THE EFFECT OF THREE SYSTEMIC CHEMICALS ON REACTION TYPE IN WINTER WHEAT INFECTED WITH *PUCCINIA* *RECONDITA* ROB. EX. DESM. F. SP. *TRITICI*

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CHAPTER I

INTRODUCTION

Puccinia recondita Rob. ex. Desm. f. sp. *tritici* is the causal agent of the leaf rust disease of wheat and occurs wherever wheat is grown. This is the most serious disease of wheat in the southern one-half of the American Central Plains Area (4). Crop losses in this region occur almost every year and in some years have been quite severe (24). Most cultivars grown in the area are susceptible, so epidemiology of the disease depends largely upon environment.

For centuries Western civilizations have depended heavily upon wheat for a major staple food. Recognition of loss in yields resulting from the leaf rust disease generated a genuine awareness of the need to control it. Limited knowledge of basic facts pertaining to the disease led early scholars to devise rather ineffective control measures. Many of them believed, for example, that environmental conditions such as hot, dry winds and other extremes in the weather made the host system frail and allowed these internal blemishes to break out (2). It is not surprising, then, that progress in the control of this disease was not experienced to any significant degree until the identity of the causal agent and certain facts relative to disease development were disclosed and accepted.

More modern control practices have developed along two major lines; host resistance and the use of chemicals. Host resistance depends upon certain inherent characteristics of plants to prevent or arrest the development of the parasite while strategic use of specific chemicals may account for the same purpose. Neither of these control measures is without shortcomings, but each has proved helpful in reducing the total damage done by this disease.

Major emphasis for control has been on the development of resistant cultivars, a practice widely accepted and employed all over the world. However, host resistance to wheat leaf rust is complicated by the occurrence of many physiologic races of the fungus and the inherent ability of the fungus to rapidly produce new races. Stakman and Piemisal (37), in 1917, first demonstrated the occurrence of physiological or pathological specialization with the stem rust disease organism. Since that time, it has been shown that the same phenomenon occurs in many pathogens including the leaf rust organism (23).

Organized investigations observing the effectiveness of chemicals in control of the leaf rust organism have been conducted for nearly eight decades. Progress along this line generally has been a result of empirical searches or relevant discoveries in closely related fields. The first investigations demonstrated effective control with elemental sulfur dust, but frequent applications of chemicals required for adequate control were not economically feasible (29). Establishment of the fact that the leaf rust organism could be chemically controlled, however, precipitated a search for compounds that might be practical. It was soon recognized that the best chemical would combine systemic activity with long duration. Action of such chemicals may inhibit infection and result in a reduced disease severity, or alter development of disease in the plant resulting in an altered disease reaction, or both.

Measurement of host-parasite interaction in the wheat leaf rust system is based on disease reaction type. Five distinct classes of reaction type are recognized. Arbitrary grouping of certain reaction types is used to measure the level of host resistance and pathogen virulence. Based on these assigned levels, the disease potential in a given combination can be assessed.

The principal objective of this investigation was to observe the effect of three systemic chemicals on reaction type in twelve host-parasite combinations grown under controlled environmental conditions. Effects of rate and time of application of chemicals in relation to infection and temperature were studied.

CHAPTER II

LITERATURE REVIEW

Disease reaction type is used in rust-host-parasite systems to describe the progress of infection after inoculation. It results from the interaction of the host gene system with the parasite gene system as affected by the environment acting upon each system and upon the interaction between these systems (8).

The system of nomenclature for the leaf rust disease reaction type was first proposed by Mains and Jackson (22), in 1926, and was based on a nomenclature proposed earlier for the stem rust disease by Stakman and Piemeisel (37). These systems used a scale from zero to four as follows:

Reaction Type	Description of Reaction Type
0	No uredia formed; small flecks chlorotic or necrotic areas more or less prevalent.
1	Uredia few, small, always in small necrotic spots. Also more or less necrotic areas produced without development of uredia.
2	Uredia fairly abundant, of moderate size, always in necrotic or very chlorotic spots. Necrotic spots seldom without uredia.
3	Uredia fairly abundant, of moderate size. No necrosis produced but sometimes slight chlorosis immediately surrounding the uredia.

Reaction Type	Description of Reaction Type
4	Uredia abundant, large. No necrosis or chlorosis immediately surrounding the uredia. Infected areas sometimes occurring as green islands surrounded in each case by a chlorotic ring.

The reaction types described above provide the basic portion of the system used today (17). Three additional reaction types have been incorporated since the system was first proposed.

A "mesothetic" reaction type was found and described by Stakman and Levine (36), in 1922, for the wheat stem rust system and later was incorporated into the wheat leaf rust system. This reaction type is denoted by the symbol "X" and describes a mixture of all reaction types or a designated combination of them occurring on the same leaf with no means of mechanical separation possible.

Stakman et al. (35), in 1944, again using the wheat stem rust system, separated a "fleck" reaction from an immune reaction. The "O" symbol was retained for the immune reaction and a "O" followed by a semicolon, "O;" was the symbol used for the fleck reaction type. This fleck reaction type described a necrotic spot without uredial development.

Heyne and Johnston (15), in 1954, described the type "Y" reaction for the wheat leaf rust system. In this reaction type, pustule type varied with the position of infection on the leaf. Exemplary of the type "Y" reaction is the occurrence of type 4 pustules toward the tip of a leaf and O or O; types toward the base of the same leaf. The reverse also is known to occur.

These reaction types provide a measure of compatibility or incompatibility of a given host-parasite combination. Thus, both resistance or susceptibility of the host and virulence or avirulence of the parasite may be measured or classified by this single criterion (34). Reaction types indicating incompatibility in a particular system would mean a resistant host, avirulent parasite, or both (21).

The host resistance involved here has been described as physiologic (12), specific or vertical (39) as opposed to morphologic, functional (13), or horizontal (39). If incompatibility exists, it is manifested only at the actual initiation of infection. It is a type of resistance governed from within the plant and is effective only after the parasite has entered (34). This would indicate that the host response is indeed physiologic or chemical in nature. Logically, there have been many investigations of the chemistry of such host-parasite systems in the rusts.

