SHORT TITLE

GENETICS OF HOST:PARASITE RELATIONS IN SUNFLOWER : RUST

BY

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GENETICS OF HOST:PARASITE RELATIONSHIPS BETWEEN SUNFLOWERS AND SUNFLOWER RUST.

by

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INTRODUCTION

The brilliant studies of Flor (1942, 1946, 1955, 1956, 1959), to account for the interaction between flax and flax rust, led him to postulate a gene-for-gene relationship in the flax and flax rust system. Later Person (1959), by an ingenious theoretical model, showed that a relationship such as postulated by Flor should occur as a general rule in host-parasite systems. And since then this hypothesis has been extended to a number of host-parasite systems (Day, 1960; Person, Samborski, and Rohringer, 1962; Person, 1965, 1966).

Besides an academic interest in understanding the genetic mechanism in host-parasite relationships, such study might be useful in breeding for disease resistant varieties of crop plants. The idea that such studies can be helpful in breeding for disease resistant varieties, has been expounded by Flor (1955), and Day (1960). Such studies might help to define the limits of variability in the pathogenic capacities of a parasitic organism as well as the differences within a host species; and they are basic to the intelligent planning for breeding programmes designed to develop varieties resistant against obligate or near obligate parasites.

The cultivated sunflower species (Helianthus annuus L.) is diploid. In this species crossing and inbreeding can be accomplished with a reasonably good seed set.

The rust (Puccinia helianthi Schw.) attacking sunflowers is autoecious and heterothallic. The sexual stages necessary for crossing and inbreeding occur readily in nature, and can often be produced with relative ease under controlled conditions.

Genetic studies conducted in Canada on rust resistance in sunflower have so far revealed two major independently inherited genes for resistance in the Canadian accessions (Putt and Sackston, 1963). With the aid of these two factors for resistance four possible physiologic races of the pathogen have been identified (Sackston, 1962). The number of interacting loci in this host-parasite system is at the moment much smaller than those identified in the flax and flax rust system. Part of its attraction as a subject for this kind of investigation is its apparent simplicity.

The sunflower and sunflower rust, possessing all these advantages, are considered to be another host-parasite system suitable for investigation of the genetic relationship between a host and a parasite. The genetics of resistance in sunflower, as mentioned before, has been studied by previous workers, but no study on genetics of pathogenicity in sunflower rust has yet been undertaken. The objectives of the present study were to investigate the nature of inheritance of pathogenicity in sunflower rust and to determine whether this host-parasite system conforms to Flor's gene-for-gene theory. However, in the course of this investigation it was discovered that the differential host varieties are heterozygous for rust reaction and that the genetics of resistance appeared to be more complex than reported by Putt and Sackston (1963). A study of the genetics of resistance in the differential host varieties was therefore included in addition to the inheritance of pathogenicity in the rust.

PART I

GENETICS OF PATHOGENICITY IN SUNFLOWER RUST

REVIEW OF LITERATURE

Genetic studies with rust fungi were made possible by the discovery of two notable phenomena. The first of these is the existence of physiologic specialization first described by Eriksson and amplified later by Stakman. Eriksson in 1894 (Johnson and Newton, 1946) demonstrated that isolates of Puccinia graminis from wheat, oats, rye, and some other grasses, although morphologically similar, were pathogenically different. He called these pathogenically distinct entities formae speciales. Stakman and associates (Stakman and Piemeisel, 1917) showed that Eriksson's formae speciales were not homogenous but included biologic forms varying in their pathogenicity on different varieties within a single cereal species. Later, from the evidence accumulated since 1917, Stakman and others (Stakman and Levine, 1922, 1938; Stakman, Levine and Loegering, 1944; Stakman, Stewart and Loegering, 1962) developed a key based on infection types for differentiating physiologic races of P. graminis, especially P. graminis f.sp. tritici. This is the foundation of present day rust classification (Johnson and Newton, 1946).

