativum and P. recondita were significantly shorter than those of the control plants (Table VII, Figure 10). For plants infected with P. recondita, the mainstems were shorter than the control but not significantly so. When the average height of all tillers was considered, plants infected with both H. sativum and P. recondita alone and in combination were all significantly shorter than the controls.

The incidence of disease for both P. recondita and H. sativum were recorded after the third inoculation of P. recondita or 170 days after planting. Plants infected with H. sativum showed dark-brown to black lesions on the stems near the soil level. Plants infected with P. recondita showed orange uredinial pustules on leaf surfaces. Leaf rust disease readings were taken from infected leaves and recorded as a percent of leaf area infected according to the Modified Cobb Scale. Infection for any single plant was expressed as the average of all leaves scored on that plant. Scores for all plants were then analyzed and summarized statistically (Table VIII). Plants infected with P. recondita alone read 27.4 percent and rust on the plants infected with the combination of the two organisms read 23.8 percent. The difference was not significant.

Correlations between all the parameters measured except

TABLE VII

MAIN[1/] MAINSTEM HEIGHT AND AVERAGE HEIGHT OF ALL TILLERS OF TRIUMPH 64 WHEAT AFTER INOCULATION WITH H. SATIVUM AND P. RECONDITA

Treatments	Mainstem Height (cm/pl)	Average Tiller Height[2/] (cm/pl)
Control	74.84	61.79
Control with alcohol	78.09	62.11
Infected with H. sativum	63.40	45.79
Infected with P. recondita	70.07	52.60
Infected with H. sativum and P. recondita	58.99	45.62
LSD .05	7.17	7.24
CV (Percent)	16.22	21.17

1/ Mean of 20 plants.
2/ Average height of all tillers.