Daly (6), in 1949, demonstrated the effect on reaction type of various nitrogen sources applied to the host. At temperatures between 65-75F, he found that the cultivar Thatcher and race 56 of Puccinia graminis tritici normally produced a mesothetic ("X") disease reaction type. When supplied with ammonium nitrogen, a low reaction type occurred, but when supplied with equivalent amounts of nitrate nitrogen, a high reaction type developed.

Thatcher (38), in 1939, proposed that host cell membrane permeability played a key role in the availability of host nutrients which an obligate parasite must have to develop. He demonstrated that compatibility could be increased if the host cells were made more permeable by cold hardening and also by the use of chloroform.

Gottlieb and Hart (10), in their investigation of the effect of growth substances on the rust disease system, found that thiamin chloride, riboflavin, nicotinic acid, ascorbic acid, and beta-indole acetic acid did not play a major role in the host-parasite interaction. They concluded that pathogenic differences in physiologic races of the stem rust pathogen which they used could not be correlated with requirements for these growth substances.

Hotson (16) found that a 300 ppm aqueous solution of sulfadiazine, plus one per cent Tween-20 as a wetting agent could completely suppress rust disease development. The rust suppression effect of sulfadiazine could be reversed, however, if the plants were sprayed with either para-amino benzoic acid or folic acid and implicated the importance of these vitamins for rust disease development.

There is an abundance of literature on physiological aspects of host-parasite interactions in this obligate parasite. However, a complete review of that literature is not relevant to the questions concerned here.

The first experimental studies for control of leaf rust with chemicals used elemental sulfur dust (3) (18) (19). These tests, with limitations, showed that sulfur dust could control the rust organism. Low feasibility of protectant chemicals, such as sulfur for the control of the cereal rusts, precipitated an extensive search for chemicals with systemic activity and greater residual.

Dickson et al. (7), in 1952, was the first to show that systemic control of a rust organism (*Puccinia graminis tritici*) was possible. He found that certain naphthoquinones and phenols at low concentrations had a fungicidal effect upon stem rust when applied prior to inoculation.

Also, that naphthoquinones applied after inoculation resulted in a reduction of pustule formation.

Livingston (20), in 1953, demonstrated, on a field basis, that the rust diseases of wheat could be controlled by a systemic compound. He determined that calcium sulfamate surpassed all other compounds tried for the control of leaf rust.

Haskett and Johnston (14) demonstrated the practicability of systemic control of the leaf rust organism with the application of Acti-dione and calcium sulfamate. They found that these systemically active compounds provided nearly 100% control of the leaf rust organism with a resultant increase in yield.

Several nickel salt amine complexes were shown to be effective in the control of the leaf rust organism under field conditions by Peterson et al. (26), in 1958, in Canada. These compounds were found to possess both protective and eradicative properties. They concluded that the nickel portion of these compounds imparted the rust control properties.

Subsequent research has revealed several other chemicals that are systemic in activity and persistent enough to provide a reasonably long range control of the rust organism. Most of these chemicals are similar in that they are of organic composition, but often represent diverse chemical families. The spectrum of activity of these compounds is quite varied. Some have a wide spectrum of fungicidal activity while others are, for all practical purposes, limited to the control of a single species.

Hacker and Vaughn (11) found a semicarbazone, and an acetate derivative of cyclohexamide, effective in inducing pre-infection resistance in wheat to the stem rust organism. These materials, unlike several other antibiotics tested, were only slightly phytotoxic at effective rates and in the absence of the disease did not reduce yields or effect seed germination.

Schmeling and von Kulka (32) introduced the 1-4 oxathiin derivatives which were shown to have systemic fungicidal activity effective against several members of the Basidiomycetes, including the stem and leaf rust organisms. Rowell (30) found that a treatment schedule with one of these compounds (DCMOD) consisting of a soil application at planting coupled with a mid-season spray resulted in yields equal to that obtained by two late season sprays of a formulation of nickel sulfate hexahydrate plus maneb.

Benomyl, under greenhouse conditions, was demonstrated to be effective in the control of wheat stem rust. However, the results of field tests were not encouraging (31).

One of the most recent developments in systemic chemicals for control of wheat leaf rust is 4-n-Butyl-1,2,4-triazol, referred to hereafter in this paper as triazol (40). It is highly specific and seems to be limited exclusively to control of wheat leaf rust. Laboratory and greenhouse assays have indicated that this chemical is an excellent protectant and systemically active material, but a poor eradicant. It persists for long periods of time in the soil and a sufficient quantity is continually taken up by wheat root absorption to impart protection against leaf rust development (Rowell, personal correspondence).

Little has been published pertaining to mode of action of the chemicals known to be systemically fungitoxic to the cereal rusts. Most of the knowledge pertaining to the mode of action of chemicals used in this investigation is a result of the work done in other disease systems. How much of this may be pertinent to the wheat leaf rust system is not known.

Schmeling and von Kulka (32), in 1966, working with two 1,4 oxathiin derivatives, found that both of these materials were partially water soluble and appeared to be readily translocated in the xylem. They demonstrated that foliar or soil sprays were effective in the control of bean rust.

Snel and Edgington (33), in 1970, disclosed the mode of uptake, translocation, and decomposition of the systemic 1,4 oxathiin derivatives in the bean plant (Phaseolus vulgaris L.). They demonstrated that one, DMOC, was taken up by root absorption better than its sulphone analog DCMOD. Radioautography of plants treated via the roots or foliage with ^{14}C-labeled DCMOD indicated apoplastic movement which resulted in marginal accumulation of label in transpiring leaves. Analysis of root tissue indicated that the carboxamide linkage was hydrolized, producing aniline which was bound to plant polymers and also resulted in the formation of highly water soluble conjugation products.

