THE UNIVERSITY OF MANITOBA

STABILIZING SELECTION IN *Puccinia graminis tritici*

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PLANT SCIENCE

WINNIPEG, MANITOBA

May 1975

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ACKNOWLEDGEMENTS

The author wishes to express his sincerest appreciation to Dr. G. J. Green for his constant guidance, advice and encouragement during the course of this study and in preparing this dissertation. Grateful appreciation is extended to Dean L. H. Shebeski, Dr. C. C. Bernier and Dr. L. E. Evans for their advice. Personal thanks are given to Mr. J. H. Campbell for his assistance during the planning of the tests.

Special thanks are due to my wife, Sarah Mong'ina, whose patient understanding, and freely given encouragement made the completion of this study possible.

The work reported herein was undertaken during the tenure by the writer of a Canadian International Development Agency Scholarship. The Ministry of Agriculture, Kenya, granted leave of absence during tenure of the scholarship.

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The competitive abilities of seven related races of *Puccinia graminis* f. sp. *tritici* were studied on three to five susceptible wheat varieties by serial inoculations in greenhouse and growth cabinets, and by natural spread from artificial infection foci in field plots. In relation to the concept of stabilizing selection, the susceptible wheat varieties met the criteria for a simple host, and races with few virulence genes and those with many met the criteria for simple and complex races, respectively. In each of three models studied three different race mixtures were made. In two models one race was a simple race and the other a complex race. The races studied in the third model had equal numbers of virulence genes. In those models with simple and complex races, complex races predominated after 4 to 10 generations in five mixtures, whereas, the simple race predominated in one mixture. In the field experiment a complex race predominated over the simple race.

It was concluded that virulence genes did not impair the competitive abilities of the races of the wheat stem rust fungus studied and that simple genetic control of virulence alone does not contribute to superior competitive ability.

Temperature did not differentially influence the competitive abilities of the races in three mixtures studied in the growth cabinets. In a fourth mixture the complex race predominated at 25^{o}C and the simple race at 15^{o}C.

Pustule age did not affect urediospore germination significantly.

Urediospore germination was slightly higher for races C18(15B-1L) and C33(15B-1L) than for races C9(15B-1L), C37(15), C38(15B-1L), C42(15) and C49(15). Temperature affected the length of the incubation period but races C18(15B-1L) and C33(15B-1L) developed faster than races C9(15B-1L), C37(15), C38(15B-1L), C42(15) and C49(15) at all temperatures. In addition, races C18(15B-1L) and C33(15B-1L) had a shorter incubation period than the other races and produced significantly more spores per pustule than races C9(15B-1L), C37(15) and C49(15). The differences in incubation period and spore production are considered to be most important and they could account for the differences in aggressiveness of the seven races studied.

1. INTRODUCTION

The hypothesis that races with wide host range lack fitness to survive in the field was proposed by Flor (1953, 1956) for *Melampsora lini* (Ehrenb.) Lev. Races with few virulence genes on the other hand were isolated frequently from field collections and were more fit to survive. Similarly, Watson (1958), in Australia, concluded that races of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and E. Henn. with wide host ranges were unable to maintain themselves in mixtures with races of narrow host range when grown on susceptible seedlings in the greenhouse. He observed that races with narrow host ranges were isolated more frequently from field collections than races with wide host ranges. Van der Plank (1968) proposed a concept of stabilizing selection to explain how reduced fitness to survive is presumed to result from unnecessary genes for virulence and this prevents virulent races from becoming predominant on simple varieties.

In van der Plank's (1968) terminology, a wide-host-range race is analogous to a complex race, and a narrow-host-range race is analogous to a simple race. He described stabilizing selection as the predominance of simple races over complex races on simple varieties. A simple race of a pathogen was described as one that can only attack varieties without genes for vertical resistance and, therefore, has few, if any, genes for virulence. A complex race has many virulence genes that enable it to attack complex varieties with many resistance genes.

According to van der Plank (1966) when plant pathogens are isolated from a field of susceptible hosts the majority of isolates should be races with few genes for virulence. Most races from a field of

resistant hosts should be relatively simple races with the minimum number of virulence genes necessary to attack those hosts. This means that the fittest races of a pathogen are those with just enough virulence to attack the predominant host variety.

Van der Plank (1968) distinguished between weak and strong resistance genes. Stabilizing selection occurs for virulence on strong genes but not on weak genes. He speculated that races of *P. graminis* f. sp. *tritici* able to attack wheat varieties with strong genes, for example *Sr6* and *Sr11*, lose fitness to survive on hosts without those genes. Races virulent on a weak resistance gene are often common and are fit to survive on hosts without that gene. Vertical resistance genes *Sr8* and *Sr15* are weak genes because all Australian races were virulent on them although no Australian variety carried them. Van der Plank believed that stabilizing selection was an important consideration in the deployment of strong resistance genes. He suggested the use of simple varieties in the southern United States and the planting of varieties with strong vertical resistance genes in northern United States and Canada. Planting simple varieties in the south would favour simple races. Complex varieties planted in the northern areas would not be damaged by simple races moving northward.

Van der Plank's proposals have stimulated research with conflicting results on the validity of his concept of stabilizing selection. The proposals, if true, have scientific and practical significance, but the contradictory results necessitate further investigations. The study described here was designed to determine whether unnecessary virulence genes are harmful to Canadian races of wheat stem rust and to investigate

the effect of environmental conditions such as temperature on the survival ability of races with different numbers of virulence genes.

2. LITERATURE REVIEW

2.1 SURVIVAL ABILITY AND STABILIZING SELECTION

Survival ability and stabilizing selection are not considered synonymous. Survival ability of a race will be used to designate the ability to survive and cause disease in competition with another race. Survival ability is used here because it is an important factor in stabilizing selection.

2.1.1 *Phytophthora infestans*

Black (1952) studied race mixtures in *Phytophthora infestans* (Mont.) de Bary and found that the wider the host range of a race, the lower its survival ability in competition with a race with a narrower host range on susceptible potato varieties. These results were similar to those obtained by Thurston and Eide (1952, 1953) who cultured races of 0 and 1 for 4 and 8 generations on susceptible potato varieties in the greenhouse. After 4 generations race 0 was the predominant race and after 8 generations the proportion of race 0 to race 1 was 90:1.

Thurston (1961), working with *P. infestans* in the greenhouse, mixed about equal proportions of race 0 and race 1, race 0 and race 4, race 0 and race 1,4, race 0 and race 2,4, and race 0 and race 1,2,4, and grew the mixtures on susceptible potato clones. After up to 9 generations race 0 predominated over the other races with wider host ranges. These results agreed with the observed high frequency of race 0 in the field.

Thurston (1961) also studied the ability of 5 races of *P. infestans* to spread in the field. He inoculated different plots of a susceptible variety separately with each one of the five races. Relative fitness

was determined by comparing rate of spread of the races in the plots. Race 2,4 with two unnecessary virulence genes, spread most rapidly. It was followed by race 1, with one unnecessary virulence gene, and then by race 0 without unnecessary virulence genes. He suggested that the difference between the greenhouse and field results was caused by different isolates of a race having different aggressiveness and he implied that virulence and aggressiveness are independent. He did not speculate on the effect of different environments on aggressiveness.

2.1.2 *Trichometasphaeria turcica*

Scheifele, Nelson and Wernham (1968) determined the survival ability of races of *Trichometasphaeria turcica* Lutt. in mixtures propagated for several generations on simple inbred lines of corn with no genes for vertical resistance. They used monoconidial isolates 13a and R58 of *T. turcica* from corn. These isolates were equally virulent on the susceptible inbred number 4. Isolate 13a had the fewest genes for virulence; R58 had more genes for virulence than 13a. In a second experiment they mixed isolates R58 and T8 on inbred number 4. Isolate T8 had more genes for virulence than isolate R58. In the first mixture, the simple isolate predominated within three generations, whereas, the complex isolate predominated in the second mixture after four generations. They suggested that other biological attributes of the organism, entirely independent of the genes for virulence, govern the success or failure of a race to survive in a given mixture during the parasitic phase of the fungus.

The idea that factors governing fitness to survive in the parasitic phase of nonobligate parasites are independent of genetic simplicity or

complexity of the pathogen was supported by the studies of Scheifele and Nelson (1970). Using similar methods and materials (isolate R60 used instead of T8) to those used in the previous experiment, they determined the differential survival abilities of three isolates of *T. turcica*. They obtained results similar to those of the previous experiment. Increased infection and sporulating efficiency and a decreased incubation period were fitness attributes associated with the predominant isolates in each mixture. They concluded that genetic simplicity for virulence could not account for the survival fitness of the simple race.

2.1.3 *Fusarium oxysporum*

Van der Plank (1968) used the example of races 1 and 2 of the tomato wilt fungus to explain stabilizing selection in nonobligate parasites. Resistance gene I confers resistance to race 1 but not to race 2. He stated that Fusarium wilt, incited by *Fusarium oxysporum* Schlect. f. sp. *lycopersici* (Sacc.) Snyd. and Hans. race 2, is unlikely to menace the tomato (*Lycopersicon esculentum* Mill.) crop as race 1 did prior to the introduction of varieties with gene I. He concluded that race 2 had occurred often and could have become common, but stabilizing selection prevented its increase. Race 2 was held in check by the tomato varieties possessing the resistance gene I. Gene I is a strong gene that enabled stabilizing selection to reduce the incidence of race 2.

Crill *et al.* (1973) demonstrated that Fusarium wilt, incited by race 2,is a very serious disease of tomato in the second year land production areas of Florida. The occurrence of race 2 of Fusarium wilt in

land which had been cropped only two seasons to the susceptible variety Homestead 24 varied from 43% to 74%. They concluded that race 2 of Fusarium wilt is as serious a menace to tomato production as race 1 had ever been. They indicated that stabilizing selection, as reported by van der Plank, did not exist with respect to Fusarium wilt of tomato caused by race 2.

2.1.4 *Puccinia striiformis*

Brown and Sharp (1970) produced evidence to show that a complex race of this rust can predominate over a simple race on the susceptible wheat varieties Lemhi and Hana. The complex race with many unnecessary genes for virulence had greater survival ability than the simple race without unnecessary genes for virulence. Their results questioned the proposal that there is always a negative correlation between survival ability and the number of genes for virulence present.

2.1.5 *Puccinia recondita*

Irish (1950) studied competition among physiologic races of leaf rust of wheat, *P. recondita* Rob. ex Desm. f. sp. *tritici*. He found that races 9 and 15 dominated in a mixture of races 9, 15, 58 and 126 on the variety Cheyenne. Race 15 has the narrowest host range followed by race 9 on the old differential hosts (Johnston, 1961).

Aslam and Browder (1971) studied the relationship of aggressiveness to pathogenicity in *P. recondita* f. sp. *tritici* in three cultures; 66 - 763, virulent at 8 out of 12 loci studied; UNO1 - 68A, virulent only at the *Plr10* locus; and UNO1 - 68B, virulent at all loci studied. Survival in mixtures, relative infectivity, and urediospore production were used as criteria for aggressiveness. Three composites of two

cultures each were grown for several generations on Bison wheat, which has no known genes for resistance. A high positive correlation was found between the cultures with few genes for virulence and survival in mixtures. The cultures also differed in infectivity on Bison with the most avirulent culture being significantly more infective. However, Watson (1970) has shown that unncessary genes for virulence are not lost from a fungus population. He reported that the most prevalent races of *P. recondita* f. sp. *tritici* in Australia are 68-1,2,3,4 and 76-2,3. Race 76-2,3 has few unnecessary genes for virulence and is able to attack the commonly cultivated wheats. Strain 68-1,2,3,4 has virulence which is not necessary for survival. He concluded that if a gene for virulence has no deleterious effect and is associated with genes for aggressiveness and survival ability in a well-adapted race, it may remain in the population whether it is necessary or not.

2.1.6 *Puccinia graminis avenae*

Leonard's results (1969) strongly support the van der Plank concept. He used a heterogenous population of *P. graminis* Pers. f. sp. *avenae* Eriks. and E. Henn. from collections of overwintered telia on orchard grass (*Dactylis glomerata* L.). Small barberry plants were inoculated in the greenhouse by suspending infected orchard grass stems over plants in a moist chamber. Susceptible Craig oat plants were inoculated with an aqueous suspension of aeciospores obtained by grinding the aecial tubes in water. He cultured the heterogenous population for eight uredial generations on the varieties Craig and Clintland A in the greenhouse. Races producing avirulent infection types on the oat stem rust differential hosts increased in the population. Races virulent on

particular varieties had survival values 14 - 46% lower than those avirulent on the same differential varieties. He concluded that the barrier to the built up of races with many genes for virulence is their inability to compete with races with few genes for virulence.

A question inherent in Leonard's work is whether a population from *D. glomerata* is representative of oat stem rust. A population from oats might have behaved differently from the one he used which may have had a long association with *D. glomerata* and barberry. Indeed, Roane *et al.* (1960) showed that those races of *P. graminis* f. sp. *tritici* isolated from aecia or from uredia in the vicinity of barberry bushes may have relatively low survival ability in comparison with other races.

Despite the relationship between survival ability and genes for virulence in *P. graminis* f. sp. *avenae*, indicated by Leonard's work, some recent reports indicate that the relationship is not real. Race 31 of oat stem rust is now the most common race in North America (Martens *et al.*, 1970; Stewart and Rothman, 1971). Since race 31 and other North American races carry several unnecessary genes for virulence Martens *et al.* (1970) concluded that unncessary genes for virulence do not necessarily reduce competitive ability. Martens (1973) studied mixtures of races of oat stem rust in growth cabinets at 15°, 20° and 25°C and in the field. The races with fewest genes for virulence maintained or increased their levels in the growth cabinets in all cases, but were consistently outperformed by races with many genes for virulence under field conditions. The data indicated that genes for virulence, other than those required for successful parasitism, are probably not important factors influencing the rise or decline of

any one race of oat stem rust in the field. He stated that an inverse relationship between the number of genes for virulence in the pathogen and its competitive ability, while it may occur in some cases, is not a general rule for host-parasite systems.

2.1.7 *Puccinia graminis tritici*

Watson (1942) selected races 17, 19, 34, 56 and 147 of wheat stem rust, *P. graminis* f. sp. *tritici*, and studied their development singly and in association with others. He cultured various mixtures of races on seedlings of susceptible varieties of wheat for five generations in the greenhouse. Watson found that race 34 with the widest host range on the standard differentials, predominated when associated with the other races, whereas, race 147 with a narrower host range was always virtually eliminated from such mixtures after five uredial generations. He suggested that the amount and character of each race in the mixture, the variety on which the mixture was cultured, and the effect of temperature on the fungus might be responsible for these changes. In field plots, where epidemics of stem rust were produced by inoculating border rows of susceptible varieties with a mixture of a large number of races, he found that less than half the races used in the initial inoculations were recovered from the plots during the season, indicating that certain races had better survival ability than others.

Loegering (1951), in a similar experiment to Watson's, obtained similar results in the greenhouse but different results in a field experiment. The field observations indicated that race 56, a race with the smallest number of virulence genes on the standard differentials (Stakman *et al.*, 1962), increased more rapidly than race 17 on Ceres wheat, and

race 19 increased more rapidly than race 17 on Mindum. Race 17 has the widest range of virulence genes on the standard differentials among the races he used. On the varieties Fulcaster and Little Club in the greenhouse, race 17 consistently increased more rapidly than races 19 and 56 when grown in mixtures for several generations. Obviously these races increased at different rates on different varieties when grown in mixtures, even though the varieties appeared to be equally susceptible. Loegering stated that the relative ability of races to increase in mixtures is due not only to observable differences in virulence but also to relatively minor ecological factors, which, when operating together, affect the success or failure of physiologic races in nature. Browder (1965) found that race 56 with a narrower host range was more aggressive than race 15B with a wider host range when grown on susceptible varieties. He stated that the relative prevalence of races 15B and 56 in the United States, particularly in the hard red winter wheat region, agreed with the hypothesized association of aggressiveness with the minimum number of virulence genes required for survival.

Keed, as cited by Watson and Luig (1968), studied the differential survival of races 21-7 and 21-1,2,3,7 of wheat stem rust in the greenhouse on host plants which were equally susceptible to both races. The two races differed in their virulence on host seedlings, having one of the following genes: *Sr6*, *Sr11*, *Sr9b*, or *Sr15*. Race 21-1,2,3,7 which is virulent on seedlings having all four of the mentioned genes in combination, was mixed in three initial amounts with race 21-7 which is virulent on seedlings with *Sr15* alone but avirulent on other combinations of these genes. The mixture was propagated for four uredial generations. Regardless of the initial mixture complex race 21-1,2,3,7

predominated after four generations. This evidence showed that, under the conditions of her studies, the race with fewer genes for virulence was the poorer competitor.

Ogle and Brown (1970) compared the survival ability of wheat stem rust races 21-2,7 and 21-2,3,7 on eight wheat varieties. Race 21-2,7 possessed virulence genes to match resistance genes *Sr11* and *Sr15* while race 21-2,3,7 carried an additional gene for virulence on *Sr9*. Four of the test varieties had no resistance genes, and two carried resistance gene *Sr15*. Race 21-2,3,7 with one extra gene for virulence predominated, and at the end of the third generation constituted 90 - 99% of the population on all test varieties. A survey in Canada (Green, 1971a) of the prevalence of races of *P. graminis* f. sp. *tritici* during a 50 year period, showed that virulent races with unnecessary virulence genes have predominated. Green concluded that there is no evidence that unnecessary virulence is harmful or that stabilizing selection is operative in Canada, nor is there evidence that virulence on resistance genes *Sr6* and *Sr11* is harmful to the rust, although according to van der Plank (1968), they are strong resistance genes.

Luig and Watson (1970) have shown that in the Western Australia area two strains of *P. graminis* f. sp. *tritici* were present in 1961 viz. 21-2 (virulent on *Sr11*) and an apparent mutant from it, 21-1,2 (virulent on *Sr6* and *Sr11*). The mutant was favoured in the severe stem rust outbreak of 1963 by the cultivation of wheats with *Sr6* and it emerged as the dominant strain in that region over the next years despite a reduction in the acreage sown to the variety Eureka with *Sr6*. The virulence of the mutant 21-1,2 on plants with *Sr6* and *Sr11* gave it little selective advantage, but Luig and Watson assumed that virulence genes were combined

with those for aggressiveness which made the mutant an effective competitor.