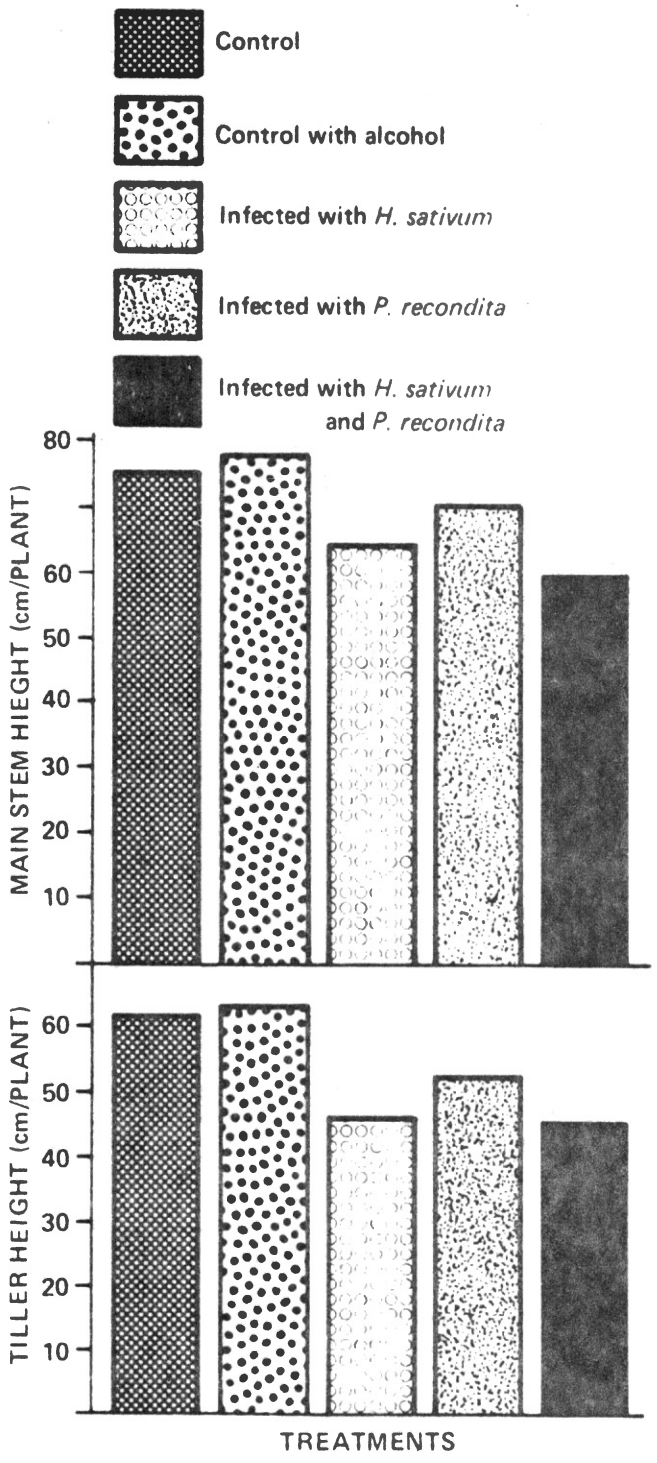


Figure 10. A Comparison of Mainstem Height and Average Tiller Height of Triumph 64 Wheat After Inoculation with H. sativum and P. recondita

TABLE VIII

DISEASE INCIDENCE OF H. SATIVUM AND P. RECONDITA ON TRIUMPH 64 WHEAT 30 DAYS AFTER THE THIRD INOCULATION OR 170 DAYS AFTER PLANTING

Treatments	Disease Incidence in Percent	
	H. sativum [1]	P. recondita [2]
Control	-	0
Control with alcohol	-	0
Infected with H. sativum	+	0
Infected with P. recondita	-	27.4
Infected with H. sativum and P. recondita	+	23.8

1/ - = no disease, + = disease symptoms present.
2/ Average of infected leaves of each tiller of 20 plants.

incidence of H. sativum were calculated. The correlations that were significant and of interest in this study are given in Table IX. Rust incidence was negatively correlated with root weight and root volume, and with main stem height and average tiller height. The coefficient for rust incidence and root weight did not quite reach significance at the five percent level and the coefficient for rust incidence and average tiller height was not significant either. Yield, as measured by either seed count or seed weight was positively correlated with mainstem and average tiller height as well as with root weight and root volume, although the latter coefficients were not significant. Yield losses due to infection with H. sativum, P. recondita, and the combination of H. sativum and P. recondita were 42.8, 43.0 and 46.4 percent respectively, compared to the control (Table X).

TABLE IX

CORRELATIONS BETWEEN PARAMETERS OF MATURE PLANTS OF THE WHEAT CULTIVAR TRIUMPH 64 FOLLOWING INOCULATION WITH H. SATIVUM AND P. RECONDITA F. SP. TRITICI

Parameters	Root Weight	Root Volume	Mainstem Height	Average Tiller Height
Rust incidence	-.249	-.417**	-.313**	-.154
Yield (seed wt)	.215	.183	.374**	.266
Seed count	.221	.140	.374**	.322**

TABLE X

PERCENTAGE OF GRAIN YIELD LOSS WITH PLANTS INFECTED WITH *H. SATIVUM* AND *P. RECONDITA F. SP. TRITICI* COMPARED WITH AN UNINFECTED CONTROL

Treatments	Average Grain Yield (g/pl)	Weight Loss (Percentage)
Control	2.84	-
H. sativum	1.62	43.0
P. recondita	1.62	43.0
H. sativum and *P. recondita*	1.52	46.4

CHAPTER V

DISCUSSION

Seedlings of the wheat cultivar Triumph 64, infected with H. sativum and P. recondita singly or in combination showed essentially the same effects in two seedling tests. There were no additive detrimental effects when plants were infected with the two organisms simultaneously; on the contrary, plants infected with one organism alone reduced leaf weights, root volumes and root weight more than when infected with the two organisms together. It would normally be thought that such a reaction reflected an antagonistic interaction between the organisms in the wheat seedlings. This is contrary to the concept of Yarwood (54) that plants infected with one organism would be so predisposed as to enhance the severity or damage caused by the subsequent infection by a second organism. The results more closely agree with Chester (11) who stated that one organism introduced earlier could weaken the effect of one introduced later. Even this is difficult to understand in this case, however, since H. sativum infects root first from infested soil and does not become systemic in above ground plant parts until after death of the plant, and P. recondita is a localized parasite of the leaves. If the interaction observed here is

real, then any interaction between these pathogens would almost have to be by some diffusable material interacting with the host as a stimulant, or with another material from the other pathogen.