Clemons and Sisler (5), in 1969, demonstrated the formation of a fungitoxic derivative from benomyl. They found that technical grade samples of the compound separated into two toxic components on silica gel (Absorbosil 1) thin layer chromatograms developed with ethyl acetate. Toxicity comparisons were made between the MBC derivative and technical grade benomyl using Sacchraromyces pastorians Han. as the assay organism. The tests revealed that ED_{50} dosage was 12 µg/ml of the derivative compared to 0.4 µg/ml for the technical grade benomyl. The highly unstable nature of benomyl in aqueous solutions would indicate that fungitoxicity at sites removed from the point of application should probably be attributed to the MBC derivative and not to benomyl as such.

Peterson and Edgington (28), in 1970, studied the transport of the systemic fungicide benomyl in bean plants and found that benomyl and its fungitoxic derivative (MBC) were taken up passively by the roots and transported to the leaf via the xylem. Accumulation of the compounds in the leaf tips and margins was observed. They concluded that the derivative was not phloem-mobile and that occurrence of it in bark tissue resulted from lateral transfer from the xylem.

Peterson and Edgington (27) recently have studied the differential transport and accumulation of benomyl in various plant organs. They found that the rate of transpiration of a given organ or tissue governed its ability to accumulate benomyl. They postulated that the marginal and apical accumulation of benomyl was a result of physical forces.

In any study of host-parasite interactions, even those when additive chemicals play a role, consideration must be given to environmental effects. Some studies of the effects of certain environmental characters have been made. Allen (1) states that the genotype of the plant ultimately determines its potentiality for becoming host to a virulent pathogen, but that this potentiality may be greatly influenced by the environment. Flor (8), in his definition of reaction type, brings into play the integral effect of the environment on the host-parasite interaction.

Newton and Johnson (25), in 1941, studied the effect of temperature and light on reaction type in several wheat leaf rust systems. Modification of temperature was demonstrated to have various effects on reaction type depending upon the specific system under study. Some were shifted toward lower reaction types when grown at low temperatures while others were shifted to higher reaction types. They concluded that temperature had a greater effect on reaction type than did light in their studies.

Williams and Johnston (41) showed that reaction types produced by some physiologic races of Puccinia recondita f. sp. tritici and seedlings of the standard wheat differential cultivars varied with temperature. As a result of their work, they recommended that three varieties be dropped from the differential set, since reaction types varied extensively with temperature variations regardless of the race used in the system.

Forward (9) established that various light photo-periods would alter the reaction type of some systems. He found that the modification was toward the lower reaction types when the periods of darkness were lengthened.

CHAPTER III

MATERIALS AND METHODS

Cultivars

Four cultivars of winter wheat, *Triticum aestivum*, commonly grown in Oklahoma, and possessing various degrees of resistance to leaf rust either in the seedling or adult growth stages were used in this investigation. They were:

Triumph 64 (Tmp 64) C.I. 13679 is one of the Triumph-type cultivars which for several years has dominated the wheat acreage of Oklahoma. This cultivar possesses no known genes for resistance to leaf rust, although it does survive and yield fairly well under moderate to heavy leaf rust infection indicating some degree of tolerance (42).

Scout 66 (Sut 66) C.I. 13996 is a cultivar becoming popular in many areas of the central plains. It possesses no known specific resistance genes but which may have some type of non-specific resistance, based on the fact that leaf rust severity has been consistently lower than that on Triumph-type cultivars in field plots in Oklahoma and elsewhere.

Sturdy (Sdy) C.I. 13684 is a relatively new cultivar that combines semi-dwarf stature and leaf rust resistance. This resistance is classified as specific resistance of the adult plant type. A mesothetic or "X" reaction type has commonly been observed in the seedling stage of growth.

Agent (Ag) C.I. 13523 is another new cultivar possessing at least one specific resistance gene. Its resistance is effective against all known leaf rust races in all plant growth stages.

Races

The races used in this investigation were isolates of races UN 2, UN 6, and UN 9. The isolate of race UN 6 is further characterized by virulence on cultivars Westar C.I. 12110 and Wesel C.I. 13090. It is referred to in this study as race UN 6B. These races have been prevalent in the central plains area for many years. For this reason they have been used extensively in screening for rust resistance. The specific isolates used in this investigation were taken from stock cultures maintained for screening purposes by the Department of Botany and Plant Pathology, Oklahoma State University. Race purity and identity were maintained by frequent inoculation of the requisite differential cultivars.

Chemicals

Three chemicals with systemic activity known to be effective in varying degrees for the control of *Puccinia recondita tritici* were used. Each of these chemicals represents a different family of fungicides. They were:

Triazol; (4-n-Butyl-1,2,4-triazol) a chemical tested under the experimental numbers RH-124 (Rohm and Haas Chemical Corp.) that has demonstrated outstanding control of the wheat leaf rust organism in both field and greenhouse trials. It is water soluble and an 80% aqueous solution was used.

Oxycarboxin; (2,3 dihydro-5-carboxanilido-6-methyl-4,4-dioxide) an emulsifiable concentrate formulation of 1 lb. active ingredient per gallon named "Plantvax" (Uniroyal Corp.). It also has been shown to be effective for leaf rust control in extensive greenhouse and field trials.

Benomyl; (1-(butylcarbamoyl)-2-benzimidizole carbamic acid) a wide spectrum systemic fungicide in a 50% wettable powder marketed as "Benlate" (Dupont Chemical Corp.). Effective control of leaf rust has been found under greenhouse conditions with this material but field trials have not been effective.

The chemicals were stored under controlled environmental conditions for the duration of the investigation. This was done to preserve their effectiveness.

The recommended application rate for each of these chemicals was as follows: triazol (RH-124) - one-half pound active ingredient per acre; oxycarboxin (Plantvax) - one pound active ingredient per acre; benomyl (Benlate) - one pound active ingredient per acre. In the tests where the application rate was a variable in the experiment, levels of one-fourth, one-half, and two times the recommended rate were used.

All chemical treatments were applied with an National Biochemical Company Universal Kit Aerosol Sprayer that used dichlorodiflouroethane as the propellent. Each sprayer was calibrated and labeled according to volume output per ten seconds for each chemical used. It was determined that ten ml of water carrier was adequate to provide thorough and uniform coverage of the test unit. Preparation of the treatments was based on this constant volume.

