REPRODUCTIVE POTENTIALS OF RACES 15B-1 (CAN.) AND 56 OF WHEAT STEM RUST

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND

RESEARCH IN PARTIAL FULFILMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

UNIVERSITY OF MANITOBA

1966

ACKNOWLEDGEMENT

The author wishes to express his gratitude to Dr. G. J. Green, Canada Department of Agriculture Research Station in Winnipeg, for suggesting the problem, providing the materials, and for his guidance and encouragement throughout the investigation and the writing of the manuscript; to Dr. T. Johnson for advice and suggestions on various aspects of the problem, and the writing of the manuscript; to Dr. R. C. McGinnis for encouragement throughout the investigation and the writing of the manuscript; to Dr. P. K. Isaac for advice and for suggestions in the preparation of the manuscript. Sincere thanks are also extended to the National Research Council of Canada for financial support.

ABSTRACT

Race 56 of wheat stem rust, Puccinia graminis Pers. f. sp. tritici Erikss. & Henn., was found to have superior competitive ability than race 15B-1 (Can.) when they were cultured in mixtures on the varieties Little Club, Red Bobs, and Marquis for 11 generations. The superiority of race 56 was most evident at high temperatures (20° and 25°). Race 15B-1 (Can.) was a much better competitor at 15°C than at 20° and 25°C.

Relative competitive abilities were found to be influenced by the density of pustules on infected leaves. On heavily infected plants of Little Club and Red Bobs at 15° and 20°C race 15B-1 (Can.) usually predominated over race 56 but on lightly infected plants race 56 predominated.

The germinability of urediospores of race 15B-1 (Can.) was slightly superior to race 56 on water agar but there was no difference on wheat leaves. There was not much difference in the longevity of spores of the two races. Spores of both races lost viability quickly at 25°C.

The successful rate of infection by single spores on Little Club seedlings was the same for races 15B-1 (Can.) and 56. The infective ability of race 56, however, was generally higher than that of race 15B-1 (Can.) on the wheat varieties Little Club, Red Bobs, Marquis, and Mindum. Marquis wheat was generally the most readily infected by the both races. Generally, the higher the temperature at which inoculated seedlings were kept, the fewer pustules developed on the plants.

The rate of growth of pustules on Little Club wheat grown at 15° and 20°C was determined by measurement with a microscope. During the early stages of rust development, the pustules of race 56 grew faster than those of race 15B-1 (Can.). Pustules of race 15B-1 (Can.), however, grew more rapidly in later stages and were larger than those of race 56 in the final stage of rust development.

Pustules of both races were smaller on heavily infected plants than on lightly infected plants.

The incubation period of race 56 was shorter than that of race 15B-1 (Can.) at 15° and 20°C. Incubation periods were shorter at 20°C than at 15°C.

Race 56 produced more urediospores more rapidly than race 15B-1 (Can.) at 15°, 20°, and 25°C.

There was no antagonistic effect between races 15B-1 (Can.) and 56 in this experiment.

The reproductive potentials of races 15B-1 (Can.) and 56 of wheat stem rust are discussed.

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INTRODUCTION

Physiologic races of various plant pathogenic fungi continuously vary in prevalence in nature. It is well-known that the introduction of new resistant varieties plays an important part in bringing about such changes. But it is also well-known that the prevalence of physiologic races changes in periods when the varieties of the host crop are static. Alterations in race prevalence that cannot be attributed to the reaction of crop varieties must be brought about by other environmental factors interacting with the capabilities of the races to develop and sporulate. Little is known about the these interactions and their effect on the competitive abilities of races in nature.

Changes in the prevalence of races 15B-1 (Can.) and 56 of wheat stem rust, *Puccinia graminis* Pers. f. sp. *tritici* Erikss. & Henn., illustrate many aspects of the problem. Race 56 was first found in Canada in 1928. It became the predominant race in 1934 and remained so until 1949 despite the widespread use of varieties resistant to it after 1935. In 1950, a new race, 15B, suddenly displaced it for the obvious reason that 15B could attack all the resistant varieties of bread wheat and durum wheat grown in the Great Plains region of North America at that time. In 1953, the variety Selkirk, with resistance to both races, was released and soon became the predominant variety in the rust area of Western Canada and in the spring wheat area of north-central United States.

Soon after the introduction of Selkirk the prevalence of race 15B declined and race 56 again became predominant despite the advantage of broader host range favoring race 15B.