The experimental data reviewed above indicate that virulence genes are not deleterious to the wheat stem rust pathogen.

2.2 FACTORS INFLUENCING SURVIVAL ABILITY

In attempting to explain the racial changes observed in nature, factors other than virulence genes and varietal reactions are very important. Knowledge of how environmental conditions affect survival abilities of different races could be helpful in explaining why one race is a better competitor than another in nature.

2.2.1 TEMPERATURE

The influence of the environment, especially temperature, on the development of *P. graminis* f. sp. *tritici* has been reported by Katsuya and Green (1967). They observed that in experiments on the competitive abilities of races C10(15B-1) and C17(56), predominance of a race in mixed infections was greatly influenced by temperature. Race C17(56) usually predominated over race C10(15B-1) in mixtures at higher temperatures but not at low temperatures.

Cassel (1939) studied the effect of temperature (2^{o}, 9^{o}, 20^{o} and 30^{o}C) on the germination of urediospores and the development of germ tubes of races 11, 34, 36, 38 and 56 of *P. graminis* f. sp. *tritici*. The optimum temperature for most races was 20^{o}C followed by 9^{o}, 30^{o} and 2^{o}C. Spores of race 34 germinated better than those of other races over a wide temperature range, but its germ tube growth was poorest at 20^{o}C. Cassel concluded that on the basis of spore germination race 36 was

adapted to high temperature and only partially tolerant to cold; race 56 was least adapted to cold; and races 38 and 11 were best adapted to low temperatures.

Line and Bugbee (1964) studied the ability of *P. graminis* f. sp. *tritici* to infect wheat seedlings at low temperatures and reported that this could be a type of aggressiveness. They selected entities of race 15B from a mixture of isolates from numerous hosts and locations by incubating inoculated seedlings at 4 - 5°C for 20 hr. The selected isolates of race 15B germinated better at low temperature (3 - 5°C) than nonselected isolates of either race 15B or 56.

In the field, isolates of race 56 spread farther than any of the isolates of race 15B regardless of the temperature. They concluded that the characteristics that make race 56 aggressive may be its rapid spread in epidemic development, prolific spore production, and high spore viability.

Johnson and Newton (1939) showed that physiologic races of the same rust differ in their sensitivity to temperature. They showed that in *P. graminis* f. sp. *tritici*, races that had been inbred by repeated selfings for two or more generations showed greater sensitivity to temperature than races collected in the field.

2.2.2 SURFACE WETNESS

Burrage (1970) showed that a minimum period of surface wetness of from 2 to 4 hr was required before plants inoculated with *P. graminis* f. sp. *tritici* became infected; thereafter the number of pustules formed increased linearly with increased duration of the period of surface wetness.

Bustamente (1972) found that race 264A of *Puccinia coronata* Cda. f. sp. *avenae* Erikss. and Henn., produced few pustules when the incubation period, or period of leaf wetness, was 4.5 hr but that the number of infections increased at 5.5 hr and 14.0 hr of leaf wetness. The number of infections of races 264B and 326 decreased more than those of race 216 when the 4.5 hr leaf wetness treatment was followed by a period of low relative humidity of 40 - 50%. He also found that the number of infections from the 14.0 hr leaf wetness treatment was very similar for races 264A and 264B but differed for races 216 and 326.

2.2.3 HOST VARIETY

Brown and Sharp (1970) found that the susceptible variety of wheat on which rust was grown had a significant effect on the survival of races of *P. striiformis* in mixtures. This result was supported by those of Beaver (1972). Beaver reported that the percentage of urediospore germination and penetration by stripe rust differed on 15 Oregon differentials. He studied competition for infection sites and sporulation within previously colonized tissue. He found that competition between two races of stripe rust can reduce the potential number of infections on a wheat leaf by 99%. In addition, competition can change the ratio of one race to another in the population from 1:1 to 3:1 in one generation.

Ogle and Brown (1970) did not observe any effect of the host variety on the survival of races 21-2,7 and 21-2,3,7 of *P. graminis* f. sp. *tritici*, although a significant increase in the proportion of race 21-2,3,7 in the mixture occurred between generations. The presence in the host variety of minor genes or major genes for resistance, or a combination

of major and minor genes, did not appear to influence the relative survival abilities of these races.

3. MATERIALS AND METHODS

3.1 GREENHOUSE INVESTIGATIONS

The possible harmful effects of virulence genes can be established unequivocably only by the use of cultures that are isogenic except for the virulence gene under investigation.

3.1.1 RUST CULTURES

This investigation into the possibility that virulence genes in *P. graminis* f. sp. *tritici* are harmful was carried out with rust cultures that appeared to be closely related. For many years race group 15B-1L has predominated in Western Canada (Green, 1971a). Within this group there have been evolutionary changes at a number of virulence loci (Table I). It seems reasonable to presume that such changes in virulence were mutational events. The chronological sequence of these changes and their nature are shown in Figure 1. The cultures selected provide an opportunity to investigate the effect of virulence on resistance genes *Sr7a*, *Sr8*, *Sr10*, *Sr11*, and *Sr15* on the aggressiveness of races of *P. graminis* f. sp. *tritici* that seem to be closely related.

Isolates of races 15 and 15B-1L of *P. graminis* f. sp. *tritici* obtained from rust collections made in Western Canada were supplied by Dr. G. J. Green, Agriculture Canada, Research Station, Winnipeg.

The nomenclature of Canadian races has been described by Green (1965, 1971a). Races C9(15B-1L), C18(15B-1L), C33(15B-1L), C37(15), C38(15B-1L), C42(15) and C49(15) appear closely related and produce the same reactions on the standard differentials (Stakman *et al.*, 1962). Race 15B attacks Lee; race 15 does not.

TABLE I. Virulence and avirulence of seven wheat stem rust races on five cultivars each carrying a single resistance gene.

Formula and race number[1]	Virulence (V) or avirulence (A) on host resistance genes					Number of avirulence genes	Number of virulence genes
	Sr7a	*Sr8*	*Sr10*	*Sr11*	*Sr15*		
C9(15B-1L)	A	A	A	V	A	4	1
C18(15B-1L)	V	A	V	V	A	2	3
C33(15B-1L)	V	V	V	V	A	1	4
C37(15)	V	A	V	A	V	2	3
C38(15B-1L)	V	A	V	V	V	1	4
C42(15)	V	A	V	A	A	3	2
C49(15)	V	V	V	A	A	2	3

[1] All races avirulent on *Sr6*, *Sr9a*, *Sr9b*, and *Sr13* and virulent on *Sr5*, *Sr9d*, *Sr14* and *Sr16*.

Race C9(15B-1L) was found in 1957 (Green 1971a). It increased to 18.9% of the isolates in 1963 then decreased to a single isolate in 1969. It is hypothesized that race C9(15B-1L) mutated for virulence on resistance genes *Sr7a* and *Sr10* to produce race C18(15B-1L). Race C18(15B-1L) proved to be aggressive and soon predominated over race C17(56) and was the main race in Canada from 1964 to 1970. In 1971 race C33(15B-1L) became the predominant race having increased from 16% of the isolates in 1970 to 56% of the isolates in 1971 (Green, 1972). Presumably race C33(15B-1L) arose by a mutation for virulence on *Sr8* in race C18(15B-1L). Race C49(15) seems to have arisen from race C33(15B-1L) by losing virulence on plants with *Sr11*. Race C37(15) resembles race C18(15B-1L) but seems to have lost virulence on *Sr11* and gained virulence on *Sr15*. Race

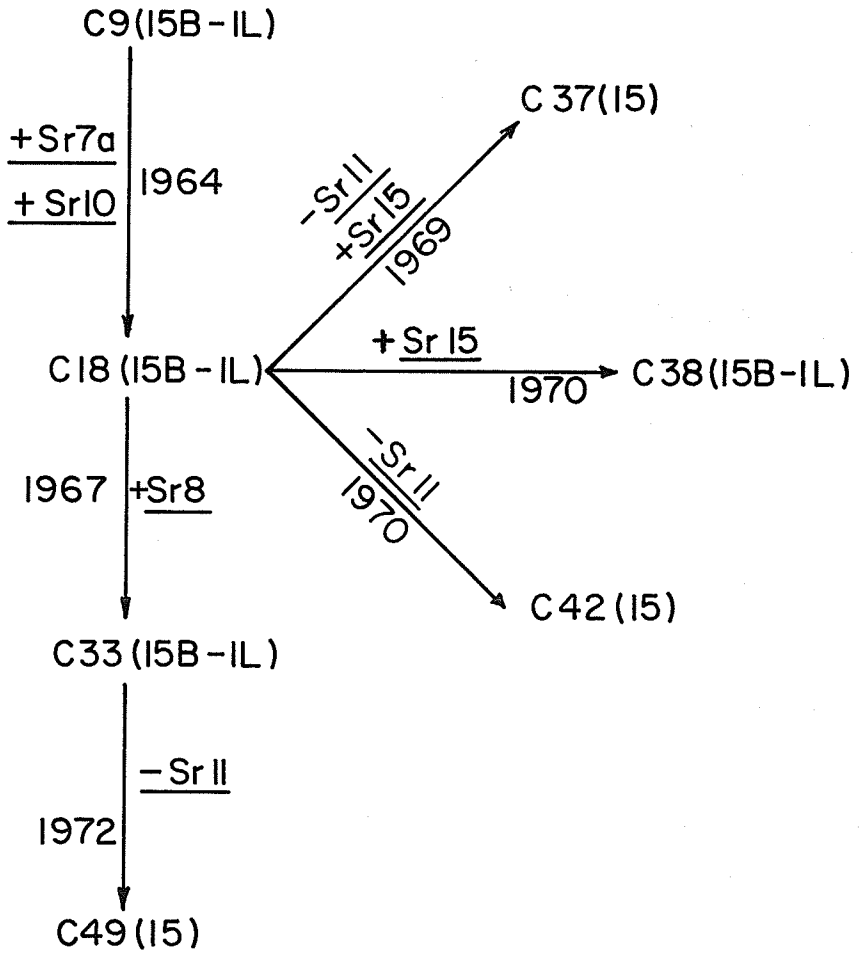

Figure 1. Suggested sequence of mutations in "Standard" race 15 for virulence or avirulence on stem rust resistance genes Sr7a, Sr8, Sr10, Sr11 and Sr15

\+ means virulence, − means avirulence

C38(15B-1L) seems to be a strain of race C18(15B-1L) that mutated to virulence on *Sr15*. Race C42(15) probably originated in race C18(15B-1L) by mutating to avirulence on plants with *Sr11*.

3.1.2 HOST VARIETIES

Wheat cultivars used in the investigation were selected for two main purposes: (1) as universal suscepts for testing the survival ability of races in mixtures; and (2) as differential hosts to establish the proportion of each race after each generation in a mixture (Table II). Lines of Marquis and Chinese Spring with single substituted resistance genes were included in the tests as universal suscepts to determine whether the survival ability of races cultured on them would be different than on those cultivars which have no known resistance genes. Seed was obtained from the Agriculture Canada, Research Station, Winnipeg.

TABLE II. Hosts used to determine the relative survival ability of wheat stem rust races.

Wheat variety and cross	C.I. Number	Purpose
Little Club	4066	Universal suscept
Marquis	3641	" "
Marquis-*Sr5* (Thatcher x Marquis^{6})	15081	" "
Marquis-*Sr10* (Egypt Na95 x Marquis^{6})	15086	" "
Marquis-*Sr8* (Red Egyptian x Marquis^{6})	15084	Differential host
Marquis-*Sr11* (Lee x Marquis^{10})	15087	" "
Chinese Spring	14108	Universal suscept
Chinese Spring - *Sr16* (Chinese Spring x Thatcher)*	14173	" "

**Sr16* transferred by chromosome substitution.

The proportion of each race in a mixture was determined by inoculating the appropriate differential host with the mixture and counting the number of virulent and avirulent infections that developed. The ratio of the two infection types represents the ratio of the two races in the mixture. Races C33(15B-1L) and C49(15) cause type 4 infections on Marquis-*Sr8*, and races C9(15B-1L), C18(15B-1L), C37(15), C38(15B-1L) and C42(15) cause type 2 infections. Races C33(15B-1L) and C9(15B-1L) cause type 4 infections on Marquis-*Sr11* and race C37(15) causes flecks and type 1 infections.

3.1.3 PURIFICATION AND INCREASE OF CULTURES

The single pustule cultures used in this study were established by transferring spores from individual uredia with a sterile scalpel to seedling leaves of Little Club wheat. The inoculated plants were then incubated at high humidity in a polyethylene chamber for 24 hr at the prevailing greenhouse temperatures. Prior to inoculation, seedlings of Little Club were grown in 4 inch pots in an air conditioned room at 20^{o}C with daylight supplemented by artificial light (cool, white fluorescent tubes) for 16 hr. The room was isolated from the rust greenhouses and contamination by rust urediospores was rare. Inoculum of each culture was collected by gently shaking infected plants over cellophane paper. These spores were suspended in oil and used to inoculate seedling plants by atomizing the suspension onto them. The inoculated seedlings were isolated in separate greenhouse compartments. The purity of each culture was determined periodically on the differential host set. Spores were stored in a refrigerator in small glass vials, or, for longer periods, were sealed into small glass ampoules and stored in liquid

nitrogen as described by Loegering *et al.* (1966).

3.1.4 INOCULATION PROCEDURES

In experiments with mixed races seedlings of Little Club wheat were inoculated first with a suspension of 10 mg of freshly collected spores of each race in 7 ml of insecticide base oil. The plants were incubated as above and then placed on greenhouse benches. Urediospores from the resulting infections were used to inoculate three pots of each of the universally susceptible host varieties Marquis, Little Club, and Chinese Spring and three pots of the differential host variety Marquis-*Sr8* (Figure 2). Inoculations subsequent to the first were made by gently shaking plants infected with the mixed cultures over seedlings that had been sprayed with water containing polyoxyethylene sorbitan monolaurate (Tween 20).

3.1.5 MODELS STUDIED

In determining whether unnecessary virulence genes are harmful to *P. graminis* f. sp. *tritici*, three models were studied. In each model three different mixtures were made.

3.1.5.1 MODEL I

Model I was intended to determine how a difference of two or three virulence genes affects competitive ability. If stabilizing selection plays a part in determining the prevalence of races of *P. graminis* f. sp. *tritici*, it should be most clearly evident here; the wide-host-range race should be displaced rapidly. In the mixtures studied race C33(15B-1L) has three virulence genes more than race C9(15B-1L) and races C49(15) and C37(15) each have two virulence genes more than race C9(15B-1L), (Table I).

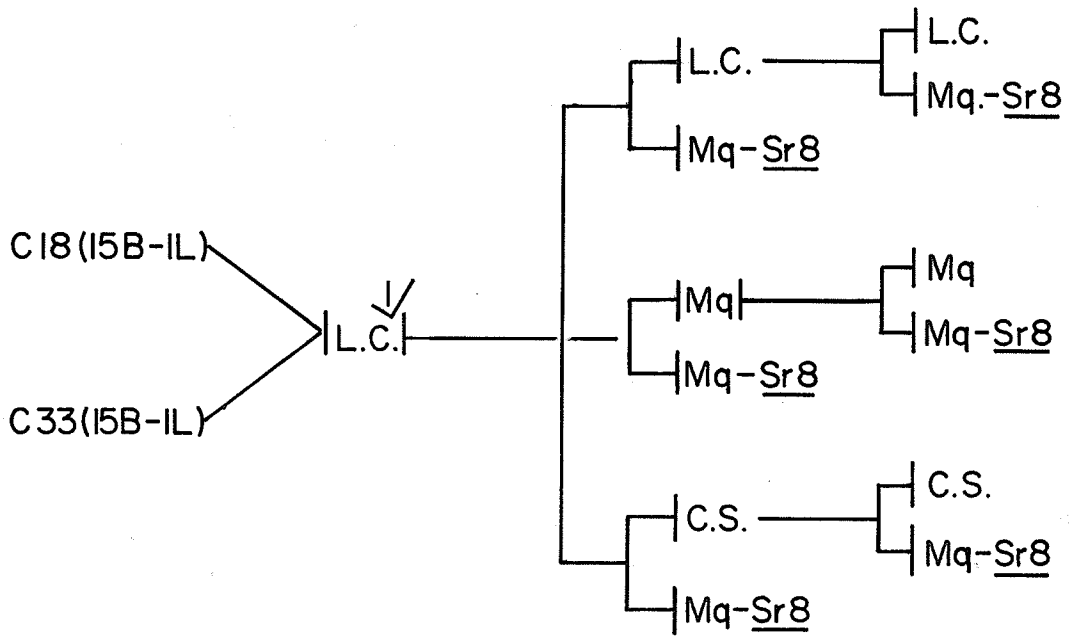

Figure 2. Serial inoculations made in studying survival through several generations of physiologic races C18(15B−IL) and C33(15B−IL) of Puccinia graminis f.sp. tritici.

↓ L.C. = Little Club, Mq−Sr8 = Marquis−Sr8, C.S.=Chinese Spring.

3.1.5.2 MODEL II

The second model was designed to determine if a difference of a single virulence gene would affect survival ability. The wide-host-range race should be gradually displaced according to the stabilizing selection concept. Race C33(15B-1L) has one virulence gene more than races C18(15B-1L) and C37(15), and race C49(15) has one virulence gene more than race C42(15).

3.1.5.3 MODEL III

The third model was planned to determine whether or not races with equal numbers of virulence genes are equally aggressive and compete without changing the initial proportion of each race in a mixture. The mixtures tested were: races C18(15B-1L) and C49(15); races C33(15B-1L) and C38(15B-1L); and races C37(15) and C49(15), (Table I).

Van der Plank (1968) has stated that *Sr8* and *Sr15* are weak genes and that *Sr11* is a strong gene, but long experience in North America (Green, 1971a) poses questions on the validity of this assumption. The status of *Sr7a* and *Sr10* are unknown.