Experimental Design and Procedures

Each test unit consisted of a wooden flat with internal measurements of 15 inches by 20 inches with a surface area of 2.08 square feet. Twenty pounds of air dried soil was placed in each flat which produced a soil depth of approximately two inches. The soil was a 1:2:1 mixture of builder's sand, sandy-loam soil and sphagnum peat, respectively. Four equidistant one-inch deep furrows were made in the soil the length of the flat. These furrows were divided into four equal parts and ten seeds of each wheat cultivar were planted in each subdivided part in a randomized fashion so that each flat contained all four varieties replicated four times.

In each experiment one flat served as a check or control and was not treated with any chemical. Also, a single flat treated with each chemical at the recommended rate was used in each experiment as a check.

All test units were maintained in chambers having temperature and light control for the entirety of the investigation, with the exception of a 12-hour period during which they were inoculated and placed in moist chambers. The growth chambers were programmed for a 12-hour-light 12-hour-dark regime with 2700 ft-c at plant height. The lights were programmed so that the incandescent lights came on 15 minutes before the florescent lights and went off 15 minutes after the florescent lights. Flats were randomized within the growth chambers.

Temperature during the light cycle was 21 $\pm$ 2C and during the dark cycle it was 18 $\pm$ 2C throughout the investigations unless temperature was a variable in a specific experiment.

Soil moisture was maintained near optimum and plants never were subjected to moisture stress. Starting with the fifth day after planting, the flats were watered with a solution containing one tablespoon of Hyponex per gallon of water.

For inoculation with rust, flats of eight day-old plants were placed in moist chambers designed to maintain free moisture on leaf surfaces and thoroughly misted with water from a hand atomizer insuring thorough coverage of foliage. Each flat was then "brush inoculated" with a designated race. For this procedure a pot of approximately 50 seedlings of a susceptible wheat cultivar that was liberally infected with rust was shaken lightly over the entire flat to disperse the loose spores. The infected leaves were then used to brush the spores evenly and thoroughly over the surface of the leaves to be inoculated. The inoculated plants were then misted again and the tops of the moist chambers covered. The flats of inoculated plants remained in the moist chambers for twelve hours before being returned to the controlled environment chambers. These procedures typically produced a rust severity on the check plants of $\pm$ 40 per cent measured on the modified Cobb scale. An untreated pot of seedlings of the cultivar Cheyenne (susceptible to all races used) was placed in each chamber that had a chemically treated test unit and was inoculated along with the test unit. These pots served to monitor the severity of inoculation that the treated test unit had received. All inoculations were made in a room maintained at 18C.

CHAPTER IV

RESULTS

The first experiment was designed to observe the effect of the three chemicals used at recommended rates on each of the host-parasite combinations. This effect was measured by disease reaction type and comparisons also were made between treated and untreated or check combinations.

It is evident from the data obtained (Table I) that the chemicals used in this investigation did affect the host-parasite interaction. This effect was most noticeable in the fully compatible host-pathogen combinations. When these chemicals were applied at their respective recommended rates, they accounted for a complete reversal in reaction type from that produced in the untreated compatible combinations. For example, the cultivars Triumph 64 and Scout 66 performed as fully susceptible hosts to all races of the pathogen in the untreated systems, but in the treated systems a low reaction type was produced indicative of incompatible combinations. In all cases where the chemicals were used at the recommended rate, the severity of infection, as indicated by the number of infections per unit area, also was reduced (Table II).

Throughout this study, an occasional pustule was produced on the cultivars Triumph 64 and Scout 66 when these systems were treated with the recommended rates of either bemonyl or oxycarboxin. These pustules, smaller in size than observed in the untreated systems, were the same

TABLE I

REACTION TYPE OF CERTAIN WHEAT LEAF RUST HOST-PARASITE COMBINATIONS TREATED WITH THREE SYSTEMIC CHEMICALS AT THE RECOMMENDED RATE[1] APPLIED AS A SOIL SPRAY AT TIME OF PLANTING

Treatment	Rate	Physiologic Race	Cultivar			
			Tmp 64	Sut 66	Sdy	Ag
Untreated		2	4	4	X	0;
		6B	4	4	X	0;-1
		9	4	4	X	0;-1
Triazol	½ lb/acre	2	0;	0;	0;	0;
		6B	0;	0;	0;	0;
		9	0;	0;	0;	0;
Oxycarboxin	1 lb/acre	2	0;[2]	0;[2]	0;	0;
		6B	0;[2]	0;[2]	0;	0;
		9	0;[2]	0;[2]	0;[3]	0;
Benomyl	1 lb/acre	2	0;[2]	0;[2]	0;	0;
		6B	0;[2]	0;[2]	0;[3]	0;
		9	0;[2]	0;[2]	0;[3]	0;

[1]Recommended rates: triazol, ½ lb/acre; oxycarboxin, 1 lb/acre; benomyl, 1 lb/acre.

[2]Occasional small compatible type pustules observed.

[3]Occasional type 1 or type 2 pustules observed.

TABLE II

SEVERITY[1] OF LEAF RUST INFECTION ON CERTAIN WHEAT CULTIVARS TREATED WITH THREE SYSTEMIC CHEMICALS AT THE RECOMMENDED RATE[2] AND INOCULATED WITH THREE DIFFERENT RACES

Treatment	Rate	Physiologic Race	Cultivar			
			Tmp 64	Sut 66	Sdy	Ag
Untreated		2	40	40	40	10
		6B	40	40	40	15
		9	40	40	40	15
Triazol	½ lb/acre	2	Tr	Tr	Tr	Tr
		6B	Tr	Tr	Tr	Tr
		9	Tr	Tr	Tr	Tr
Oxycarboxin	1 lb/acre	2	2-5	2-5	2-5	Tr
		6B	2-5	2-5	2-5	Tr
		9	2-5	2-5	2-5	Tr
Benomyl	1 lb/acre	2	2-5	2-5	2-5	Tr
		6B	2-5	2-5	2-5	Tr
		9	2-5	2-5	2-5	Tr

[1]Based on modified Cobb scale.