The ability of the cereal rusts to change and produce new races, some of which can attack widely grown resistant varieties, poses plant breeders and pathologists with a continuing problem in controlling the cereal rusts. But new races that appear to be equally threatening from the standpoint of virulence do not have equal abilities to increase and menace crops. Several races, such as 29-1 (Can.), 15B-3 (Can.) and 15B-5 (Can.) can attack Selkirk but they were unable to develop and threaten crops in the Great Plains region of North America. Nevertheless, when they were discovered they were viewed as serious potential threats and breeding programs were initiated to counteract them. If the factors, other than virulence, that govern the prevalence of races in the field were understood much time and effort could be saved. These factors, therefore, have great practical importance.

A knowledge of how environmental conditions affect the competitive abilities of different rust races would be helpful, also, in understanding the epidemiology and ecology of the rusts. Studies on the competitive ability of rust races reported in the literature have been concerned mainly with the ability of races to increase at the expense of other races in mixed uredial infections over several generations. Little is known about the factors that make one race a better competitor than another in a mixture.

This investigation was undertaken to elucidate some of the factors that may have been responsible for the increase of race 56 and the decline of race 15B after 1953. The factors governing the competitive ability of races in mixed uredial infections under artificial conditions can be broadly classified into two groups - 1) the inherent capabilities of the races or internal factors and 2) environmental conditions. The internal factors include urediospore viability, infective capability, rate of growth, and sporulating capacity. Environmental factors include host variety, infection density, and temperature and other meteorological conditions. The inherent capabilities of races 15B-1 (Can.) and 56 of wheat stem rust were compared under several different environmental conditions to learn what factors favored one race or the other and thus explain the marked changes in their prevalence in the field.

REVIEW OF LITERATURE

Studies on the relative survival ability in mixed infections of races of pathogenic fungi and studies on the reproductive potential of cereal rust races have been reported in the literature. In this review, only papers considered relevant to this investigation will be discussed.

In studies on competitive ability in wheat stem rust, *P. graminis* f. sp. *tritici*, Watson (59) found that race 34 always grew well and maintained itself, or increased, in percentage of the mixture when associated with races 17, 19, 56, and 147, whereas race 147 was always virtually eliminated from such mixtures after several uredial generations. He suggested that the amount and character of each race in mixture, the effect of temperature on the fungus, and the variety on which the mixture was cultured might be responsible for the changes.

Loegering (38) reported that, in a mixture of races 17 and 56 on the wheat varieties Fulcaster and Little Club, race 17 became predominant by a wide margin, while on Ceres it predominated only slightly. In a mixture of races 17 and 19, on Fulcaster and Little Club, race 17 predominated after a very few generations, while on Mindum race 17 predominated only slightly after six generations.

Bromfield and Broyles (3) found by studying survival rates in mixed cultures that the survival ability of many races might be determined by comparing races in mixtures with one or more marker races.

In leaf rust of wheat, *Puccinia recondita* Rob. ex Desm., Irish (23) found that race 58 is the weakest competitor of races 9, 15, 58, and 126 on the variety Cheyenne.

Hassebrauk (19) reported that race 52 of leaf rust predominated over race 20 in mixed infections on Michigan Amber for 50-60 uredial generations.

In studying mixtures of races of *Phytophthora infestans* (Mont.) de Bary, Black (1) found that the wider the host range of a race, the less prolific it was on varieties susceptible to many races, and the lower its survival values in competition with races with narrower host ranges. These results were supported by Thurston and Eide (57, 58) in studying *P. infestans*. They reported that an isolate from the resistant variety Cherokee lacked survival ability when grown on the susceptible variety Cobbler in mixtures with a field isolate.

Thurston (56) found that race 0 of *P. infestans* predominated or entirely displaced the other races with which it was mixed after perpetuation of the mixture on susceptible potato clones for 2-9 generations. When compared singly, isolates of race 0 were usually more infectious than the other races. He concluded that survival ability is the result of a complex of factors, and suggested that these factors may be influenced by the various interactions of host, parasite, and environment; and probably include ability of a fungus to overseason; to grow after overseasoning; to sporulate; to survive dissemination until arrival on a susceptible host; to germinate rapidly; and finally to penetrate and infect the host.

Rodenhiser and Holton (46), in studying differences in capacity for survival in interspecific and inter-race mixtures of *Tilletia caries* (DC.) Tul. and/or *T. foetida* (Wallr.) Liro, found that the species and races of the bunt fungi differ in their ability to develop in a susceptible host in mixed populations. They reported that no explanation can be offered for the demonstrated differential ability of species and races of *T. caries* and *T. foetida* to survive passage through the host in combination with each other. Rate of spore-germination may be at least a contributing factor in some cases. Antagonistic effects on mycelial growth apparently had no influence.