3.2 FIELD INVESTIGATIONS

Stabilizing selection is said to be operative when strong vertical resistance genes are involved. Races virulent on strong resistance genes are said to be unfit to survive in the absence of those genes. Vertical resistance genes are considered to be weak when races with virulence genes to match them are fully fit to survive in the absence of those genes (van der Plank, 1968). To test this hypothesis, a field experiment was performed to study: (a) relative fitness of races by comparing

their spread within plots; and (b) the harmfulness of virulence on the strong resistance gene *Sr11* (van der Plank, 1968).

3.2.1 RUST CULTURES

Races C9(15B-1L), C33(15B-1L) and C37(15) were selected for the field plot experiment. Race C33(15B-1L) is considered to be very aggressive because of its predominance in Canada in 1971, 1972 and 1973 (Green, 1972, 1973) when it comprised 56.4%, 57.1% and 69.2% of the isolates, respectively. In greenhouse studies race C33(15B-1L) predominated in all mixtures containing it. Race C9(15B-1L) is considered to be moderately aggressive because of its moderate prevalence in commercial fields. It never exceeded 18.9% of the total isolates in any season (Green, 1971a). Race C37(15) is considered to be nonaggressive because it was isolated only twice in a single season. Races C9(15B-1L) and C33(15B-1L) are virulent on resistance gene *Sr11* but race C37(15) is not.

3.2.2 HOST VARIETY

The wheat variety Red Bobs (*Triticum aestivum* L.) was chosen because it is very susceptible to stem rust, *P. graminis* f. sp. *tritici*, in Western Canada. Red Bobs carries a gene for seedling resistance at the *Sr7* locus that resembles *Sr7b* of Marquis, and resistance gene *Sr10* (Dyck and Green, 1970). The three races selected are virulent on both genes carried by Red Bobs except race C9(15B-1L) that is avirulent on *Sr10*. Gene *Sr10* does not confer a high level of resistance.

3.2.3 FIELD PLOT DESIGN

Three plots, 20 rows wide with rows 30 cm apart and 6.1 m in length, were planted with Red Bobs on May 9, 1973. They were seeded early in the

season to avoid natural stem rust infection. Plot to plot spread of rust was reduced by sowing 10 rows of the resistant variety Neepawa between the plots of Red Bobs. A control plot, planted to Red Bobs, was located 800 m from the inoculated plots to indicate the severity of natural infection and to serve as a source of rust for the identification of naturally occurring races.

3.2.4 INOCULATION PROCEDURES

Approximately equal amounts of an aqueous suspension of freshly collected urediospores of each race were hypodermically injected into five plants at the center of each plot on June 16, 1973.

3.2.5 SAMPLING PROCEDURES

One hundred and eighty collections were made from the experimental plots and the races were identified in the greenhouse. As far as possible, samples consisted of single pustules. Samples were taken at four different periods to determine which race spread most rapidly. The first collections were made randomly within 1.2 m of the inoculated plants on July 16, 1973. The second lot of samples were taken randomly from all parts of the plots. The third and the fourth collections were from the margins of the plots. Forty-five samples were taken at each collection interval (15 per plot). Twenty-five samples were taken from the control plot.

3.2.6 RACE IDENTIFICATION

The samples were increased on seedlings of the variety Little Club. A plastic bag was placed around each pot to prevent contamination during the inoculation and incubation periods. The urediospores that developed

on Little Club were shaken over the differential hosts in a plastic chamber. The chamber was washed with water between inoculations. After incubation, plants were placed on greenhouse benches and grown at normal greenhouse temperatures. A short differential set of three varieties (Marquis-*Sr7a*, Marquis-*Sr8*, and Marquis-*Sr11*) was used to identify the samples as to race (Table III). For every five samples identified by a short differential set, a full set of differential hosts (Green, 1972) was used for one sample. Samples from the control plot were identified using all differential hosts. Infection types were recorded as described by Stakman *et al.* (1962). Races in mixed collections were separated by establishing cultures from single pustules. Each race was considered an isolate.

TABLE III. Infection types produced on the short differential set by races C9(15B-1L), C33(15B-1L), and C37(15).

Differential varieties	Physiologic race		
	C9(15B-1L)	C33(15B-1L)	C37(15)
Marquis-*Sr7a*	3	4	3
Marquis-*Sr8*	2	4	2
Marquis-*Sr11*	4	4	1

3.3 GROWTH CABINET STUDIES

The preliminary greenhouse and field plot experiments indicated that races differed in their rate of increase. A series of experiments was, therefore, carried out to determine what factors affect the rate of increase of one race in relation to another. The factors investigated

were: temperature, urediospore viability, incubation period, urediospore production, and urediospore germination.

3.3.1 EFFECT OF TEMPERATURE ON SURVIVAL ABILITY

The affect of temperature on survival ability was studied in growth cabinets using races C9(15B-1L), C18(15B-1L), C33(15B-1L), C37(15), and C49(15). These races were chosen because of their performance in greenhouse and field studies. The methods of inoculation and recording results were similar to those used in greenhouse studies. Inoculated seedlings were incubated for 24 hr in a water-saturated atmosphere in growth cabinets at 15^{o} and 25^{o}C. The plants were grown in the growth cabinets under a 16 hr day length with light from cool, white, fluorescent tubes. Serial inoculations (Figure 3) were carried out using generation times of 12 and 18 days for 25^{o} and 15^{o}C, respectively. A thermograph was used to monitor the temperature.

3.3.2 PUSTULE AGE AND SPORE VIABILITY

Urediospores were collected from 30 plants that had been inoculated at the same time. The first collection was made 3 days after the appearance of the first pustules. Subsequent collections were made at 3 day intervals, until the leaves senesced and few, if any, spores were produced. After each collection the plants were sprayed with water to remove any remaining spores from the leaf surface. Spore germination was determined after 3 hr in the dark at 20^{o}C on 1% water agar.

3.3.3 EFFECT OF TEMPERATURE ON UREDIOSPORE VIABILITY

Freshly collected spores were incubated in chambers at 2^{o}, 5^{o}, 10^{o}, 15^{o}, 20^{o}, 25^{o}, and 30^{o}C and per cent germination was determined after

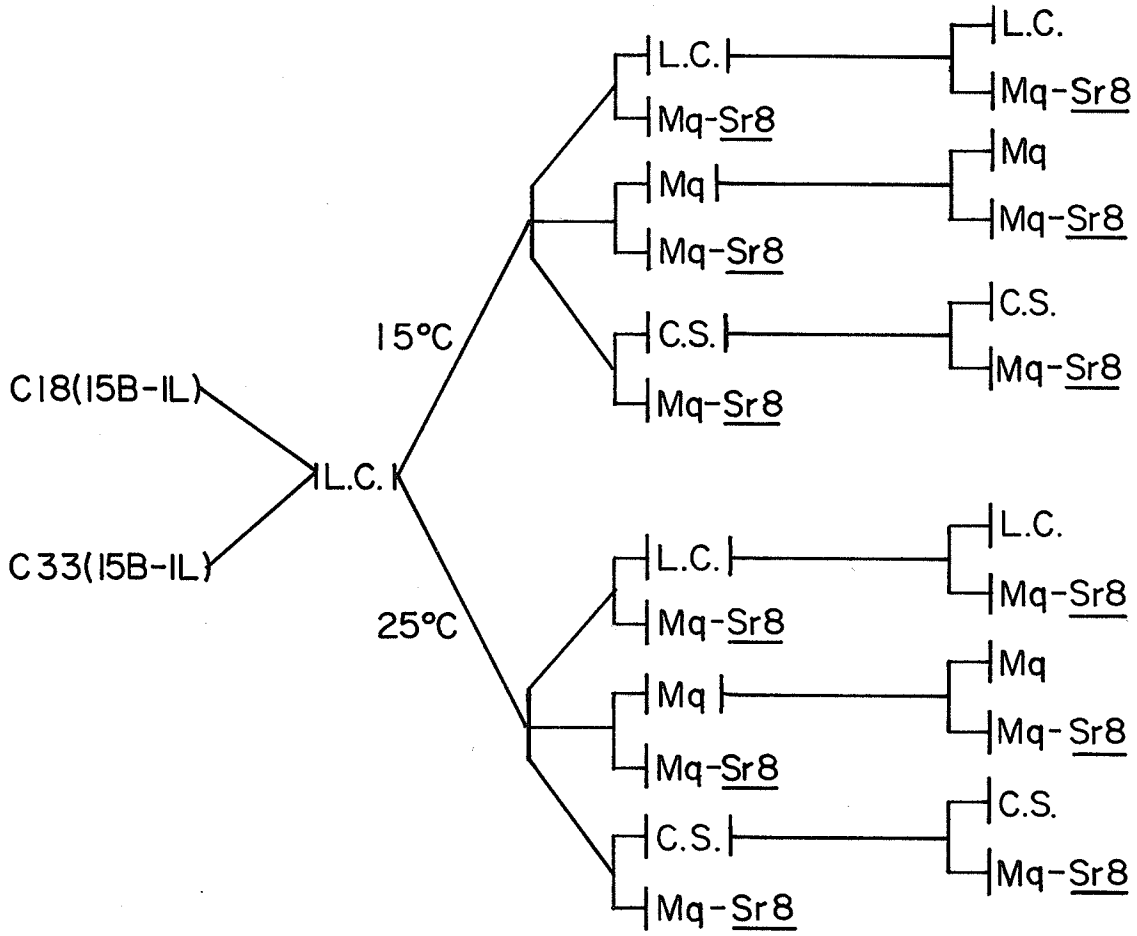

Figure 3. Serial inoculation in a growth cabinet study of a mixture of races C18(15B-IL) and C33(15B-IL).

3 hr at temperatures from 10^{o} to 30^{o}C and after 8 hr at 2^{o} and 5^{o}C.

3.3.4 INCUBATION PERIOD AND SURVIVAL ABILITY

The incubation period, that is the time interval between inoculation and the breaking of the host epidermis, was determined on sparsely infected seedlings of Little Club at 15^{o}, 20^{o}, and 25^{o}C. Observations were made every day from 4 to 15 days after inoculation with the seven races.

3.3.5 SPORE PRODUCTION AND SURVIVAL ABILITY

Seven day old seedlings of Little Club wheat were inoculated with a very dilute suspension of urediospore of races C9(15B-1L), C18(15B-1L), C33(15B-1L), C37(15), and C49(15). After incubation the plants were moved to a growth cabinet kept at 21 ± 1^{o}C and a 16 hr day length. Seven days after inoculation, seedlings with a maximum of four pustules on the first leaf were selected and each was placed in an open ended glass tube. Urediospores were collected by gently tapping the leaf so that mature spores fell in the tube. The spores were collected at intervals at 4 days beginning at the end of the incubation period 10 days after inoculation. The spores were suspended in oil and counted by means of Levy and Levy-Hausser corpuscle counting chamber.

4. EXPERIMENTAL RESULTS

4.1 GREENHOUSE INVESTIGATIONS

4.1.1 MODEL I

The races used in mixtures in this model differ by two or three virulence genes. This model was designed to determine whether races with many genes for virulence would quickly displace or be displaced by races with few genes for virulence.

4.1.1.1 RACES C9(15B-1L) AND C33(15B-1L)

Urediospore mixtures of races C9(15B-1L) and C33(15B-1L) were prepared as described previously. The number of pustules of each race in the mixture after culturing on the varieties Little Club, Marquis, Marquis-*Sr5*, Chinese Spring, and Chinese Spring-*Sr16* are given in Table IV and on Little Club in Figure 4. Race C33(15B-1L) predominated over race C9(15B-1L) after the first generation and comprised more than 90% of the pustules in the fourth generation. Race C33(15B-1L) which has three more virulence genes than race C9(15B-1L) (*Sr7a*, *Sr8*, and *Sr10*) was more aggressive on all five host varieties. The presence of resistance genes *Sr5* and *Sr16* in Marquis and Chinese Spring had no effect on the relative survival ability of these races. The mixture was intended to determine whether race C9(15B-1L) would displace the more virulent race C33(15B-1L) in the mixture but the opposite occurred. These results show that simple races are not always the fittest to survive in competition with complex races.

TABLE IV. Number of pustules of races C33(15B-1L) and C9(15B-1L) on the differential host Marquis-*Sr8* after each of four generations on five wheat varieties.

Generation	Susceptible host varieties and races				
	Little Club C33:C9	Marquis C33:C9	Chinese Spring C33:C9	Marquis-*Sr5* C33:C9	Chinese Spring-*Sr16* C33:C9
1	371:399				
2	441:157	420:163	344:104	287:82	281:74
3	350:102	273: 54	203: 49	352:45	258:45
4	504: 55	514: 49	679: 61	340:21	612:42

4.1.1.2 RACES C9(15B-1L) AND C49(15)

The numbers of pustules of each race, after culturing this mixture on the varieties Little Club, Marquis, Chinese Spring, Marquis-*Sr5* and Chinese Spring *Sr16*, are shown in Table V and on Little Club in Figure 5. Race C9(15B-1L) with two virulence genes less than race C49(15) (Table I), comprised more than 90% of the pustules in the tenth generation. Race C49(15) increased in the mixture from 52% in the first generation to about 68% in the third generation but then decreased steadily until the tenth generation. In this mixture the simplest race predominated over a more complex race at the conclusion of the experiment. It would be an oversimplification to conclude that race C9(15B-1L) predominated over race C49(15) because it possessed fewer genes for virulence. Stabilizing selection is said to be operative when strong genes such as *Sr6* and *Sr11* (van der Plank, 1968) are involved and, therefore, race C9(15B-1L) which is virulent on *Sr11*, would not be expected to compete

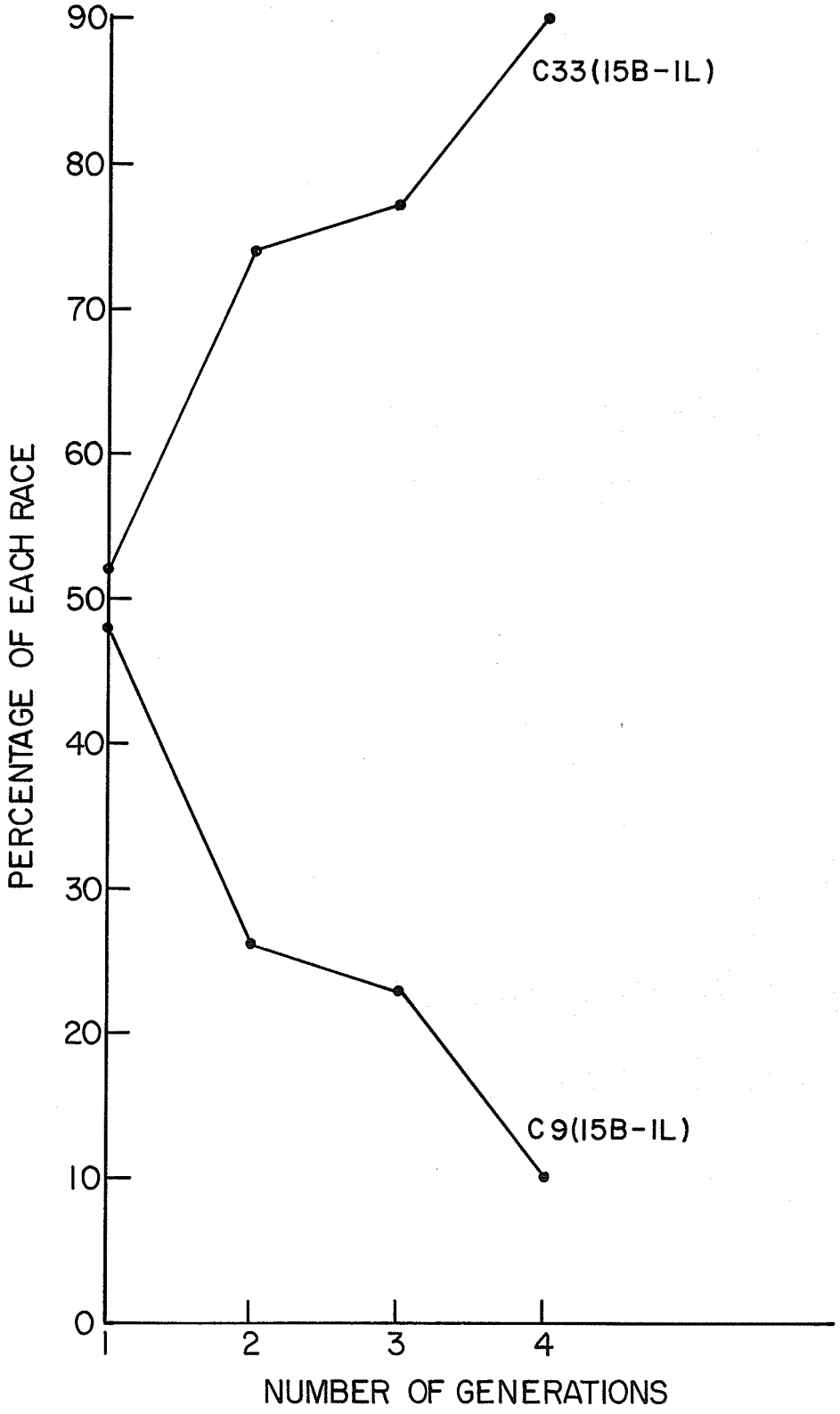

Figure 4. Change in percentage of races C9(15B-IL)and C33(15B-IL) in a mixture cultured for four generations on the variety Little Club.

favourably with race C49(15) despite its avirulence on other resistance genes. Although race C9(15B-1L) ultimately predominated in the mixture it required 7 generations to gain a distinct advantage.

TABLE V. Number of pustules of races C9(15B-1L) and C49(15) on the differential host Marquis-*Sr8* after each of ten generations on five wheat varieties.