[2]Recommended rates: triazol, ½ lb/acre; oxycarboxin, 1 lb/acre; benomyl, 1 lb/acre.

reaction type; similar to type 3 without the usual associated chlorosis. The cultivar Sturdy, on which the mesothetic reaction was typically produced in untreated combinations, had an occasional pustule which was type 1 or 2 in untreated combinations. No fruiting infections were observed with chemically treated systems involving the cultivar Agent nor with host-parasite combinations treated with triazol at the recommended rate.

Another test was then designed to observe the effect of different rates of each of the three chemicals studied. The recommended rates used in the first experiment were designated as x, and the rates used in this experiment were ¼x, ½x, and 2x. Disease response resulting from treatments made at these rates were then compared to the typical disease response produced in the untreated systems and to the response produced with the treatments at the recommended rate.

Varying the rates of application did effect the reaction type in certain combinations (Table III). The lowest rate (¼x) of benomyl, however, had no measurable effect on reaction type. The reaction types observed in systems treated with this rate of benomyl were the same as those observed in the untreated host-parasite combinations. Oxycarboxin at the ¼x rate likewise had no effect on reaction type in the combinations that involved Triumph 64, Scout 66, and Agent, but a slight change in reaction type (from "X" to "Y") in the combinations with Sturdy was noted. At the ¼x rate, triazol produced reaction types in all combinations very similar to those found when the recommended rate was used. The only deviation was the production of occasional small compatible pustules, whereas no such pustules were observed in any of the combinations treated at the recommended rate.

TABLE III

REACTION TYPE OF CERTAIN WHEAT LEAF RUST HOST-PARASITE COMBINATIONS TREATED WITH ONE-FOURTH, ONE-HALF, AND TWICE THE RECOMMENDED RATE x[1] OF THREE SYSTEMIC CHEMICALS APPLIED AS A SOIL SPRAY AT TIME OF PLANTING

Cultivar	Physiologic Race	Treatment and Rate												
		Untreated	Triazol				Oxycarboxin				Benomyl			
			¼x	½x	x	2x	¼x	½x	x	2x	¼x	½x	x	2x
Triumph 64	2	4	0[2]	0[2]	0;	0	4	0;	0;	0	4	0;[2]	0;[2]	0
	6B	4	0[2]	0[2]	0;	0	4	0;[2]	0;[2]	0	4	0;[2]	0;[2]	0
	9	4	0[2]	0[2]	0;	0	4	0;[2]	0;[2]	0	4	0;[2]	0;[2]	0
Scout 66	2	4	0;[2]	0;	0;	0;	4	0;	0;	0	4	0;[2]	0;[2]	0
	6B	4	0;[2]	0;	0;	0;	4	0;	0;[2]	0	4	0;[2]	0;[2]	0
	9	4	0;[2]	0;[2]	0;	0;	4	0;	0;[2]	0	4	0;[2]	0;[2]	0
Sturdy	2	X	0;[3]	0;	0;	0	Y	0;	0;	0	X	0;[3]	0;	0
	6B	X	0;[3]	0;	0;	0	Y	0;[3]	0;	0	X	0;[3]	0;	0
	9	X	0;[3]	0;	0;	0	Y	0;[3]	0;[3]	0	X	0;[3]	0;[3]	0
Agent	2	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;
	6B	0;[3]	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;
	9	0;[3]	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;

[1]Recommended rates: triazol, ½ lb/acre; oxycarboxin, 1 lb/acre; benomyl, 1 lb/acre.
[2]Occasional small compatible type pustules observed.
[3]The severity was reduced with these treatments compared to the check.

The ½x rate of all three chemicals affected the reaction type in a manner very similar to the effect observed when the recommended rates were used. However, occasional small compatible pustules occurred more frequently at the ½x rate than at the recommended rate.

The effect of doubling the recommended rate of application was to produce a near immune response in most host-parasite combinations. Zero fleck reaction types were observed only when triazol at 2x was used with the combinations involving Scout 66 and Agent. Benomyl at this rate caused a slight general chlorosis of the leaf. In several instances this resulted in a chlorotic leaf with small green flecks scattered over the surface. The frequency of these green flecks was comparable to that of the pustule severity recorded on the inoculum density check leaves.

Following this study, an experiment was made to test the effect of the time of application of the chemicals in relation to the time of inoculation. Application of the chemicals was made in the same manner as described above. Times of application were as follows: 96 hours prior to inoculation, 12 hours after inoculation, and 96 hours after inoculation. Contact of the spray materials directly on the plants was minimized by covering the plants with waxed paper hoods.

Time of application in relation to time of inoculation did effect the progression of infection in several combinations (Table IV). Application of the recommended rate of the test chemicals 96 hours prior to inoculation produced the same effect upon reaction type as did application at the time of planting.

Application of the recommended rate within twelve hours after inoculation had no affect upon the host-parasite interaction in any combination treated with benomyl or triazol compared to the reaction

TABLE IV

REACTION TYPE OF CERTAIN WHEAT LEAF RUST HOST-PARASITE COMBINATIONS TREATED WITH THREE SYSTEMIC CHEMICALS AT THE RECOMMENDED RATES[1] APPLIED AT DIFFERENT TIMES WITH RESPECT TO INOCULATION