The second major step which made genetic studies of rust fungi possible was the discovery by Craigie of the function of pycnia (Craigie, 1927) and the existence of heterothallism in certain rust fungi (Craigie, 1928). The first experimental hybridization between races of wheat stem rust was reported by Waterhouse (1929). He mixed pycnial nectar of races 34 and 43 and obtained two races which were new to the rust population of Australia. Stakman, Levine and Cotter (1930) confirmed the role of barberry in the production of new races of wheat stem rust. Newton,

Johnson and Brown (1930a) reported selfing of several races of wheat stem rust. Of the eight races selfed, only one proved to be homozygous for all pathogenic characters expressed on 12 differential varieties.

Johnson and his associates (Newton, Johnson and Brown, 1930a, 1930b; Newton and Johnson, 1932; Johnson and Newton, 1940a, 1940b) have reported since 1930 the results of extensive studies on selfing and hybridization of physiologic races of cereal rusts which have contributed to much of our knowledge of rust genetics. They demonstrated that the pathogenic properties of Puccinia graminis are generally inherited in accordance with Mendelian laws. They presented evidence of dominance, recessiveness, and independent segregation of pathogenic factors. Avirulence was generally dominant and virulence recessive. This has been found to be true with rusts in general (Flor, 1946, 1955, 1956; Loegering and Powers, 1962; McCain, 1963). However Johnson and Newton (1946) and Johnson (1954) have shown that in some cases virulence can be dominant over avirulence.

Newton et al. (1930a), Johnson, Newton and Brown (1934), Flor (1942, 1946), Johnson (1954), Vakili (1958), Wilcoxson and Paharia (1958), Flangas and Dickson (1961a) and Samborski (1963) found most rust races were highly heterozygous for pathogenic factors and upon selfing produced progenies with different pathogenic properties from the parents. Johnson and Newton (1940a, 1940b) showed that some races of wheat stem rust and oat stem rust contained both homozygous and heterozygous lines.

Johnson and associates (Newton and Johnson, 1927; Newton et al., 1930b; Johnson et al, 1934; Johnson and Newton, 1938, 1940a; Johnson, 1949) studied the inheritance of spore colour and found that it obeyed Mendelian laws. Newton et al. (1930b) also reported an association between spore colour and pathogenicity.

Newton et al. (1930b), Newton and Johnson (1932) and Johnson and Newton (1946) considered pathogenicity in some cases to be controlled by cytoplasmic factors. Certain crosses with wheat stem rust races showed matroclinal inheritance while other races gave strictly Mendelian ratios. Johnson (1949) found a similar phenomenon in crosses between some oat stem rust races.

Flor (1942, 1946, 1955, 1956, 1959) working with flax and flax rust conducted a more detailed investigation, studying the genetics of both host and pathogen simultaneously, which led him to propose a gene-for-gene theory. For each gene conditioning resistance in the host there is a specific and complementary gene conditioning virulence in the pathogen. Flor found that resistance in flax was inherited as a dominant character and virulence in the rust was recessive except in one variety, Williston Brown, where virulence appears to be dominant. Rust resistance genes in flax have been shown to occur as multiple alleles at 5 loci and some resistance genes were found to be linked. Virulence genes in M. lini on the other hand, were found to be non-allelic and unlinked.

The gene-for-gene theory as postulated by Flor was elaborated by Person (1959). A theoretical consideration of a gene-for-gene relationship led Person to conclude that such relationships as proposed by Flor for the Linum-Melampsora system should occur in host-parasite systems in general. Person's analysis of Flor's data showed, however, that most of Flor's "single-gene" differential varieties actually possess two or more genes for resistance and that resistance genes in these varieties need not fall into allelic or closely linked groups.

Person's analytical method, developed for ideal systems with one-for-one relationship, has been shown to be also valid for systems with two-for-one relationships, that is, where more than one gene (duplicate or complementary loci) in the host conditions resistance to a single gene for virulence in the parasite and vice-versa (Person, 1959). It was pointed out by Person that lack of knowledge of the genotype, either in the host or in the pathogen to which segregating progenies are exposed, may impose a severe limitation on genetic work. However, properties of gene-for-gene relationships as revealed in the analysis can overcome these difficulties. Person discussed the pattern of physiologic races that can be generated by a system of host genes with the concept of gene-for-gene relationships and showed by this analysis how the concept may be used tentatively to determine the number of genes involved in each differential host variety and which gene they have in common. Person's analysis of the published data showed which kind of host-parasite system may be suitable for demonstrating a gene-for-gene relationship. The Solanum-Phytophthora system is relatively simple because the number of interacting loci is small and the information on the host-parasite is complete. But the Linum-Melampsora is complex because of the discovery of a large number of races, the number of interacting loci is relatively large and the information on host-parasite interactions is incomplete. This is expected to become more complex with the detection of undiscovered genes which can further resolve pathogenicity of the fungus.