Generation	Susceptible host varieties and races				
	Little Club C9:C49	Marquis C9:C49	Chinese Spring C9:C49	Marquis-*Sr5* C9:C49	Chinese Spring-*Sr16* C9:C49
1	535:589				
2	217:435	202:320	221:408	384:654	227:483
3	458:814	393:849	416:641	287:572	268:374
4	231:505	230:406	269:438	141:410	190:328
5	171:183	231:210	224:223	149:241	62: 37
6	320:242	287:212	369:228	320:194	379:246
7	466:156	402:167	240:117	226: 85	274: 86
8	305:147	315:126	287: 84	336:119	357:160
9	464: 98	392: 97	445: 88	428: 89	460: 59
10	517: 46	484: 36	501: 36	498: 30	505: 31

4.1.1.3 RACES C9(15B-1L) AND C37(15)

In this mixture race C37(15) was a better competitor than race C9(15B-1L), (Table VI and Figure 6). Race C37(15), which has two more genes for virulence than race C9(15B-1L), increased from 55% of the mixture in the first generation to over 90% in the fourth generation.

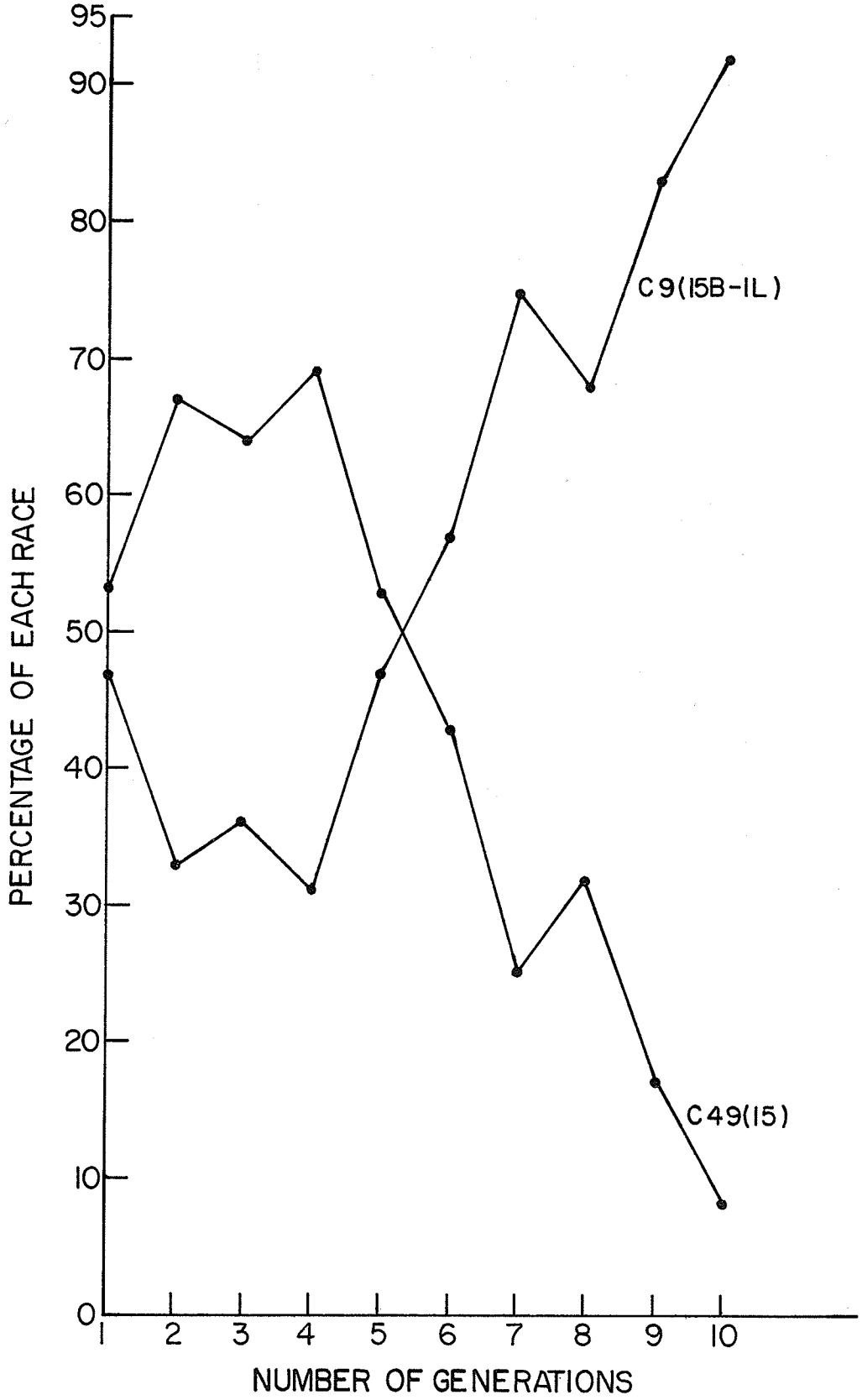

Figure 5. Change in percentage of races C9(15B-1L)and C49(15) in a mixture cultured for ten generations on the variety Little Club.

Apparently the extra virulence on resistance genes *Sr7a* and *Sr15* did not impair the ability to race C37(15) to compete with race C9(15B-1L). It would not be justified to conclude that race C37(15) predominated in the mixtures because, unlike race C9(15B-1L), it is avirulent on the strong resistance gene *Sr11*. Despite its virulence on *Sr11* race C9(15B-1L) predominated over avirulent race C49(15) in an earlier mixture.

TABLE VI. Number of pustules of races C37(15) and C9(15B-1L) on the differential host Marquis-*Sr8* after each of four generations on three wheat varieties.

Generation	Susceptible host varieties and races		
	Little Club C37:C9	Marquis C37:C9	Chinese Spring C37:C9
1	398:314		
2	498:430	522:390	516:367
3	496:211	455:223	418:177
4	713: 67	741: 43	699: 44

4.1.2 MODEL II

The races used in mixtures in this model differed by a single virulence gene. The mixtures were designed to show whether there are large or small differences in the competitive abilities of these races.

4.1.2.1 RACES C18(15B-1L) AND C33(15B-1L)

Race C33(15B-1L) predominated over race C18(15B-1L) after ten generations (Table VII and Figure 7). The proportions of races in the mixture changed only slightly in the first three generations but then

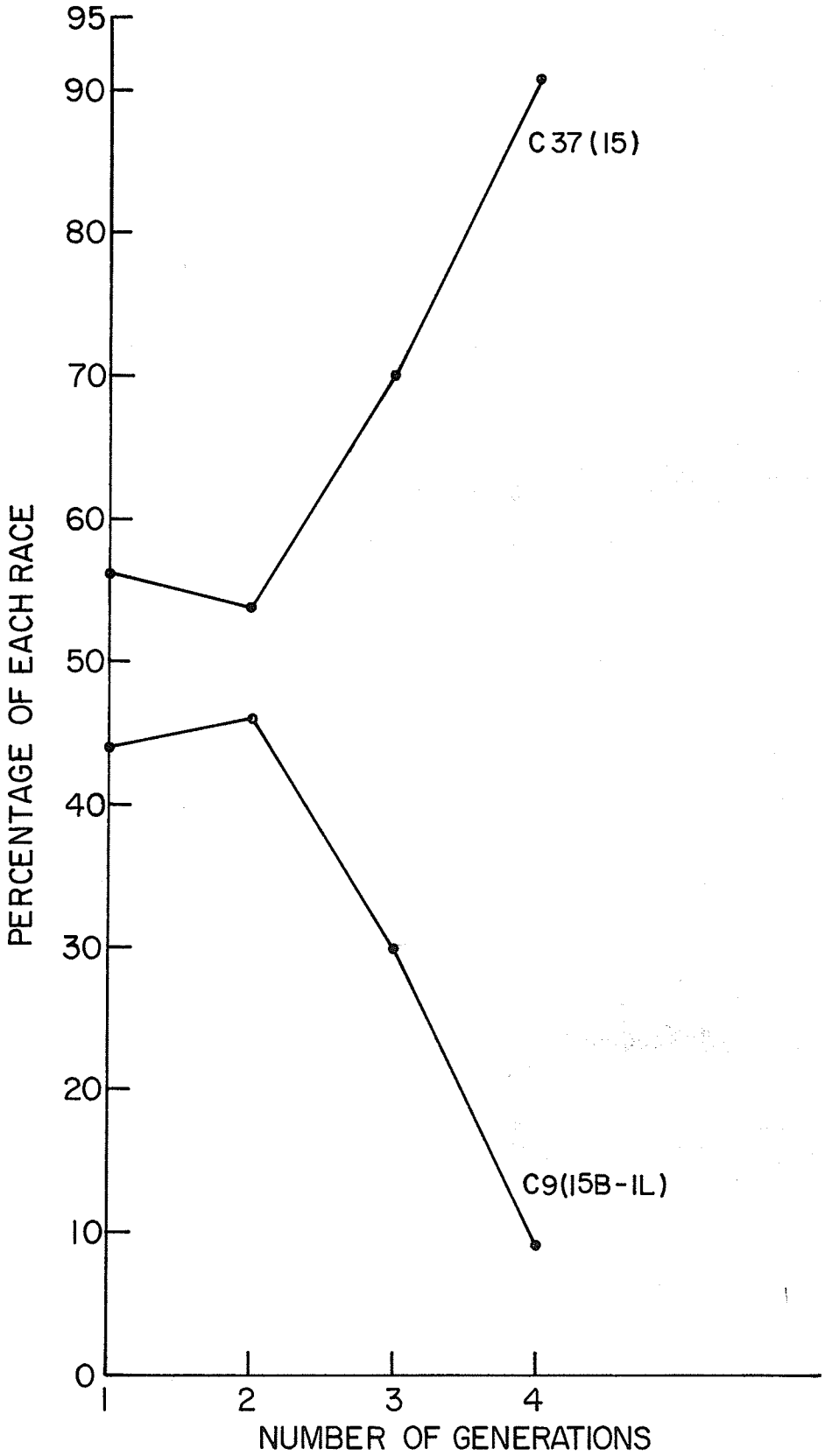

Figure 6. Change in percentage of races C9(15B-IL) and C37(15) in a mixture cultured for four generations on the variety Little Club.

race C18(15B-1L) decreased markedly. At the tenth generation race C33(15B-1L) comprised 92% of the pustules in the mixture on all varieties. Both races are virulent on the strong resistance gene *Sr11*. Races C33(15B-1L) and C18(15B-1L) differ only in that race C33(15B-1L) is virulent and C18(15B-1L) avirulent on gene *Sr8*. Van der Plank (1968) stated that *Sr8* is a weak gene and would not greatly affect competitive ability. However in this mixture, and in the mixture of races C9(15B-1L) and C49(15), virulence on *Sr8* did not seem to be even slightly harmful.

TABLE VII. Number of pustules of races C18(15B-1L) and C33(15B-1L) on the differential host Marquis-*Sr8* after each of ten generations on four wheat varieties.

Generation	Susceptible host varieties and races			
	Little Club C33:C18	Marquis C33:C18	Chinese Spring C33:C18	Marquis-*Sr11* C33:C18
1	691:925			
2	295:334	256:363	323:430	395:409
3	394:378	285:331	279:391	279:391
4	253:177	263:153	231:170	303:195
5	643:205	443:189	448:179	602:208
6	228:111	212:157	253: 80	273: 73
7	317: 63	316:116	417:105	372: 47
8		NOT SCORED		
9	431: 47	460: 52	447: 43	416: 50
10	467: 23	442: 24	470: 26	441: 24

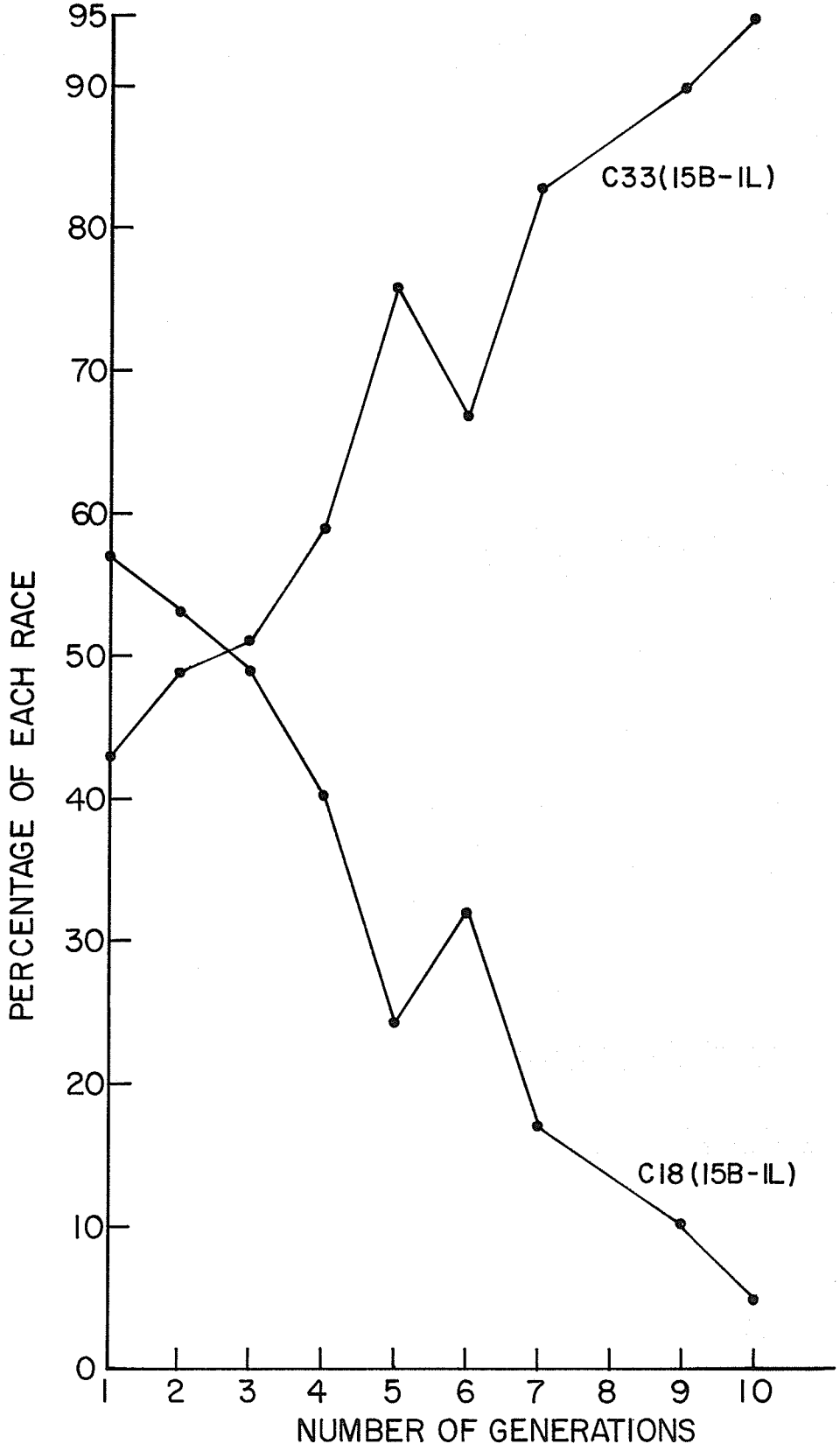

Figure 7. Change in percentage of races C18(15B-IL) and C33(15B-IL) in a mixture cultured for ten generations on the variety Little Club.

4.1.2.2 RACES C42(15) AND C49(15)

Data in Table VIII and Figure 8 show that race C49(15) predominated over race C42(15) after four uredial generations. At the conclusion of the experiment race C49(15) comprised more than 80% of the pustules. Race C49(15) has a gene for virulence on resistance gene *Sr8* not carried by race C42(15) and should be the weaker competitor, but it displaced race C42(15) in only four generations.

TABLE VIII. Number of pustules of races C42(15) and C49(15) on the differential host Marquis-*Sr8* after each of four generations on four wheat varieties.

Generation	Susceptible host varieties and races			
	Little Club C49:C42	Marquis C49:C42	Chinese Spring C49:C42	Marquis-*Sr10* C49:C42
1	527:512			
2	259:114	216: 79	208: 85	206: 52
3	467:122	486:140	469:127	474:129
4	432: 99	482: 24	492: 34	529: 60

4.1.2.3 RACES C33(15B-1L) AND C37(15)

The competitive ability of races C33(15B-1L) and C37(15) was compared on seedlings of three susceptible varieties for six generations (Table IX and Figure 9). Race C33(15B-1L) has one gene for virulence more than race C37(15). These races were about equally represented in the first generation on Little Club and there was little change in their proportions during the first three generations. In the fourth generation

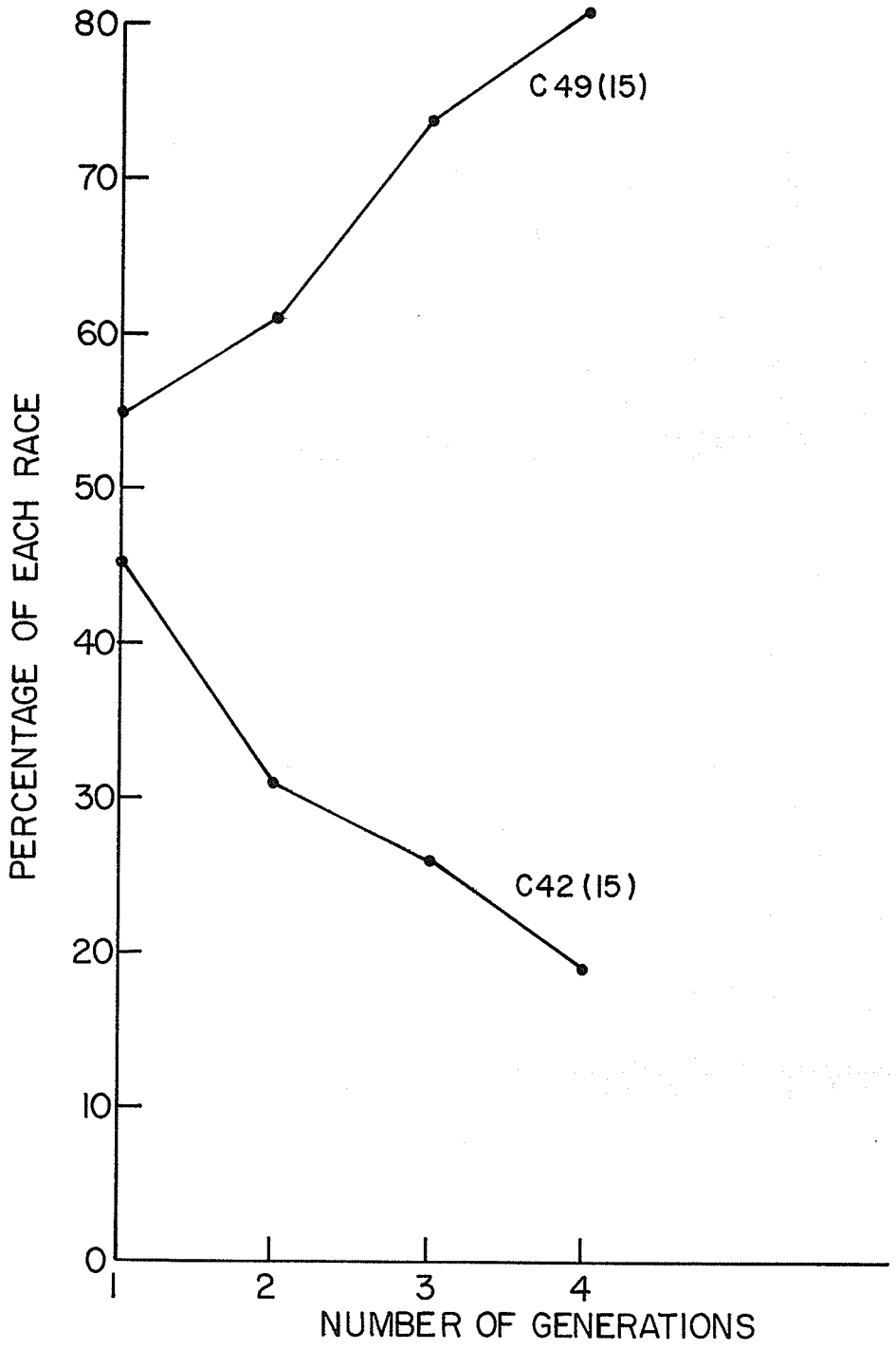

Figure 8. Change in percentage of races C42(15) and C49(15) in a mixture cultured for four generations on the variety Little Club.

race C33(15B-1L) increased over race C37(15) and it continued to increase to the sixth generation when it constituted over 80% of the pustules.