Treatment	Physiologic Race	Cultivar and Time of Application											
		96 hrs before inoculation				12 hrs after inoculation				96 hrs after inoculation			
		Tmp 64	Sut 66	Sdy	Ag	Tmp 64	Sut 66	Sdy	Ag	Tmp 64	Sut 66	Sdy	Ag
Untreated	2	4	4	X	0;	4	4	X	0;	4	4	X	0;
	6B	4	4	X	0;	4	4	X	0;	4	4	X	0;
	9	4	4	X	0;	4	4	X	0;	4	4	X	0;
Triazol	2	0;	0;	0;	0;	4	4	X	0;	4	4	X	0;
	6B	0;	0;	0;	0;	4	4	X	0;	4	4	X	0;
	9	0;	0;	0;	0;	4	4	X	0;	4	4	X	0;
Oxycarboxin	2	0;[2]	0;[2]	0;[3]	0;	0;[2]	0;[2]	0;[3]	0;	4	4	X	0;
	6B	0;[2]	0;[2]	0;[3]	0;	0;[2]	0;[2]	0;[3]	0;	4	4	X	0;
	9	0;[2]	0;[2]	0;[3]	0;	0;[2]	0;[2]	0;[3]	0;	4	4	X	0;
Benomyl	2	0;[2]	0;[2]	0;	0;	4	4	X	0;	4	4	X	0;
	6B	0;[2]	0;[2]	0;	0;	4	4	X	0;	4	4	X	0;
	9	0;[2]	0;[2]	0;	0;	4	4	X	0;	4	4	X	0;

[1]Recommended rates: triazol, ½ lb/acre; oxycarboxin, 1 lb/acre; benomyl, 1 lb/acre.

[2]Occasional small compatible type pustules observed.

[3]Occasional type 1 or type 2 pustules observed.

types produced by the same combinations untreated. Application of oxycarboxin at this time, however, produced a disease response identical to that produced by an application of this chemical four days prior to inoculation.

There was no effect on the reaction type observed in the systems to which the recommended rate of the chemical was applied four days after inoculation. All host-parasite combinations produced a disease reaction typical of that combination in untreated systems.

The effect of temperature on disease response of both treated and untreated host-parasite combinations was studied. Comparisons were made between the disease response of systems grown at 21C during the light phase and those grown at 27C during the light phase. In both cases, the dark phase temperature was 18C. The rates of chemicals used in this test were the recommended rate x and ¼x, ½x, and 2x. As in the first tests, the chemicals were applied to the soil immediately following planting.

In the untreated systems, a differential response to temperature was observed only with Sturdy where a mesothetic reaction type was found at 21C and a 0;-2 type at 27C with all races (Table V). This cultivar also was the only one with which an obvious response to temperature was observed in the treated systems. A characteristic type "X" reaction occurred when benomyl at ¼x was used at 21C, but at 27C a type "Y" reaction was found with 0;-2 reaction types occurring at the tip and three to four pustule types occurring at the base of the leaf. Similarly, with oxycarboxin at ¼x at 21C, a type "Y" reaction developed in combinations involving Sturdy, but at 27C at the same rate a type zero fleck (0;) reaction developed.

TABLE V

REACTION TYPE OF CERTAIN WHEAT LEAF RUST HOST-PARASITE COMBINATIONS TREATED WITH FOUR RATES[1] OF THREE SYSTEMIC CHEMICALS APPLIED AS A SOIL SPRAY AT PLANTING TIME AND GROWN AT TWO TEMPERATURES

Cultivar	Physiologic Race	Treatment, Temperature (light phase), and Rate																									
		Untreated		Triazol								Oxycarboxin								Benomyl							
		21C	27C	21C				27C				21C				27C				21C				27C			
				¼x	½x	x	2x	¼x	½x	x	2x	¼x	½x	x	2x	¼x	½x	x	2x	¼x	½x	x	2x	¼x	½x	x	2x
Tmp 64	2	4	4	0;	0;	0;	0	0;	0;	0;	0	4	0;[2]	0;	0	4	0;[2]	0;	0	4	0;[2]	0;[2]	0	4	0;[2]	0;[2]	0
	6B	4	4	0;	0;	0;	0	0;	0;	0;	0	4	0;[2]	0;[2]	0	4	0;	0;	0	4	0;[2]	0;[2]	0	4	0;[2]	0;	0
	9	4	4	0;	0;	0;	0	0;	0;	0;	0	4	0;[2]	0;	0	4	0;	0;	0	4	0;[2]	0;[2]	0	4	0;[2]	0;[2]	0
Sut 66	2	4	4	0;[2]	0;	0;	0	0;	0;	0;	0	4	0;[2]	0;[2]	0	0;[2]	0;[2]	0;	0	4	0;[2]	0;[2]	0	4	0;[2]	0;[2]	0
	6B	4	4	0;[2]	0;	0;	0	0;	0;	0;	0	4	0;[2]	0;	0	0;[2]	0;[2]	0;	0	4	0;[2]	0;[2]	0	4	0;[2]	0;[2]	0
	9	4	4	0;[2]	0;	0;	0	0;	0;	0;	0	4	0;[2]	0;	0	0;[2]	0;[2]	0;	0	4	0;[2]	0;[2]	0	4	0;[2]	0;[2]	0
Sdy	2	X	0;-2	0;[3]	0;	0;	0	0;	0;	0	0	Y	0;[3]	0;[3]	0	0;[3]	0;[3]	0	0	X	0;[3]	0;[3]	0	0;[3]	0;[3]	0	0
	6B	X	0;-2	0;[3]	0;	0;	0	0;[3]	0;	0;	0	Y	0;[3]	0;[3]	0	0;[3]	0;[3]	0	0	X	0;[3]	0;[3]	0	0;[3]	0;[3]	0	0
	9	X	0;-2	0;[3]	0;	0;	0	0;[3]	0;	0	0	Y	0;[3]	0;[3]	0	0;[3]	0;[3]	0	0	X	0;[3]	0;	0	0;[3]	0;[3]	0	0
Agent	2	0;[3]	0;	0;	0;	0;	0;	0;	0;	0;	0	0;	0;	0;	0	0;	0;	0	0	0;	0;	0;	0	0;	0;	0;	0
	6B	0;[3]	0;[3]	0;	0;	0;	0;	0;	0;	0;	0	0;	0;	0;	0	0;	0;	0	0	0;	0;	0;	0	0;	0;	0;	0
	9	0;[3]	0;	0;	0;	0;	0;	0;	0;	0;	0	0;	0;	0;	0	0;	0;	0	0	0;	0;	0;	0	0;	0;	0;	0

[1]Recommended rates: triazol, ½ lb/acre; oxycarboxin, 1 lb/acre; benomyl, 1 lb/acre.