Antagonism between the two pathogens when they are together often has been reported. Ledingham (29) reported such action with H. sativum and Fusarium culmorum, and Boossalis (2) also reported existence of an antagonism between H. sativum and Fusarium. Young (52) grew H. sativum and Fusarium spp. on artificial medium in the laboratory and observed that H. sativum grew much slower when Fusarium was present. Ledingham (29) conducted comparative studies of H. sativum and F. culmorum and reported that the two pathogens showed antagonism toward one another when associated on wheat seed. Greaney and Machacek (21) in 1935 reported that the pathogenicity of H. sativum was reduced in the presence of Trichothecium (Cepalothecium) roseum. In all of these cases, however, the organisms involved were associated either in the soil substrate or infected with same plant parts in a similar manner.

In the experiment with adult plants, grain yields of plants inoculated with H. sativum 0, 90 and 120 days after sowing, with P. recondita after 15, 110 and 140 days and in combination, differed significantly from control plants. Grain weight was much reduced, mainly by extreme shriveling caused by leaf rust, while the number of seeds per plant was much reduced by Helminthosporium. When the two organisms

occurred together in the plants for a longer period of time, additive effects were indicated in contrast to what occurred when measurments were made in the seedling stage only 45 days after planting. Yarwood (53) also observed that the longer his virus was established in the wheat plants, the greater was the susceptibility to later rust infection.

In the mature plant study the additive effects of the two pathogens were also found in plant height, number of tillers and number of heads per plant. Plants inoculated with both fungi were shorter than those inoculated with either alone and had fewer heads. Strangely, however, the number of tillers per plant was higher than with plants inoculated with either pathogen alone and was higher than the controls. This would indicate some stimulatory effect that could be related to some effect upon growth regulators.

The correlation between disease incidence and grain yields, and rust incidence and mainstem height were negatively related. Disease incidence involving both rust and root rot and number of tillers were positively correlated. It indicated that infection lowered the grain yield, shortened the mainstem and increased the number of non-heading tillers.

It can be concluded also that leaf rust in the presence of root rot caused by H. sativum will cause more damage than leaf rust alone, and vice versa. There is also some evidence of cross protection, since the incidence of rust on plants also infected with H. sativum was lower than on plants not infected with that fungus. It should be noted in this regard that inoculation with H. sativum was done first in all cases.

CHAPTER VI

SUMMARY

Plants of the hard red winter wheat cultivar Triumph 64 were infected in the greenhouse with H. sativum and P. recondita f. sp. tritici singly and in combination. The following effects were noted:

1. Significant reductions in dry leaf weight, root volume, and dry root weight resulted from infection with either H. sativum or P. recondita when measurements were made within 60 days of planting.

2. When compared to uninoculated controls plants inoculated with both pathogens caused no reduction in either leaf weight, root volume or root weight when measured within 60 days of planting.

3. Plants infected from the seedling stage through maturity sustained yield reductions in terms of seed weight per plant of 43 percent from P. recondita alone, 42.8 percent from H. sativum alone, and 46.4 percent from the combined effects of H. sativum and P. recondita. The losses were significant when compared with uninoculated control plants, but they did not differ significantly from each other.

4. P. recondita had a greater number of seeds per plant than the combination of the two organisms. However, the difference in weight of seeds per plant between these two treatments was not different significantly.

5. Concurrent infection with H. sativum and P. recondita from seedling through mature plant stages increased the number of heads per plant, and the average tiller height and the height of the mainstems.

6. Disease incidence was negatively correlated with reduced root volume, plant height, and yield; but was positively correlated with an increased number of tillers.

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