TABLE IX. Number of pustules of races C33(15B-1L) and C37(15) on the differential host Marquis-*Sr8* after each of six generations on three wheat varieties.

Generation	Susceptible host varieties and races		
	Little Club C33:C37	Marquis C33:C37	Chinese Spring C33:C37
1	496:446		
2	303:265	322:262	274:226
3	442:391	391:238	504:320
4	423:267	437:247	478:275
5	395:194	387:191	433:167
6	305: 60	369: 54	380: 70

4.1.3 MODEL III

The races used in this model have equal numbers of different virulence genes. The mixtures were designed to show whether the races used have equal competitive abilities and survive equally well in mixtures.

4.1.3.1 RACES C18(15B-1L) AND C49(15)

Race C18(15B-1L), despite its virulence on the strong resistance gene *Sr11*, increased from 40% of the pustules in the first generation to over 98% in the eighth generation (Table X and Figure 10). It was thought that, since race C18(15B-1L) is virulent on *Sr11*, it would not

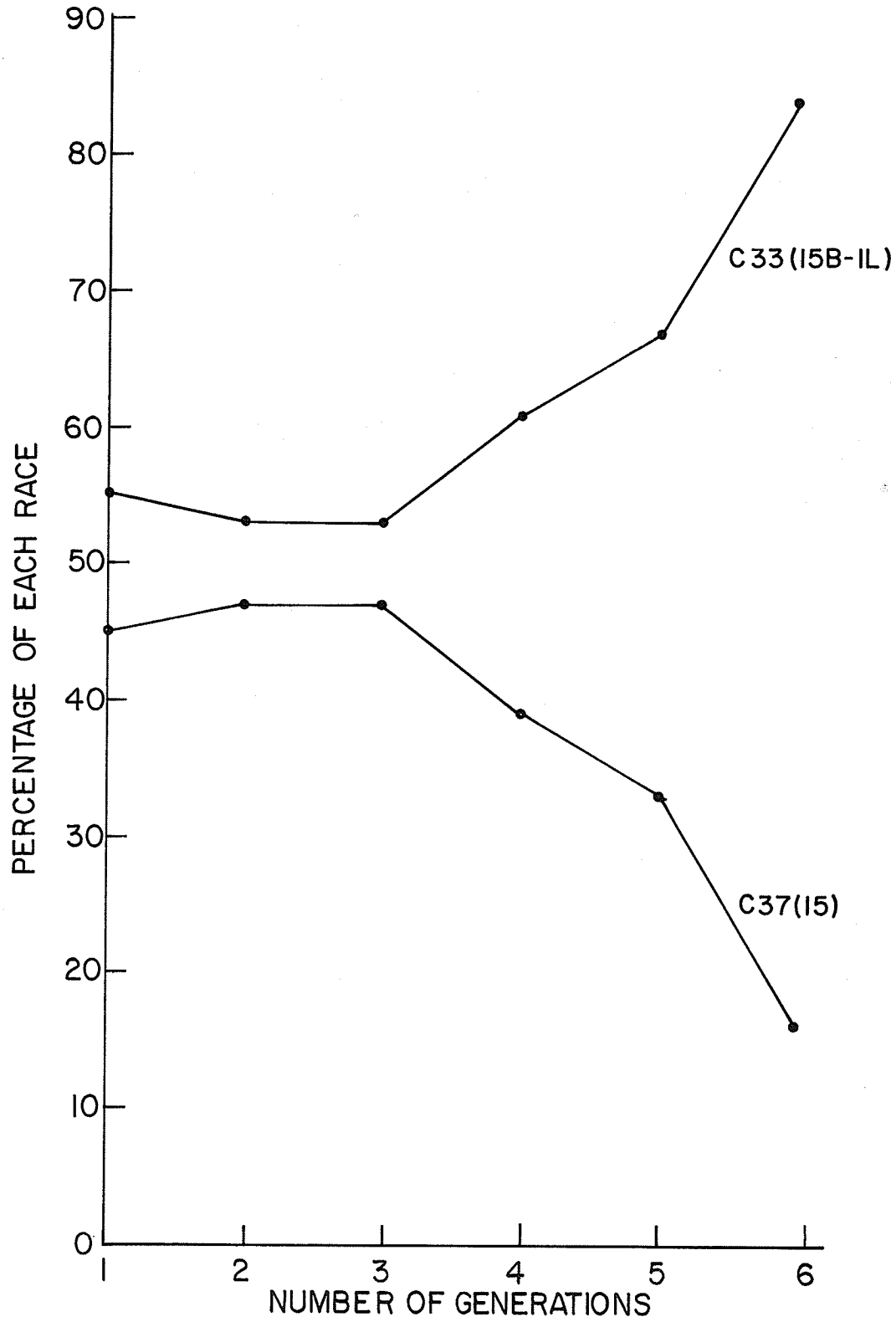

Figure 9. Change in percentage of races C33(15B-IL) and C37(15) in a mixture cultured for six generations on the variety little club.

be fit to compete on susceptible hosts with race C49(15) which is avirulent on *Sr11*. However, there was no evidence to show that virulence on *Sr11* was harmful to race C18(15B-1L).

TABLE X. Number of pustules of races C18(15B-1L) and C49(15) on the differential host Marquis-*Sr8* after each of eight generations on four wheat varieties.

Generation	Susceptible host varieties and races			
	Little Club C18:C49	Marquis C18:C49	Chinese Spring C18:C49	Marquis-*Sr5* C18:C49
1	741:491			
2	273:211	337:233	367:305	97: 69
3			NOT SCORED	
4	732:320	695:277	634:240	750:282
5	344: 76	361:100	299: 62	262: 59
6	362: 33	253: 27	366: 32	264: 21
7			NOT SCORED	
8	832: 14	811: 11	819: 9	837: 22

4.1.3.2 RACES C33(15B-1L) AND C38(15B-1L)

The data on the competitive abilities of races C33(15B-1L) and C38(15B-1L) on four host varieties (Table XI and Figure 11) show that these races survived in the mixture without significant change in the racial composition for seven uredial generations. During the ninth and tenth generations, race C33(15B-1L) became predominant. Race C33(15B-1L) is virulent on the weak gene *Sr8* and race C38(15-1L)

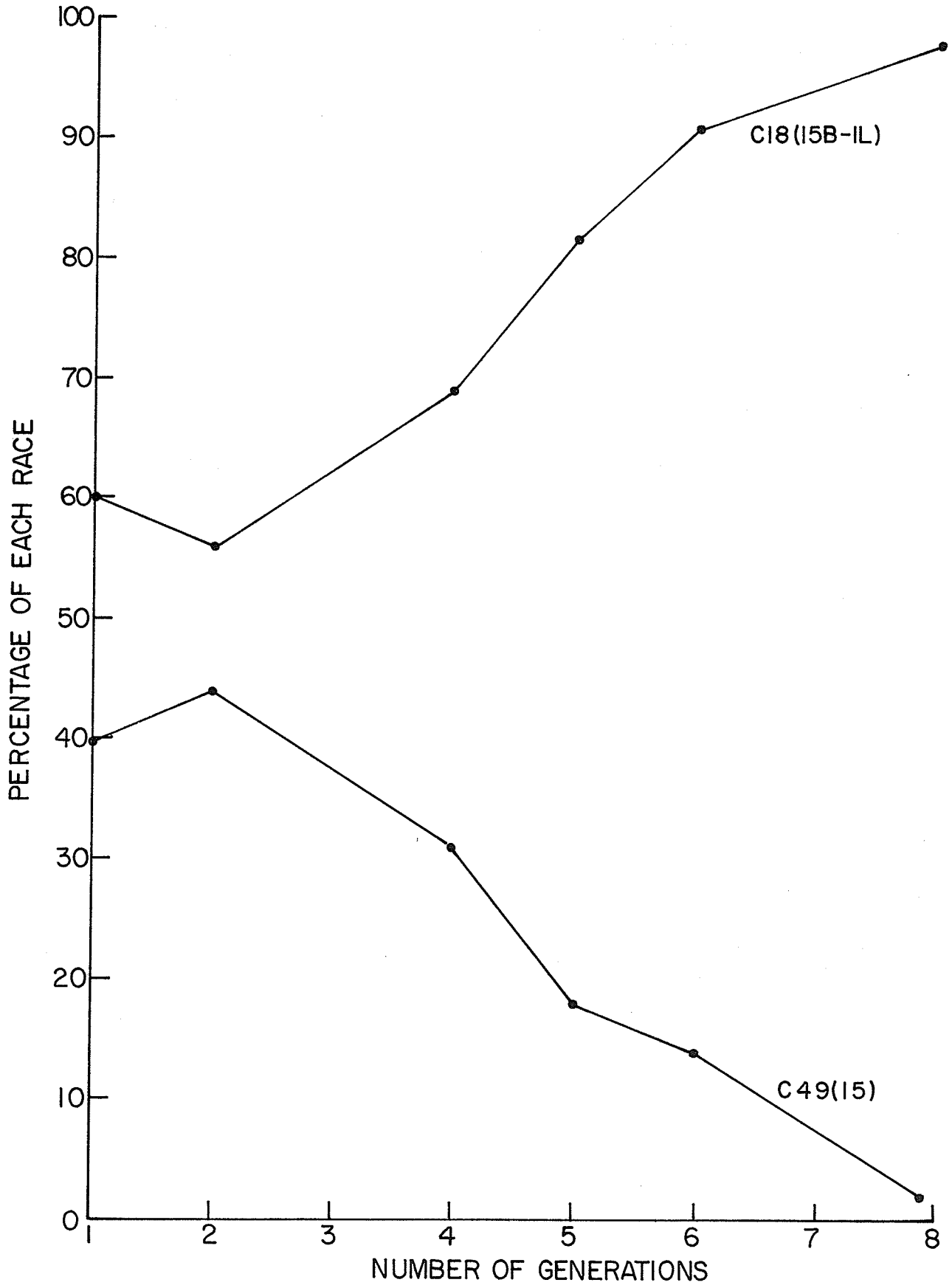

Figure 10. Change in percentage of races C18(15B-IL) and C49(15) in a mixture cultured for eight generations on the variety Little Club.

is not. Race C38(15B-1L) is virulent on *Sr15*, also a weak gene, and race C33(15B-1L) is avirulent. Both genes should have the same effect on survival ability according to the stabilizing selection concept. It would be inconsistent to conclude that race C38(15B-1L) decreased in the mixture because of its virulence on gene *Sr15* when race C37(15), virulent on the same gene, predominated over races C49(15) and C9(15B-1) which are avirulent.

TABLE XI. Number of pustules of races C33(15B-1L) and C38(15B-1L) on the differential host Marquis-*Sr8* after each of ten generations on four wheat varieties.

Generation	Susceptible host varieties and races			
	Little Club C33:C38	Marquis C33:C38	Chinese Spring C33:C38	Marquis-*Sr10* C33:C38
1	449:488			
2	282:187	214:182	170:155	136:132
3	429:403	444:262	526:336	557:413
4	269:248	285:209	335:251	214:153
5	349:275	363:250	338:269	289:194
6	245:258	354:244	329:253	313:254
7	368:281	349:272	385:241	243:148
8		NOT SCORED		
9	408:301	379:287	425:261	254:160
10	422:173	420:151	422:167	446:131

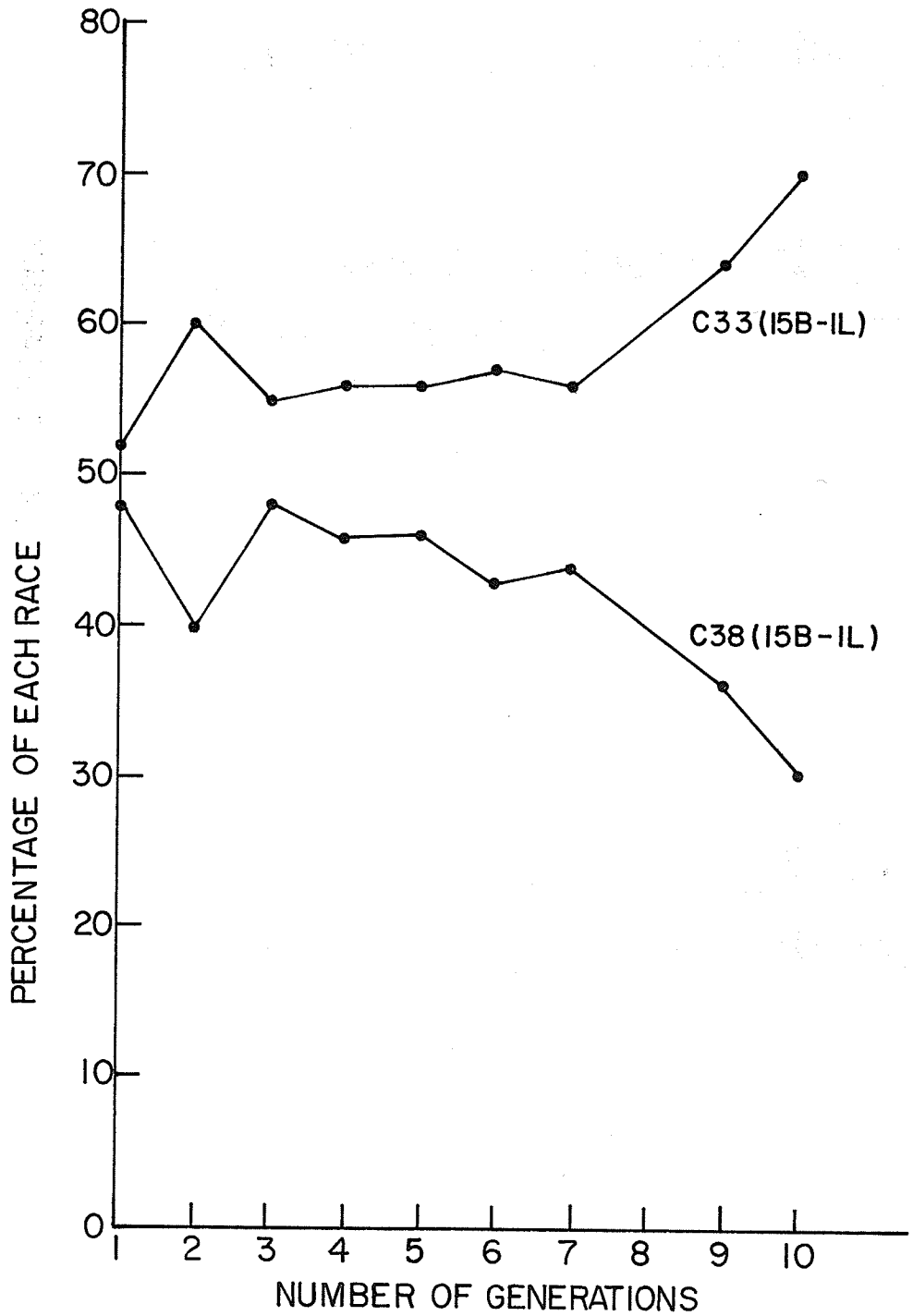

Figure 11. Change in percentage of races C38(15B-IL) and C33(15B-IL) in a mixture cultured for ten generations on the variety Little Club.

4.1.3.3 RACES C37(15) AND C49(15)

After maintaining the mixture of races C37(15) and C49(15) on four host varieties for five generations (Table XII and Figure 12), race C37(15) became dominant over race C49(15), notwithstanding their equal numbers of virulence genes. Race C49(15) declined in the mixture from 53% of the pustules in the first generation to less than 10% in the fifth generation on all four host varieties. Race C37(15) quickly predominated over race C49(15) in a mixture in which these races were expected to compete equally well.

TABLE XII. Number of pustules of races C37(15) and C49(15) on the differential host Marquis-*Sr8* after each of five generations on four wheat varieties.