[2]Occasional small compatible type pustules observed.

[3]Occasional type 1 or type 2 pustules observed.

Another major differential response to temperature was observed with Scout 66 treated with oxycarboxin at ¼x. A type 4 response was found at 21C and a 0; response at 27C. In two instances involving Agent, oxycarboxin at the recommended rate and triazol at twice the recommended rate, a 0; reaction was produced at 21C, but in both cases an immune reaction developed at 27C. In all cases observed, the production of an occasional pustule of higher than normal reaction types was observed less frequently in the treated systems at the higher temperature.

Throughout the study, occasional small but compatible type pustules developed in treated systems where the vast majority of reaction types were low or indicative of incompatible systems. It seemed relevant to check the viability of the spores produced in such pustules. Therefore, spores from these small pustules were transferred to healthy untreated seedlings of the cultivar Cheyenne, a susceptible check cultivar. The plants were inoculated in the usual manner described previously. In all cases, abundant infection of the inoculated plants occurred with a normal high infection type indicative of compatible systems (Table VI).

TABLE VI

SEVERITY AND REACTION TYPE PRODUCED ON THE CULTIVAR CHEYENNE INOCULATED WITH SPORES FROM UNUSUAL PUSTULES DEVELOPED IN CERTAIN TREATED SYSTEMS

Pustule Source		Severity	Reaction Type
Benomyl	½x[1]	10	4
	x	10	4
Oxycarboxin	½x	10	4
	x	10	4
Triazol	¼x	10	4
	½x	10	4

[1]x = recommended rates as follows: triazol, ½ lb/acre; oxycarboxin, 1 lb/acre; benomyl, 1 lb/acre.

CHAPTER V

DISCUSSION

Application of the recommended rate of the chemicals used in this investigation did effect disease reaction type in most host-parasite combinations as well as disease severity. However, the cultivars used did not have a differential effect on the races in the untreated system, and treatment with these chemicals failed to induce any differential response. The effect of treatment at the recommended rate was to induce a lower infection type indicative of a more resistant host, a less virulent parasite, or both. Hosts possessing no known genes for resistance to leaf rust exhibited the greatest over-all change in reaction type. Triumph 64 and Scout 66 were fully compatible with all test races in untreated systems but treatment with these chemicals changed the reaction to a completely incompatible system, indicative of a host possessing a gene or genes for specific resistance, an avirulent pathogen, or both.

The effect of triazol was the same regardless of the rate of applications used in this study. Benomyl and oxycarboxin, however, failed to induce any appreciable change in reaction on type at one-fourth of the recommended rates for these chemicals. In all cases increasing the rates of application produced a more incompatible type of reaction. Also, in all treated systems at all rates a few pustules developed on infected plants that had a higher infection type than usual for that system. Increasing the rates of application tended to reduce the number of such pustules.

Altering the time of treatment in relation to time of inoculation also affected the progress of infection in several host-parasite combinations. Applications four days prior to inoculation produced the same effect as applications at planting time. When applied within 12 hours after inoculation, the effect was dependent upon the material used. Neither benomyl nor triazol had any observable effect upon the progress of infection applied at this time. Oxycarboxin applied within 12 hours after inoculation produced nearly the same effect as it did when applied at planting time, or four days prior to inoculation. It is evident that this material moves into the plant and becomes effective at a much more rapid rate than the others.

Increasing the temperature at which these systems were held during development affected disease response by a general shift toward lower infection types. Also, observable signs of infection were less common in systems held at the higher temperature. An intermediate reaction type ("X" or "Y") was produced on the cultivar Sturdy treated with either benomyl or oxycarboxin at one-fourth the recommended rate when incubated at 21C but incubated at 27C a zero fleck (0;) reaction type was typically produced regardless of the race involved in the system. None of the other host-parasite systems exhibited such a drastic shift in response.

As stated previously, occasional pustules were found in certain treated systems even though most of the infections did not develop a fruiting lesion. Spores from such pustules were tested for viability using an untreated susceptible cultivar (Cheyenne). Regardless of treatment system from which the spores were obtained, they appeared to possess the same viability as stock cultures of the same race. The occurrence of these occasional pustules in certain treated systems could result from a mechanism similar to that involved in the "X" reaction in some untreated systems, such as those involving the cultivar Sturdy in this study.

CHAPTER VI

SUMMARY

1. Recommended rates of triazol, benomyl, and oxycarboxin applied as a soil amendment at planting time altered both the disease reaction type and the disease severity of wheat leaf rust.
2. Reaction types of compatible systems were altered by chemical treatment more than normally incompatible systems.
3. Reaction types shifted toward the lower types and disease severity was lowered as rates were increased.
4.
    a. Recommended rates of the chemicals used as soil amendments applied 96 hours prior to inoculation altered disease reaction type in a manner similar to treatments applied at planting time.
    b. The effect of applications 12 hours after inoculation was dependent upon the chemical involved. Oxycarboxin was effective in lowering infection types but benomyl and triazol were not.
    c. None of the chemicals applied as soil amendments 96 hours after inoculation altered disease reaction type.
5. Infected plants held at 27C had lower disease reaction types than those held at 21C.
6. Spores formed in occasional pustules produced in certain treated systems were virulent on the susceptible cultivar Cheyenne.

LITERATURE CITED

(1) Allen, P. J. 1954. Physiological aspects of fungus diseases of plants. Ann. Rev. Plant Phy. 5:225-248.

(2) Arthur, J. C. 1929. The plant rusts. John Wiley and Sons, New York. p. 30-72.

(3) 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.).

(4) Chester, K. Starr. 1947. Nature and prevention of plant diseases. Second edition. McGraw and Hill Book Co., Inc. p. 34.

(5) Clemons, G. P., and H. D. Sisler. 1969. Formation of a fungi-toxic derivative from Benlate. Phytopathology 59:705-706.