Cassell (7) studied the effect of temperature on urediospore germination and germ tube development of several races of wheat stem rust. He found that, in general, 20°C was most favorable for spore-germination. The races did not behave alike. Spores of race 34 germinated better than those of the other races over a wide range of temperature, but germ-tube growth was poorest at 20°C. Germ-tubes of race 56 developed faster than those of all other races at 20°C, closely followed in this respect by race 11 and race 38. He concluded that on the basis of spore-germination, race 36 was one of those best adapted to high temperature and was only partially tolerant to cold, race 56 was next to race 36 in its ability to develop at high temperature and was the least adapted to cold, and races 38 and 11 were best able to tolerate low temperature.

Manners (39), in studying *Puccinia glumarum* (Schm.) Erikss. & Henn., found that urediospores of race 2 germinate less well than those of races 5 or 8, other races being intermediate, and that the optimum temperature for spore-germination of race G (isolated from Dactylis glomerata L.) is 22.5°C, that of all other races is 10-13°C.

Line and Bugbee (37) compared the germination ability of isolates of race 15B of wheat stem rust selected at low incubation temperature (4-5°C.) for more than 20 generations and nonselected isolates. At 3-5°C, urediospores of selected isolates germinated sooner, produced longer germ tubes, and were higher in percentage of germination than spores of nonselected isolates of races 15B and 56. At 20-25°C, urediospores of nonselected isolates germinated better. When urediospores of isolates selected at 4-5°C were transferred for 4 and 8 generations at 20-25°C, they germinated better at 3-5°C than spores of isolates always transferred at 20-25°C, but less well than spores of selected isolates always transferred at 4-5°C.

In studying infectibility of hosts to rust, Hayden (20) found that the spread of race 15B differed on the wheat varieties Lee, Marquis, Mida, Carlton, Nugget, and Sentry. Rust severity reached 35-50% in an area approximately 3 feet in diameter on Lee but the area of spread was more extensive and severity was greater on Marquis and Mida. Determinations of temperature range or moisture requirements for infection of seedlings of individual varieties which might account for differences in infectibility and rate of development of rust to epidemic proportions were inconclusive.

In studying the effect of temperature on the development of rust races, Melander (40) found that physiologic races of wheat stem rust differed in ability to produce uredia at low temperature. At 0-1°C, race 36 produced normal uredia readily, races 15 and 35 produced only a few minute pustules.

Johnson and Newton (26) showed that physiologic races of the cereal rusts differ considerably in their response to high temperatures, and the higher the temperature at which the host plants were kept, the fewer pustules developed on the plants. Cassell (6), in comparative tests between races 36 and 56 of wheat stem rust on Ceres wheat, found great difference at different temperatures. Race 36 caused the heaviest infection at moderate to low temperatures, while race 56 caused the heaviest infection at moderate to high temperatures. He also reported that mycelia of race 15B survived in the host for 85 days at low temperatures, and for 42 days at high temperatures.

Yarwood (60) first reported acquired immunity to rust after studies in which he inoculated plants a second time with the same or with different rust species. He stated that a gaseous metabolite of the pathogen is responsible for immunization, and that immunization is restricted to the germination and penetration phases of the infection. He found that the tissue adjacent to old uredial infections of bean rust, *Uromyces phaseoli* (Rebent.) Wint. on *Phaseolus vulgaris* L., was not infected by the second inoculation. The zone of inhibition was wider distally than proximally from the infected area, and its width varied directly with the age of the first infection. He concluded that a gaseous substance formed by *U. phaseoli* is toxic to the germinating spores of certain rusts but is not toxic to certain other plant pathogens.

In double inoculations with different rusts, Yarwood (61) found that when adequate dosages of urediospores of *U. phaseoli* were placed on sunflower leaves before inoculation of the leaves with *Puccinia helianthi* Schw., or along with the inoculum, the sunflower leaves were protected from infection. Johnston and Huffman (34) pointed out that on leaf rust susceptible wheat seedlings inoculated with urediospores of oat crown rust prior to inoculation with the wheat leaf rust, the leaf rust pustules were fewer in number and of a different infection type than were those on similar plants inoculated only with the leaf rust. They suggested that local antagonism may be one of contact inhibition by the action of substances produced by the latent mycelium of an organism that was not able to establish itself parasitically, or that it may result from a reduction in the number of possible infection courts caused by killing or plugging of many of the stomata.