Generation	Susceptible host varieties and races			
	Little Club C37:C49	Marquis C37:C49	Chinese Spring C37:C49	Marquis-*Sr10* C37:C49
1	422:478			
2	378:224	348:199	334:235	320:265
3			NOT SCORED	
4	458:106	446: 94	456:109	462: 95
5	493: 39	616: 36	557: 36	515: 43

4.2 FIELD PLOT RESULTS

Flecking was observed nine days after inoculation on plants inoculated June 16, 1973. It was evident that all three races had caused almost equal amounts of infection. Secondary infection was observed

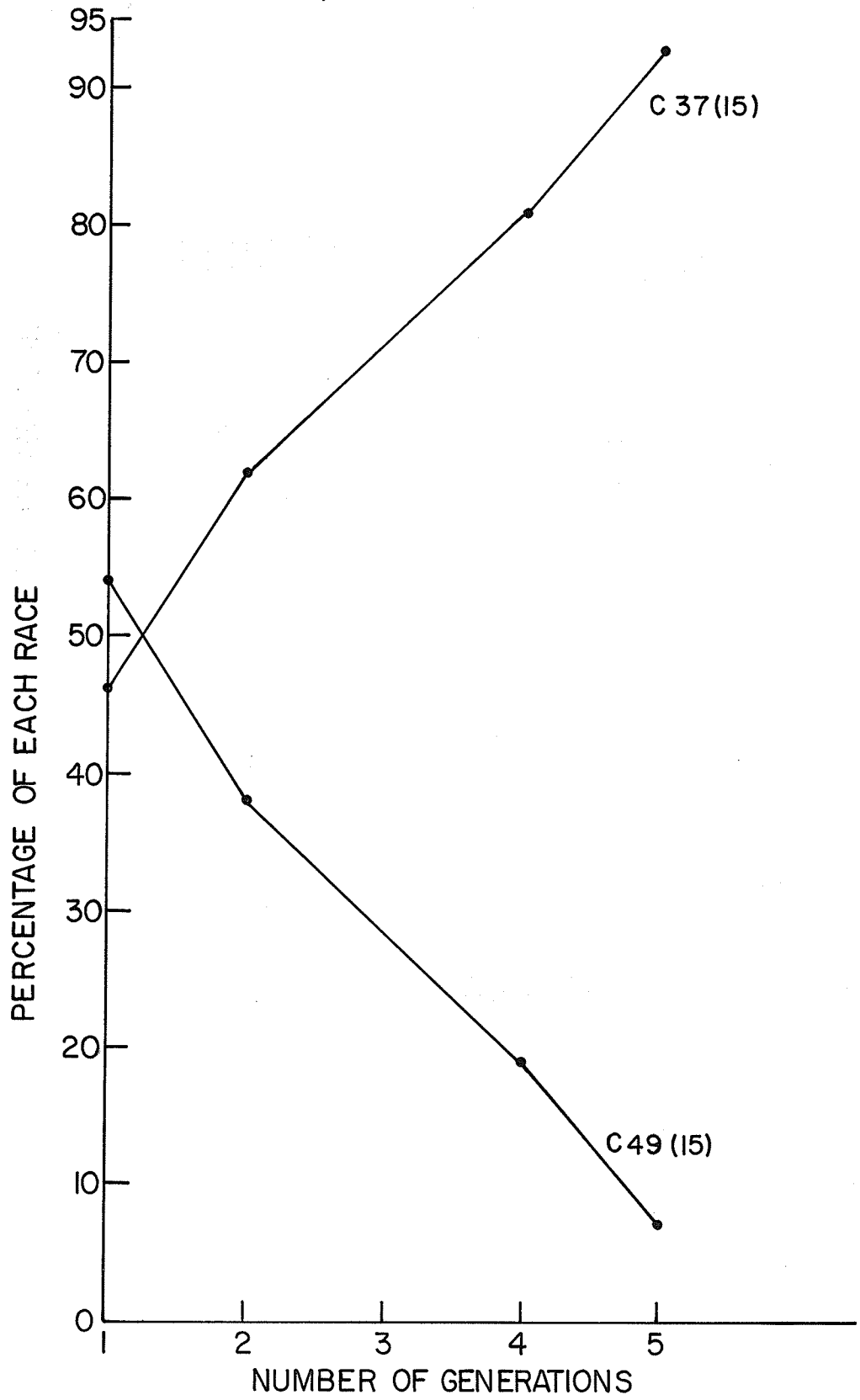

Figure 12. Change in percentage of races C 37(15) and C 49(15) in a mixture cultured for five generations on the variety Little Club.

21 days after inoculation, mostly within .6 m of the artificially inoculated plants. Four weeks after inoculation a few pustules appeared at the margins of all plots. Warmer weather after July 16 increased the rate of disease spread. Rust developed equally in all three plots and was found throughout the plots by July 26.

Control plots were regularly checked for natural infection. A few rust pustules were found in the control plots on July 27. Twenty-five samples were collected from these plots and identified in the greenhouse on full differential sets. Nineteen samples were identified as race C33(15B-1L), four as race C37(15), and two as race C9(15B-1L). These are the same races released in the artificially infected plots and they may have originated there.

Two hundred and one single-pustule isolates were obtained by the end of the experiment. Of these, 106 were identified as race C33(15B-1L), 53 as race C37(15), and 42 as race C9(15B-1L), (Table XIII and Figure 13). Thus, after four collection periods, or about 5 generations, race C33(15B-1L) with many genes for virulence comprised 52% of the isolates, race C37(15) 26%, and race C9(15B-1L) 22%. Race C33(15B-1L) had increased appreciably, race C37(15) had increased slightly, and race C9(15B-1L) had decreased sharply. Chi-square was used at the end of the experiment to test a 1:1:1 distribution. Differences between the frequency of isolation of these races were highly significant.

Van der Plank (1969), in analyzing Katsuya and Green's data, indicated that race 15B has more virulence genes than race 56, which allowed it to attack varieties such as Hope which race 56 cannot attack, but has less aggressiveness on varieties such as Little Club, Marquis, or Red Bobs which are susceptible to both races. In gaining the virulence needed

to overcome the resistance of Hope race 15B lost aggressiveness on susceptible varieties. According to his hypothesis this is the essence of resistance given by strong genes. It makes the pathogen lose aggressiveness as it acquires virulence.

TABLE XIII. Number of isolates of three races of *Puccinia graminis* f. sp. *tritici* from artificially inoculated field plots at four collection periods.

Collection Period	Physiologic race		
	C9(15B-1L)	C33(15B-1L)	C37(15)
I (July 16)	21	21	10
II (July 20)	8	28	11
III (July 25)	8	27	16
IV (July 30)	5	30	16
TOTAL	42	106	53

Races C9(15B-1L) and C33(15B-1L) can attack varieties with the strong resistance gene *Sr11*. Race C37(15) cannot attack varieties with gene *Sr11* and, therefore, races C9(15B-1L) and C33(15B-1L) should not be able to compete as well as race C37(15) on the susceptible variety Red Bobs, but race C33(15B-1L) was a better competitor than either race C9(15B-1L) or race C37(15). The poor competitive performance of race C9(15B-1L) may have been influenced by resistance gene *Sr10* carried by Red Bobs. Gene *Sr10* is effective against race C9(15B-1L) but ineffective against races C33(15B-1L) and C37(15). Plants with *Sr10* become more susceptible at high temperatures and the gene usually does not

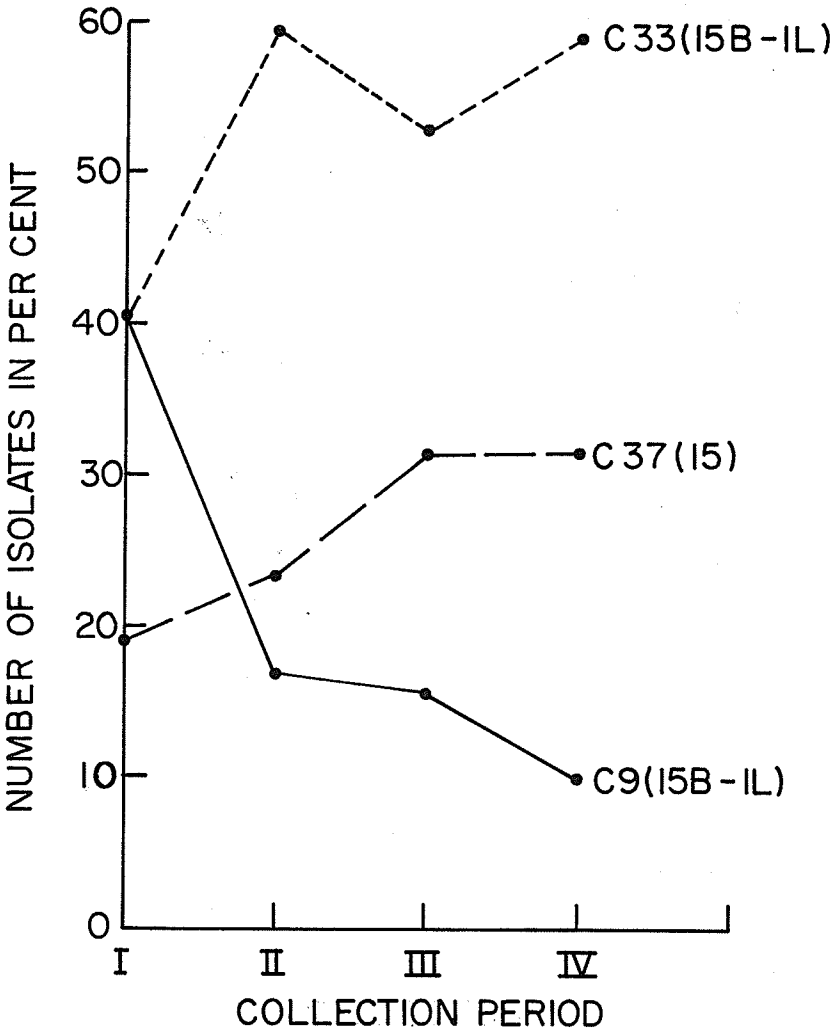

Figure 13. Recovery in percent of races used to artificially inoculate field plots.

confer a high level of resistance. However, the concurrence of the field and greenhouse results, in which race C33(15B-1L) predominated over race C37(15) which predominated over race C9(15B-1L) on three susceptible varieties, suggests that gene *Sr10* did not greatly alter the relative aggressiveness of the races in the field. It is more likely that changing temperature (Table XX) was more important in altering the relative prevalence of the three races in the plots.

The field plot results do not support the suggestion (van der Plank, 1968) that the planting of simple varieties in the southern United States, would increase simple races avirulent on northern varieties at the expense of complex races and hence render the resistance of complex northern varieties effective for extended periods. The evidence obtained here shows that complex races often have superior aggressiveness and, therefore, planting susceptible varieties in the south would not reduce their prevalence but could seriously threaten both southern and northern wheat production.

4.3 GROWTH CABINET INVESTIGATIONS

4.3.1 COMPETITIVE ABILITIES

Four mixtures were studied in growth cabinets to investigate the influence of temperature on the relative competitive abilities of races C9(15B-1L), C18(15B-1L), C33(15B-1L), C37(15), and C49(15).

4.3.1.1 RACES C9(15B-1L) AND C33(15B-1L)

Race C33(15B-1L) has the widest virulence range and race C9(15B-1L) has the narrowest of the races used. In the growth cabinets race

C33(15B-1L) soon predominated as it did in the greenhouse studies, particularly in the 25°C growth cabinet (Tables XIV and XV and Figure 14). The difference in competitive ability at 15°C did not become clear until the fifth generation. Race C9(15B-1L) was a poor competitor at 25°C and at the end of the fifth generation had decreased to less than 10% of the mixture, while at 15°C it was a better competitor and only decreased to 27% of the mixture.

TABLE XIV. Number of pustules of races C9(15B-1L) and C33(15B-1L) on the differential host Marquis-*Sr8* after each of five generations on three wheat varieties at 15°C.

Generation	Susceptible host varieties and races		
	Little Club C33:C9	Marquis C33:C9	Chinese Spring C33:C9
1	475:500		
2	487:351	415:320	356:275
3	479:301	493:330	551:258
4	592:429	608:486	610:498
5	675:265	666:248	668:226

TABLE XV. Number of pustules of races C9(15B-1L) and C33(15B-1L) on the differential host Marquis-*Sr8* after each of four generations on three wheat varieties at 25°C.

Generation	Susceptible host varieties and races		
	Little Club C33:C9	Marquis C33:C9	Chinese Spring C33:C9
1	443:338		
2	396:197	467:121	551:122
3	521:145	463:146	469:107
4	599: 51	585: 64	608: 33

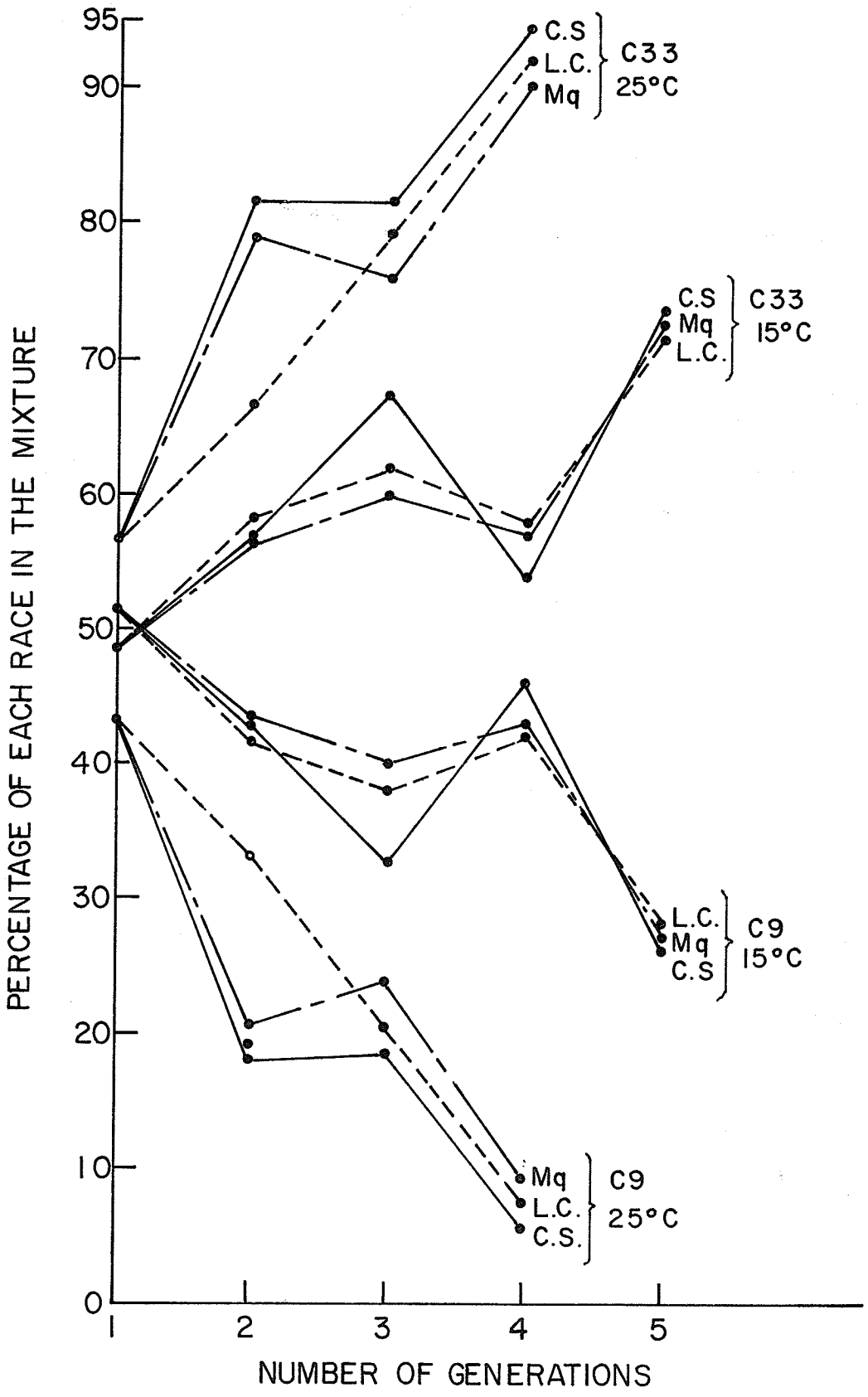

Figure 14. Survival ability of races C9(15B-IL) and C33(15B-IL) at 15° and 25° C.

That race C33(15B-1L) can increase in mixtures under a wide range of temperatures was also indicated by data from the field experiment. Race C33(15B-1L) rapidly became predominant after the first collections were made on July 16, 1973, and continued to predominate until the end of the experiment on July 30, when temperatures were higher. The field plot results suggest that race C9(15B-1L) is a better competitor at lower temperatures than at higher temperatures. About equal numbers of isolates of the races C9(15B-1L) and C33(15B-1L) were obtained in the first collection period when the temperature was low, but as temperature rose the number of isolates of race C9(15B-1L) decreased.

The results from the growth cabinet experiments might explain why race C9(15B-1L) never became a predominant race in the field. The maximum prevalence of race C9(15B-1L) was only 18.5% of the isolates in 1963. These isolates could have been produced early in the growing season when temperature was low, and as temperature became higher there was no further increase of race C9(15B-1L).

4.3.1.2 RACES C18(15B-1L) AND C33(15B-1L)

A mixture of races C18(15B-1L) and C33(15B-1L) was studied in growth cabinets because both races occurred commonly in nature. In greenhouse studies race C33(15B-1L) predominated over race C18(15B-1L) after eight generations. It was thought that race C18(15B-1L) should be a good competitor because of its long predominance in nature. It was therefore decided to study mixtures of these races at 15^{o} and 25^{o}C to learn whether race C18(15B-1L) would be a better competitor in a growth cabinet than in the greenhouse.