(6) Daly, J. M. 1949. The influence of nitrogen source on the development of stem rust of wheat. Phytopathology 39:387-94.

(7) Dickson, J. G., G. R. Grimm, and A. L. Hooker. 1952. Stem rust control with fungicides. Phytopathology 42:465 (Abstr.).

(8) Flor, H. H. 1959. Complementary gene interaction in the rusts. Recent advances in Botany. Toronto: University of Toronto Press. pp. 567-571.

(9) Forward, D. F. 1932. The influence of altered host metabolism upon modification of the infection type with *Puccinia graminis tritici* p. f. 21. Phytopathology 22:498-555.

(10) Gottlieb, D., and H. Hart. 1948. Growth substances and the rust fungi. Phytopathology 33:724-28.

(11) Hacker, R. G., and J. R. Vaughn. 1957. Chemically induced resistance to stem rust by derivatives of cyclohexamide. Plant Disease Reptr. 41:442-46.

(12) Hart, Helen. 1949. Morphologic and physiologic studies on stem rust resistance in cereals. Ann. Rev. of Microbiology 3:289-316.

(13) __________. 1931. Morphologic and physiologic studies on stem rust resistance in cereals. U.S.D.A. Tech. Bul. 266.

(14) Haskett, W. C., and C. O. Johnston. 1958. Chemical control of stem and leaf rust of wheat in Kansas. Plant Disease Reptr. 42:5-14.

(15) Heyne, E. C., and C. O. Johnston. 1954. Inheritance of leaf rust reaction and other characters in crosses among Timstein, Pawnee, and Red Chief wheats. Agronomy Journal 46:81-85.

(16) Hotson, H. H. 1952. The inhibition of wheat stem rust by sulfadiazine and the reversal of this effect by para-amino benzoic acid and folic acid. Phytopathology 42:11 (Abstr.).

(17) Johnston, C. O., and L. E. Browder. 1966. Seventh revision of the internation register of physiologic races of Puccinia recondita f. sp. tritici. Plant Disease Reptr. 50:756-760.

(18) Knightlinger, C. V., and H. H. Whetzel. 1926. Second report on dusting for cereal rusts. Phytopathology 16:64 (Abstr.).

(19) Lambert, E. B., and E. C. Stakman. 1926. Effect of sulfur dust on the development of black stem rust of wheat in a natural epidemic. Phytopathology 16:64 (Abstr.).

(20) Livingston, J. E. 1953. The control of leaf and stem rust of wheat with chemotherapeutants. Phytopathology 43:496-99.

(21) Loegering, W. Q., and H. R. Powers, Jr. 1962. Inhibitance of pathogenicity in a cross of physiological races III and 36 of Puccinia graminis f. sp. tritici. Phytopathology 52:547-554.

(22) Mains, E. B., and H. S. Jackson. 1926. Physiologic specialization in the leaf rust of wheat Puccinia triticina Erikss. Phytopathology 16:89-120.

(23) ____________ and ____________. 1921. Two strains of Puccinia triticina on wheat in the United States. Phytopathology 11:40 (Abstr.).

(24) Melchers, L. E. 1917. Puccinia triticina Erikss. leaf rust of winter wheat causes damage in Kansas. Phytopathology 7:224.

(25) Newton, M., and T. Johnson. 1941. Environmental reaction of physiologic race of Puccinia triticina and their distribution in Canada. Canada J. Res. C. 19:121-133.

(26) Peterson, B., F. R. Forsyth, and C. B. Lyon. 1958. Chemical control of cereal rusts II. Control of leaf rust of wheat with experimental chemicals under field conditions. Phytopathology 48:655-57.

(27) Peterson, C. A., and L. V. Edgington. 1971. Transport of benomyl into various plant organs. Phytopathology 61:91-92.

(28) Peterson, C. A., and L. V. Edgington. 1970. Transport of the systemic fungicide benomyl in bean plants. Phytopathology 60:475-478.

(29) Rowell, J. B. 1968. Chemical control of the cereal rusts. Ann. Rev. Phyto. 6:242-263.

(30) ____________. 1967. Control of leaf and stem rust of wheat by an 1,4 oxathiin derivative. Pl. Dis. Reptr. 51:336-39.

(31) Ryker, T. C. 1967. New fungicide for experimental testing. Dupont Information Bulletin. p. 2.

(32) Schmeling, B., and M. von Kulka. 1966. Systemic fungicidal activity of 1,4-oxathiin derivatives. Science 152:659-60.

(33) Snel, M., and L. V. Edgington. 1970. Uptake, translocation, and decomposition of systemic oxathiin fungicides in bean. Phytopathology 60:1708-1716.

(34) Stakman, E. C. 1936. The problem of specialization and variation in phytopathogenic fungi. Genetica XVIII:372-89.

(35) ____________, M. N. Levine, and W. Q. Loegering. 1944. Identification of physiologic races of <u>Puccinia graminis tritici</u>. U.S.D.A. Agr. Res. Adm., Bureau of Entomology and Plant Quarentine.

(36) ____________, and ____________. 1922. The determination of biologic forms of <u>Puccinia graminis</u> on Triticum spp. Minn. Agr. Expt. Sta. Tech. Bul. 8, p. 10.

(37) ____________, and F. J. Piemeisel. 1917. Biologic forms of <u>Puccinia graminis</u> on cereals and grasses. Jour. Agr. Res. 10:429-495.

(38) Thatcher, F. S. 1939. Osmotic and permeability relations in the nutrition of fungus parasites. Am. J. Botany 26:449-458.

(39) van der Plank, J. E. 1968. Disease resistance in plants. Academic Press. pp. 9-11.

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

(41) Williams, E. Jr., and C. O. Johnston. 1965. Effect of certain temperatures on identification of physiologic races of <u>Puccinia recondita</u> f. sp. <u>tritici</u>. Phytopathology 55:1317-19.

(42) Young, H. C., Jr. 1967. Variation in virulence and its relation to the use of specific resistance for the control of wheat leaf rust. Proceedings, International Symposium on Plant Pathology. Indian Phytopathology. New Delhi.

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