MATERIALS AND METHODS

Isolates of races 15B-1 (Can.) and 56 of wheat stem rust, *P. graminis* f. sp. *tritici*, obtained from rust collections made in Western Canada and a grayish-brown isolate of race 15 obtained in hybridization studies in the greenhouse, were supplied by Dr. G. J. Green, Canada Department of Agriculture Research Station, Winnipeg. Monourediospore cultures were established and used in this study to avoid errors resulting from the use of cultures consisting of more than one genotype. The cultures were established by picking up a single spore on a sterile needle under a microscope and placing it on a seedling leaf of the wheat variety Little Club. Seedlings inoculated in this way were incubated under lantern chimnies in a growth chamber at 20°C for 24 hours. The lantern chimnies were then removed and the results of inoculation were observed 10 days later. The number of successful infections was 2.3 per cent for race 15B-1 (Can.) (15 infected plants/640 plants inoculated), and also 2.3 per cent for race 56 (15 infected plants/655 plants inoculated). The percentage of single spore infections of the grayish-brown isolate was not calculated. The grayish-brown culture was used, in addition to races 15B-1 (Can.) and 56, because in studying competitive ability in spore-mixtures, uredia of races 15B-1 (Can.) and 56 are not distinguishable on varieties susceptible to both races, while the grayish-brown race can be distinguished from races 15B-1 (Can.) and 56 on the susceptible varieties. All the cultures were grown on Little Club seedlings in different growth chambers to avoid contamination, and occasionally their purity was confirmed by testing on the differential host varieties.

Seeds of the wheat varieties Little Club, Red Bobs, Marquis, Mindum, Spelmar, and Arnautka were obtained from stocks at the Canada Department of Agriculture Research Station, Winnipeg. The varieties Little Club, Red Bobs, and Marquis are susceptible to the three rust cultures used but Mindum, Spelmar, and Arnautka are resistant to race 56 and susceptible to race 15B-1 (Can.) (Table 1).

Table 1. Rust reaction of five wheat varieties to three races of stem rust.

Race	Little Club	Marquis	Red Bobs	Mindum	Arnautka
15B-1 (Can.)	4	4-	4	4=	4=
56	4	3+	4	1=	1=
15 (grayish-brown mutant)	4	4-	4	4=	4=

The seedlings used in the experiments were grown in 4 inch clay pots in a growth chamber at 20°C, and in a few cases at 25°C, at the Canada Department of Agriculture Research Station. Temperatures in the growth chambers varied ± 1°C. Relative humidity was maintained at about 40% (20-60%). Light, supplied 16 hours per day by cool white fluorescent tubes, was about the same intensity in all growth chambers.

The varieties Little Club and Red Bobs were used because they are completely susceptible to the races used, while Marquis was slightly more susceptible to race 15B-1 (Can.) than to race 56. Mindum, Arnautka, and Spelmar were used to determine the proportions of races 15B-1 (Can.) (susceptible) and 56 (resistant) in urediospores harvested from mixed infections.

In most experiments, seedlings in the first leaf stage were inoculated by spraying with a suspension of urediospores in water containing 1 per cent polyoxyethylene sorbitan monolaurate (Tween 20). The addition of Tween 20 was necessary to suspend the spores uniformly in the water and to produce a uniform infection on the seedlings. The suspensions were prepared by adding weighed quantities of urediospores of the different cultures to the water-Tween 20 solution. Throughout the tests care was taken to apply approximately the same amount of suspension to each plant although the amount was not measured. The seedlings inoculated were incubated for 24 hours under glass lamp chimnies in the growth chambers used for the particular experiment and then the lamp chimnies were removed. A second method of inoculation, used mainly for increasing cultures, was the application of spores to seedlings with carefully washed fingers. Seedlings were sprayed with water containing 1% Tween 20, and the spores were applied by drawing the moistened leaves between carefully washed thumb and forefinger. The seedlings were incubated as described above.

Tests on the effect of polyoxyethylene sorbitan monolaurate on spore germination showed no difference between germination in a solution of 1% Tween 20 and in water.

The urediospores of single spore cultures used in each experiment were grown on seedlings of Little Club wheat at 20°C. In each test of competitive ability, seedlings of Little Club, Red Bobs, Marquis, Mindum, Spelmar and/or Arnautka were inoculated with the same 1% Tween 20 suspension of spores prepared with 25 mgs of urediospores of the races being compared per 100 cc. Subsequent generations were produced by collecting spores from the mixed infections on each variety by shaking the seedling leaves over a sheet of cellophane and inoculating new seedlings with the mixture (Figure 1a). The spores from the mixed infections were collected 18 days after inoculation at 15°C and 14 days after inoculation at 20° and 25°C.

Experiments on the effect of pustule density on competitive ability were carried out in a similar manner (Figure 1b). A suspension of 40 mgs of urediospores of each race in 50 cc of 1% Tween 20 solution was used to produce heavy infections (over 100 infections