The competitive abilities of the two races were similar at 25^{o} and 15^{o}C (Tables XVI and XVII, and Figure 15) with race C33(15B-1L)

predominating at both temperatures. These results show that race C33(15B-1L) was more aggressive than race C18(15B-1L) and that it is a race that can adapt readily to various temperatures. It is suggested that the poor performance of race C18(15B-1L) in competition with race C33(15B-1L) is not due to temperature alone, and consequently temperature is not responsible for its declining prevalence in the field in recent years.

TABLE XVI. Number of pustules of races C18(15B-1L) and C33(15B-1L) on the differential host Marquis-*Sr8* after each of three generations on three wheat varieties at 15°C.

Generation	Susceptible host varieties and races		
	Little Club C33:C18	Marquis C33:C18	Chinese Spring C33:C18
1	430:476		
2	426:362	448:361	473:336
3	614:182	635:191	647:161

TABLE XVII. Number of pustules of races C18(15B-1L) and C33(15B-1L) on the differential host Marquis-*Sr8* after each of four generations on three wheat varieties at 25°C.

Generation	Susceptible host varieties and races		
	Little Club C33:C18	Marquis C33:C18	Chinese Spring C33:C18
1	566:576		
2	406:227	427:210	426:219
3	424:158	457:146	446:134
4	477: 87	506: 83	501: 95

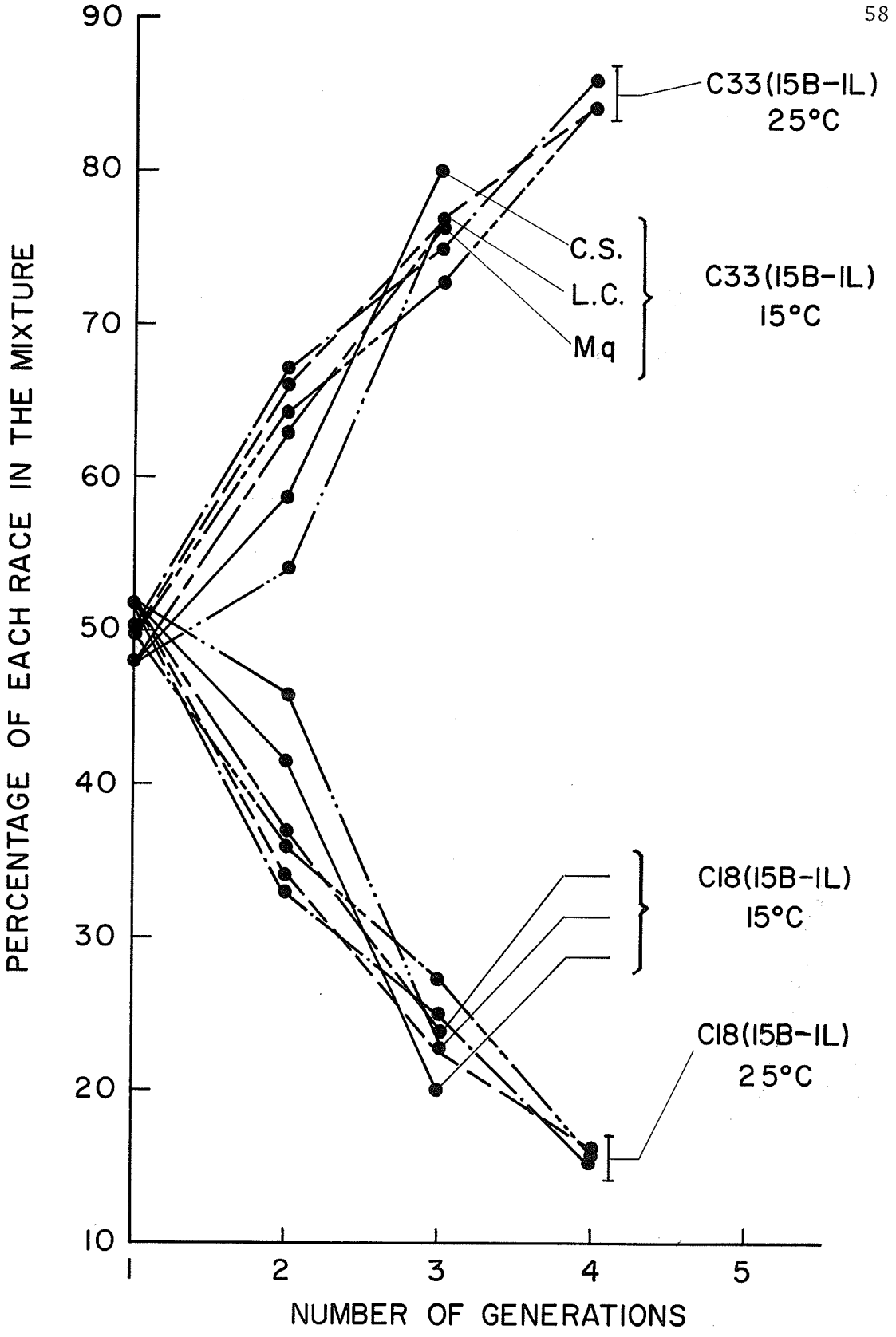

Figure 15. Survival ability of races C33(15B–IL) and C18(15B–IL) at 15°C and 25°C

4.3.1.3 RACES C9(15B-1L) AND C37(15)

When these races were mixed in the greenhouse, race C37(15), an unimportant race in Canada, predominated over race C9(15B-1L) possibly because of a temperature response. The data in Tables XVIII and XIX and Figure 16 on the competition between these races at 15° and 25°C show that race C9(15B-1L) predominated at 15° while race C37(15) predominated at 25°C. It was shown with a mixture of races C33(15B-1L) and C9(15B-1L), that race C9(15B-1L) was a better competitor at 15°C even though it comprised the smaller part of the mixture at both temperatures. Apparently race C37(15) was a better competitor at 25°C than at 15°C because the data suggest strongly that high temperature was of greater benefit to race C37(15) than to race C9(15B-1L). It is possible that race C37(15) could have become an important race in the field if temperatures over 20°C had persisted during the growing season.

TABLE XVIII. Number of pustules of races C9(15B-1L) and C37(15) on the differential host Marquis-*Sr8* after each of four generations on three wheat varieties at 15°C.

Generation	Susceptible host varieties and races		
	Little Club C9:C37	Marquis C9:C37	Chinese Spring C9:C37
1	455:192		
2	412:191	389:219	426:198
3	432:361	451:363	473:339
4	520: 88	539:100	545: 72

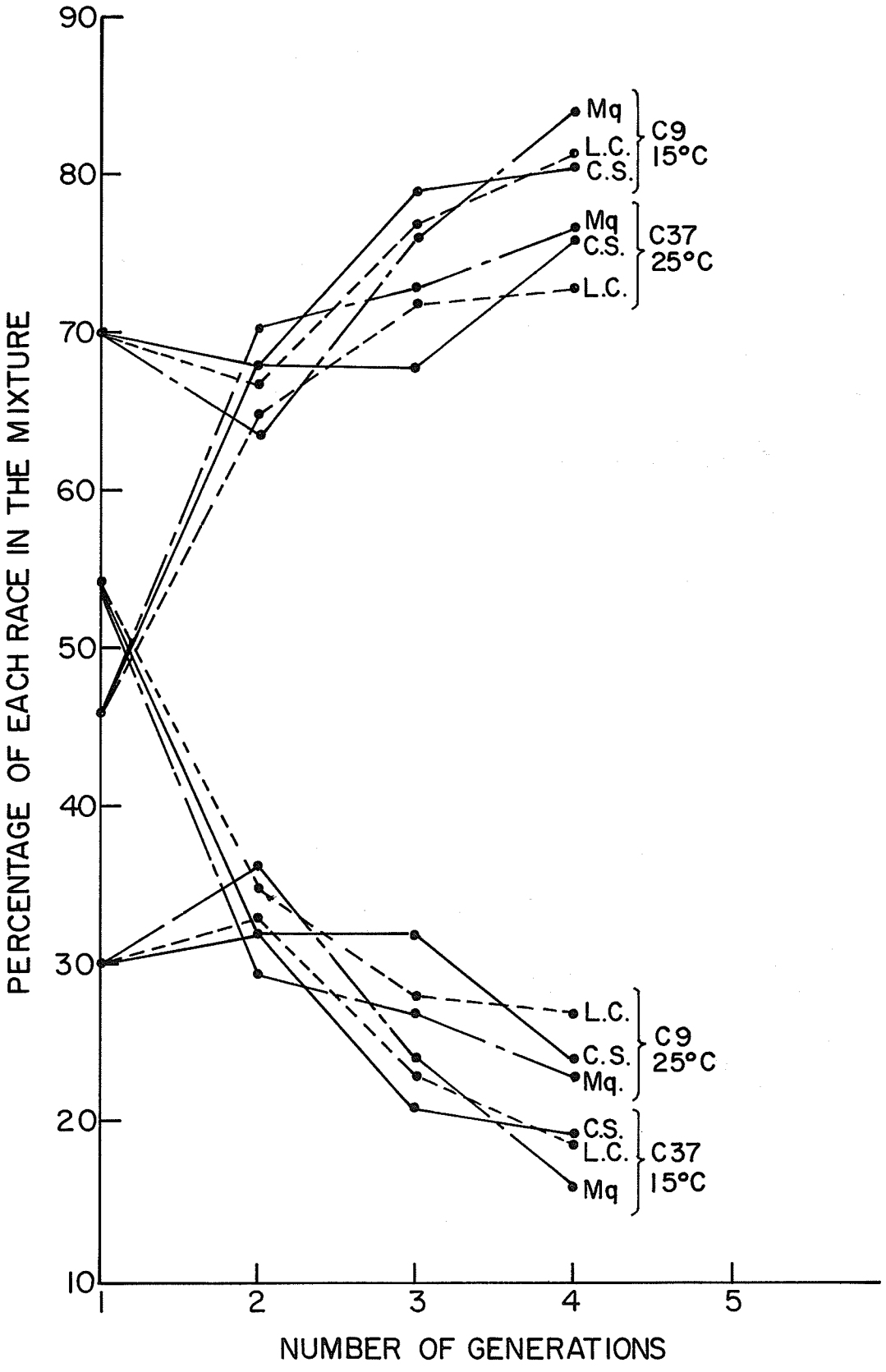

Figure 16. Survival ability of races C 9 (15B-1L) and C37 (15) at 15° and 25° C.

TABLE XIX. Number of pustules of races C9(15B-1L) and C37(15) on the differential host Marquis-*Sr8* after each of four generations on three wheat varieties at 25°C.

Generation	Susceptible host varieties and races		
	Little Club C37:C9	Marquis C37:C9	Chinese Spring C37:C9
1	246:241		
2	279:151	311:119	298:142
3	415:242	435:182	442:208
4	844:185	850:180	867:174

The effect of higher temperature on the performance of race C37(15) was confirmed in the field experiment where race C37(15) was isolated more frequently than race C9(15B-1L) towards the end of the experiment. The temperatures during June and early July when the rust was developing were low (Table XX) and isolates of race C37(15) were few. In the first collection period about 20% of the isolates were race C37(15), about 40% were race C9(15B-1L), and about 40% were race C33(15B-1L). As the temperatures rose after July 16, race C37(15) increased to about 31% of the isolates at the last collection period, and race C9(15B-1L) decreased from 40% to 23%.

TABLE XX. Mean temperatures observed in May, June, and July, 1973, at Glenlea Research Station.*

Month	Temperature in °F	
	Maximum	Minimum
May	65.0	40.0
June	70.3	50.9
July	76.0	53.0

*Data supplied by Dr. J. D. Truscott.

4.3.1.4 RACES C9(15B-1L) AND C49(15)

In the previous two mixtures with races C33(15B-1L) and C37(15), race C9(15B-1L) was a better competitor at low temperature than at high temperature. In competition with race C49(15), race C9(15B-1L) also was better at low temperature than at high temperature (Tables XXI and XXII and Figure 17). In the first generation at 15°C both races were equally represented (Table XXI). In the second generation race C9(15B-1L) increased to about 75% of the pustules and in the fourth generation comprised over 80%. The rate of change at 25°C was slower (Figure 17). Race C9(15B-1L) comprised about 59% of the pustules in generation one and did not increase for the next two generations. It increased about 7% in the fourth generation and suddenly in the fifth generation increased to 80% of the mixture. It required only four generations for race C9(15B-1L) to increase to 80% of the mixture at low temperature while at high temperature it required five generations.

TABLE XXI. Number of pustules of races C9(15B-1L) and C49(15) on the differential host Marquis-*Sr8* after each of four generations on three wheat varieties at 15°C.

Generation	Susceptible host varieties and races		
	Little Club C9:C49	Marquis C9:C49	Chinese Spring C9:C49
1	388:360		
2	446:158	445:159	439:149
3	1077:261	1034:244	951:283
4	659:149	802:141	805:125

TABLE XII. Number of pustules of races C9(15B-1L) and C49(15) on the differential host Marquis-*Sr8* after each of five generations on three wheat varieties at 25°C.

Generation	Susceptible host varieties and races		
	Little Club C9:C49	Marquis C9:C49	Chinese Spring C9:C49
1	486:334		
2	403:320	427:277	352:224
3	348:268	402:256	406:248
4	601:311	579:290	625:261
5	960:230	1056:194	958:263

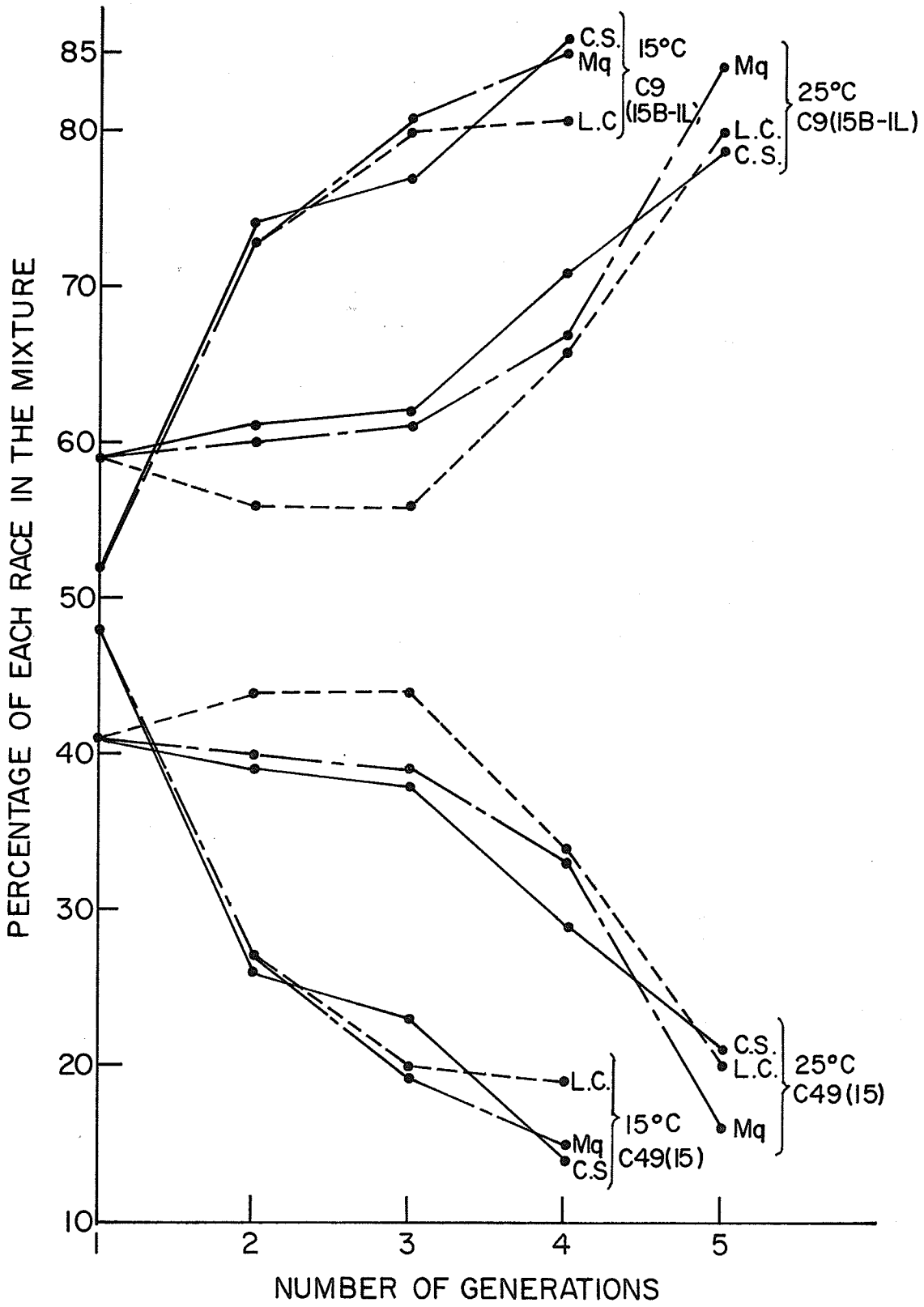

Figure 17. Survival ability of races C9(15B-IL) and C49(15) at 15° and 25° C.

4.3.2 FACTORS AFFECTING COMPETITIVE ABILITY

4.3.2.1 EFFECT OF PUSTULE AGE ON UREDIOSPORE VIABILITY

Viability of urediospores of seven races collected from 10 to 22 days after inoculation was similar (Table XXIII). Evidently pustule age during this period had little or no effect on spore germination. Spores of race C49(15) collected 25 days after inoculation did not germinate well after 3 hrs incubation but after 24 hrs reached about the same level as earlier collections. Apparently spores produced over a fairly long time interval by a single pustule have equal viability and pustule age is not an important factor in competitive ability insofar as it affects spore viability.

TABLE XXIII. Effect of pustule age on viability of urediospores of seven races of *Puccinia graminis tritici* at 20°C.

Race	Percent germination after 3 hours incubation					
	Days after inoculation					
	10	13	16	19	22	25
C9(15B-1L)	94.6	90.0	95.0	98.6	92.6	81.3
C18(15B-1L)	94.3	96.3	95.3	98.6	93.6	96.6
C33(15B-1L)	90.3	96.0	96.0	98.3	96.0	96.3
C37(15)	97.6	92.0	86.0	97.3	89.0	90.6
C38(15B-1L)	99.0	94.6	92.0	96.3	90.0	85.6
C42(15)	95.0	94.0	92.6	97.3	88.0	82.3
C49(15)	95.6	94.6	89.6	96.6	86.0	40.7

The slower germination of spores collected 25 days after inoculation may result from any one of a number of factors such as increased inhibitor concentration or slower water uptake. It probably would not affect the rate of rust spread at this late date of pustule development, possibly just prior to telial development, but it could be an important mechanism contributing to survival of the race under adverse conditions. However, delayed germination in one test could be characteristic of a particular spore collection rather than of the race.

4.3.2.2 EFFECT OF TEMPERATURE ON UREDIOSPORE VIABILITY

Samples were collected from freshly ruptured pustules to obtain spores of uniform age and to reduce contamination. Germination percentages were obtained using fresh spores, or spores stored for less than 7 days. Spores were seeded at low density on 10 ml of 1% water agar in plastic petri dishes (9 cm diameter). Seeding was accomplished by dipping a sterile camel's hair brush into the urediospores and brushing over water agar in a petri dish. Before seeding the spores, all dishes containing agar were equilibrated at the test temperature. Water agar was preferred to sterilized water because urediospores tend to aggregate in water making counts of germinated spores difficult. The position of the urediospores when seeded lightly on water agar, is fixed, and counts can be made accurately. Water agar also tends to offset the effect of endogenous germination inhibitors. Three hundred spores were counted in the initial test and 500 when the test was repeated (Test 2). A spore was considered to have germinated when the germ tube length was equal to or longer than half the width of the spore. Clumped spores were not included in the assessment. Spores

were considered clumped when three spores touched each other.

There was no germination at 2° and 5°C after 3 hrs and germination percentages at these temperatures were obtained in Test 1 after 8 hrs and in Test 2 after 6 hrs. Germination percentages for all races were still low after these periods (Table XXIV).

TABLE XXIV. Percent germination of urediospores of *Puccinia graminis tritici* at seven temperatures in two tests.

Test 1	Temperature °C						
Race	2	5	10	15	20	25	30
C9(15B-1L)	30.6	68.6	76.0	90.6	91.0	92.0	81.6
C18(15B-1L)	34.3	71.0	78.0	91.6	93.0	92.3	92.0
C33(15B-1L)	50.3	72.0	82.7	89.7	91.7	91.6	93.0
C37(15)	25.0	67.0	79.7	84.6	87.0	88.0	72.0
C38(15B-1L)	26.3	60.7	76.0	82.6	90.0	93.6	81.0
C42(15)	12.0	56.6	77.0	90.0	87.6	91.6	77.6
C49(15)	23.3	50.0	76.0	86.0	91.6	88.3	89.3
Test 2							
Race							
C9(15B-1L)	21.0	48.0	70.0	90.8	81.0	50.8	80.6
C18(15B-1L)	34.0	62.0	78.0	91.0	95.0	91.0	81.8
C33(15B-1L)	46.0	65.0	79.0	92.0	94.6	91.6	87.4
C37(15)	24.0	40.0	76.0	90.0	90.6	90.4	82.0
C38(15B-1L)	28.0	64.0	76.0	89.6	94.0	90.2	87.0
C42(15)	24.0	48.0	78.0	90.0	91.8	86.0	84.0
C49(15)	14.0	36.0	58.0	81.0	62.6	32.0	31.6

The pattern of germination was similar in both tests (Table XXIV). Races C33(15B-1L) and C18(15B-1L) reached 50% and 34% germination, respectively, at 2°C in Test 1. In Test 2 the percentage of germinated spores at 2°C was the same for race C18(15B-1L), slightly less for race C33(15B-1L) (46%), and 28% or less for the other races, with race C49(15) being lowest. In Test 1, race C42(15) was lowest followed by race C49(15). The same pattern was observed at 5°C although germination was better. At temperatures from 10° to 30°C differences in viability almost disappeared but races C49(15) and C9(15B-1L) germinated poorly in some tests, especially at 25°C. Race C49(15) germinated better in Test 1 than in Test 2 suggesting variability in the germinability of different spore lots.

4.3.2.3 INCUBATION PERIOD AND SURVIVAL ABILITY

The incubation period of the seven races was compared by counting ruptured pustules on seedlings of Little Club wheat in three pots, each containing approximately ten seedlings, at 15°, 20°, and 25°C. Three temperatures were used to determine whether or not races react differentially to temperature.

Pustules of races C18(15B-1L) and C33(15B-1L) broke through the epidermis about one day earlier than those of races C9(15B-1L), C37(15), C38(15B-1L), C42(15), and C49(15) at all three temperatures (Table XXV). The incubation period at 15°C was longer by 4 to 5 days than that at 20°C and 5 to 6 days than that at 25°C.

These observations indicate that the predominance of races C18(15B-1L) and C33(15B-1L) in nature as well as in experimental work could be partly due to a shorter incubation period.

TABLE XXV. Percentage of open pustules of *Puccinia graminis tritici* on Little Club wheat on successive days after inoculation at three temperatures.

Temperature	Race	Days after inoculation									
		6	7	8	9	10	11	12	13	14	15
	C9(15B-1L)							27	25	19	17
	C18(15B-1L)						14	28	23	35	
	C33(15B-1L)						26	38	28	19	
15°C	C37(15)							25	28	28	12
	C38(15B-1L)							26	23	28	15
	C42(15)							15	26	43	16
	C49(15)							7	21	41	23
	C9(15B-1L)			26	48	17	7				
	C18(15B-1L)		21	45	21	14					
	C33(15B-1L)		23	37	26	13					
20°C	C37(15)			15	54	22	9				
	C38(15B-1L)			24	37	30	8				
	C42(15)			18	44	29	9				
	C49(15)			4	54	25	17				
	C9(15B-1L)		26	50	24						
	C18(15B-1L)	14	68	17							
	C33(15B-1L)	31	52	17							
25°C	C37(15)		35	45	20						
	C38(15B-1L)		24	47	29						
	C42(15)		21	44	35						
	C49(15)		16	50	34						

4.3.2.4 SPORE PRODUCTION AND SURVIVAL ABILITY

Seven days after inoculation, seedlings of Little Club wheat with a maximum of 4 infections were placed in an open ended, glass, "sporulation" tube 15 cm long and 18 mm in diameter. Preliminary experiments revealed that significantly more spores, about 30%, could be collected from leaves in the sporulation tube than from leaves exposed to the normal airflow in growth cabinets. To support the seedlings in the tube a circular opening about 18 cm in diameter was made in the top of a cardboard box which measured 35 x 35 x 15 cm. The lower side of the box was removed so that the pots stood on the growth cabinet benches. Three pots with lightly infected seedlings were placed in the box. "Sporulation" tubes containing infected leaves were placed horizontally on top of the carboard box. Open ended glass tubes were used to avoid condensation by permitting free movement of air in the tube.

Variability in spore production was reduced to a reasonable level by selecting pustules of the same age and size on plants kept under the same environmental conditions. The growth cabinet temperature was 21^{o}C with a slight variation not exceeding 2^{o}C. It was monitored by means of a thermograph and checked by a thermometer. The host leaves in the sporulation tubes produced spores for almost 26 days. The growth of the host apparently was unaffected by the glass tube.

Analysis of variance (Table XXVII) showed that race C33(15B-1L) produced most spores but the differences between successive races in Table XXVI were not significant. Average spore production for all races was greatest between 19 and 22 days after inoculation. These results agree with those of Browder (1965) who found that peak spore production occurred between 16 and 24 days after inoculation.

TABLE XXVI. Spore production by five races of *Puccinia graminis tritici*.

Race	Spores per pustule					
	Days after inoculation					
	14	18	22	26	Total	Average
C33(15B-1L)	47,200	83,320	120,000	85,000	335,520	83,880
C18(15B-1L)	61,000	85,000	90,000	75,000	311,000	77,750
C9(15B-1L)	48,500	67,500	55,000	61,250	232,250	58,062
C37(15)	45,000	39,000	85,000	55,000	224,000	56,000
C49(15)	30,500	40,540	66,660	81,100	218,800	54,700
					1,321,570	

TABLE XXVII. Analysis of variance of the spore production capability of five races of *Puccinia graminis tritici*.

Sources of variation	D.F.	S.S.	M.S.	F
Races	4	2993.6800	748.4200	3.4366*
Time	3	3607.3063	1202.4354	5.5214*
Time x Races	12	2613.2826	217.7735	

*Significant at the 5% level.

All races produced 1,321,570 spores in 16 days, a daily average production of 82,600 spores. Race C33(15B-1L) produced 20,970 spores daily, race C18(15B-1L) 19,437, race C9(15B-1L) 14,515, race C37(15) 14,000 and race C49(15) 13,675. These numbers agree with those calculated from the finding of Katsuya and Green (1967), that a well

developed uredium produces about 31 ug of spores per day, and that of Rowell (1972), that there are about 500 spores in a ug, indicating a daily production of 15,000 spores per uredium.

The differences in the numbers of spores collected after each interval may be caused by differences between races in the speed of spore release and time required for spores to mature. The differential rate at which spores are released by different races could be a very important factor in the speed at which races spread in the field. The average number of spores produced for all collection intervals for each race (last column Table XXVI) shows the differences between the spore producing abilities of the races. When these results are compared with the results obtained in the experiments on competitive ability in the greenhouse and growth cabinets, there is a close correlation between the number of spores produced by the races and their ability to predominate. In both sets of experiments race C33(15B-1L) predominated over the other races, race C18(15B-1L) predominated over race C49(15), race C37(15) predominated over races C9(15B-1L) and C49(15), and race C9(15B-1L) predominated over race C49(15).

In a mixture, starting with approximately the same amount of each race, race C33(15B-1L) did not increase significantly over race C18(15B-1L) in the greenhouse in the first 4 generations, indicating that spore production by these races does not differ greatly. But race C33(15B-1L) produces more spores than race C18(15B-1L) and, therefore, in each of the following generations the effect of a difference in spore production increases and this could explain why race C33(15B-1L) predominated over race C18(15B-1L) in later generations. Race C33(15B-1L) displaced race C9(15B-1L) after only four generations possibly because

race C33(15B-1L) produces considerably more spores than race C9(15B-1L). Spore production capability seems to be an important factor in aggressiveness.

5. DISCUSSION

The hypothesis of stabilizing selection proposed by van der Plank has stimulated investigations that have produced conflicting results. The hypothesis is based on the premise that virulence genes are harmful and that strains of plant pathogens with many virulence genes (complex races) are less fit to survive than strains with few or no virulence genes (simple races). The hypothesis is qualified by the stipulation that host resistance genes are "strong" if virulence on them seriously reduces pathogen aggressiveness and "weak" if they cause little harm. Virulence on a strong resistance gene is usually found only in those races isolated from varieties with that particular resistance gene. Virulence on weak genes is less harmful and may occur in a pathogen population without the advantage of selection pressure from host varieties. This qualification greatly reduces the potential usefulness of the original hypothesis which could be of value in breeding for resistance if it could be proved that virulence is harmful to the stem rust fungus.

The purpose of this study was to establish clearly whether or not stabilizing selection was operative in wheat stem rust in North America. The approach to the problem was to demonstrate whether or not virulence genes are harmful. The hypothesis of stabilizing selection rests on this point and although there is evidence that virulence is not harmful much of the evidence is circumstantial or can be criticized because investigations were performed with races not shown to be alike except for virulence or avirulence on a particular host resistance gene. Obviously, evidence cannot be regarded as valid if it is obtained using

races that differ in genes, other than virulence genes, that govern aggressiveness. The ideal test is to compare the aggressiveness of two pathogen races that are isogenic except for a single virulence gene. The reasons for selecting the cultures used in this study have been given previously. There are good reasons for believing they are closely related although there may be some differences among them, other than virulence, that affect aggressiveness. In testing the hypothesis, it seems reasonable to assume, using this kind of material, that if virulence genes are harmful the accumulation of virulence should usually reduce fitness (or aggressiveness) and the loss of virulence should increase fitness. If the results are otherwise it would be difficult to regard virulence genes as being harmful.

The results with eight out of nine mixtures studied in the greenhouse do not support van der Plank's (1963, 1968, 1969) hypothesis and one does (C9(15B-1L) and C49(15), Table XXVIII). In six mixtures involving simple and complex races, a complex race predominated in five mixtures while a simple race predominated in one mixture. In mixtures involving races with equal numbers of virulence genes (Model III) there was no evidence of races competing equally well. Races used to study this model differed in competitive ability: one race increased at the expense of the other. These results are in general agreement with those of Keed (as reported by Watson and Luig, 1968) and Ogle and Brown (1970). They used closely related races and in both cases a race with many genes for virulence predominated in the mixtures. The evidence obtained here indicates that genes determining competitive ability are independent and randomly distributed in the rust population. Their association with avirulence genes in some races is by chance, although in some instances

there may be linkage.

TABLE XXVIII. Predominant races in mixtures in greenhouse studies and some of their characteristics.

Race Mixture	Predominant Race	Generations to predominance	Daily spore production	Incubation period at 20°C
Model I				
C9+C33	C33	4	20,970	7 days
C9+C49	C 9	10	14,515	8 days
C37+C9	C37	4	14,000	8 days
Model II				
C18+C33	C33	10		
C33+C37	C33	6		
C42+C49	C49	4	13,675	8 days
Model III				
C18+C49	C18	8	19,437	7 days
C33+C38	C33	10		
C37+C49	C37	5		

The hypothesized series of mutations in the 15B-1L group that produced the races selected for this study has been described previously. It is not clear, however, whether these mutations altered aggressiveness. Presumably, race C18(15B-1L) evolved from the less aggressive race C9(15B-1L), and race C33(15B-1L) evolved from the less aggressive race

C18(15B-1L). The failure in this study to find aggressiveness associated with avirulence leads to the conclusion that mutations at virulence loci do not alter aggressiveness directly. However, they may cause changes if aggressiveness depends on gene interactions. A second explanation for altered aggressiveness in races that apparently evolved from one another by single gene mutations is that the original race consisted of strains of different aggressiveness. A mutation to virulence on *Sr8* in an aggressive strain of race C18(15B-1L) would produce race C33(15B-1L).

The concept of stabilizing selection as presented by van der Plank is a proposal to explain the relative prevalence of races of plant pathogens in nature. As far as it is known, changes in races of plant pathogens are caused by two main factors: a change in host resistance genes, or a change in the environment. A change in race prevalence could be a direct result of the introduction of a resistant variety, which caused avirulent genotypes of the pathogen to decrease and virulent genotypes to increase. In the second case, changes in race prevalence could result from the effect of changing environmental conditions on characteristics of races other than virulence. It has been shown (Johnson, 1953; Green, 1971a) that probably the most important factor favouring race 15B in 1950 was the enormous acreage of common and durum wheats resistant to other races but susceptible to race 15B. Johnson and Newton (1941), Stakman, Loegering and Cotter (1942) and Green (1971a) produced evidence to show that the widespread cultivation of the susceptible varieties Ceres and Marquis favoured the increased prevalence of race 56. However, there are many factors besides varietal reactions that affect the survival ability and prevalence of physiologic

races. Katsuya and Green (1967) showed that the rise of race 15B in 1950 was favoured by low temperature. Cool, rather moist, summer weather prolonged the 1950 growing season and gave race 15B additional time to develop as well as favouring its spread. The results obtained in these studies, both in growth cabinets and field plots showed that environmental factors, particularly temperature, have a strong influence on the competitive ability of wheat stem rust races. Race C9(15B-1L) in growth cabinets was favoured by low temperature ($15^{o}C$) while race C37(15) was favoured at high temperature ($25^{o}C$). Race C33(15B-1L) tolerated high and low temperatures. In view of the complexity of the selection pressures operating in nature, the concept of stabilizing selection as the principal factor governing race prevalence seems inadequate.

A race with superior urediospore viability would be expected to produce more infections than a race with average viability. The results of the experiment on spore germination indicate that races differ somewhat in spore viability (Table XXIV). Races C18(15B-1L) and C33(15B-1L) had slightly higher viability than other races, especially at low temperature. The superior urediospore viability of these races could contribute to their generally superior aggressiveness, although its influence would not be expected to be large.

There is evidence that the length of the incubation period is an important factor in the relative aggressiveness of races (Browder, 1965; Katsuya and Green, 1967). In the experimental work described here the length of the incubation period of races C18(15B-1L) and C33(15B-1L) was found to be one day shorter than that of the other races. The early production of urediospores is probably one of the main reasons for the

superior aggressiveness of these races.

There is good evidence (Table XXVI) that the sporulating ability of races C18(15B-1L) and C33(15B-1L) contributes greatly to their aggressiveness. They produced more urediospores per pustule than races C9(15B-1L), C37(15), and C49(15), and there is a good correlation between the number of urediospores produced and the relative competitive abilities of these races. Other reports confirm that races differ in sporulating ability and that the differences are important in determining relative aggressiveness. Katsuya and Green (1967) found that race 56 produced about twice as many urediospores as race 15B-1 (Can.). Broyles (1955) demonstrated a close relationship between the number or urediospores produced and the relative rate of increase of races in mixtures. He also found that races differ in the time required for urediospores to mature and the ease with which they are shed. Differences in the morphology of the uredia may also affect aggressiveness (Kak *et al.*, 1963). It is concluded that spore producing capacity is an important factor, possibly the most important one, determining the relative aggressiveness of races.

The results of this study show that virulence genes are not harmful to the wheat stem rust fungus and consequently, that the concept of stabilizing selection, as proposed by van der Plank, is invalid with respect to *P. graminis* f. sp. *tritici* in North America. Nelson (1972) came to a similar conclusion and commented that "the idea of stabilizing selection by vertical resistance genes never passed the hypothetical phase since it was never put to the test before it was presented as a concept fortified with the self-evident axiom that simplicity equals fitness and buffered by the strong-and-weak gene rationale." It seems

evident that the factors most strongly influencing race prevalence are the resistance of widely grown varieties, environmental conditions that differentially affect races, and the characteristics of the races in the population such as incubation period and sporulating capacity.

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