Besides the Solanum-Phytophthora and Linum-Melampsora systems, gene-for-gene relationships have been demonstrated or invoked in the explanation of genetic data arising out of a number of other host-parasite systems (Boone and Keitt, 1957; Moseman, 1959; Person et al, 1962; Person, 1965, 1966).

Mode (1958) discussed the consequences of complementary genetic systems where random mating occurs in both host and pathogen populations over a long period of time, the significance of linkage of genes for resistance in the host, and the relationship of such systems to the co-evolution of obligate parasites and their hosts. Through a mathematical model he suggested a state of dual balanced polymorphism between the host and pathogen populations was a necessary condition for the co-evolution of obligate parasites and their hosts.

The existence of gene-for-gene relationships as applied to the resistance of wheat to Puccinia graminis tritici has been questioned by Laubscher (1963) on the grounds that the number of identified resistance genes in wheat is relatively small in comparison to the number of races isolated. In addition, resistant plants can be predisposed to susceptibility in the absence of a pathogen. In his view, a more satisfactory explanation would be that wheat resistance is conditioned by regulator genes able to ~~suppress~~ repress the biosynthesis of metabolites specifically necessary for particular rust races. The induction of susceptibility by high temperature may be due to inactivation of the repressor protein by inductor metabolites formed in excess by the host under these conditions.

MATERIALS AND METHODS

Four physiologic races of Puccinia helianthi Schw., described by Sackston (1962), were subjected to selfing and crossing studies in this work. The selfed and hybrid progenies of these races were tested on three differential varieties and three supplemental lines. The three differential varieties were selected by Sackston (1962) and their genetic constitution determined by Putt and Sackston (1963). The varieties are: (1) S37-388, having no known resistance to rust; (2) Morden Cross 29, possessing the resistance factor; R_2, of source 88 and (3) Morden Cross 69 possessing the resistance factor, R_1, of source 22. The differential varieties Morden Cross 29 and Morden Cross 69 will be hereafter called simply by the names Morden 29 and Morden 69 respectively; in order to avoid confusing use of the word "Cross" to describe both host varieties and rust hybrids.

Differential varieties

S37-388 (Universal suscept). The inbred line S37-388, having no known resistance to this pathogen, was designated by Sackston (1962) as 'universal suscept' for the sunflower and sunflower rust system based on Person's (1959) gene-for-gene concept in host-parasite relations.

Morden 29. According to Sackston (1962) and Putt and Sackston (1963), this variety carries the resistance gene designated R_2, of source 88. It is attacked by races 2 and 4 and is resistant to races 1 and 3. The seeds of this variety were first supplied as single-head lines by Dr. Sackston from the stock raised at Winnipeg and later by Dr. Putt at Morden, Manitoba. However, at the start of this work, none of the seedlings raised from any

of the heads supplied, gave constant results. Thereafter, different lines of this variety were grown at the Macdonald College farm and selfing followed by selection was practiced for three years for types with desirable rust reactions. Seeds from selected heads have been employed in this study. However, the seedlings raised from the selected heads also in some cases gave aberrant or unusual reactions.

Morden 69 or CM9ORR. Morden 69 was selected to represent the source of resistance possessing gene R_1. According to Sackston (1962) and Putt and Sackston (1963) this variety is attacked by races 3 and 4 and is resistant to races 1 and 2. As in Morden 29, the seed of this variety was obtained from individual heads. In routine tests at the beginning of this work it was found that these heads were heterozygous for rust reactions. Three other lines, S37-388RR, CM5RR and CM9ORR, which were also supposed to carry the resistance factor of source 22 (Putt, personal communication) were tested for possible use as a differential. CM9ORR was selected for use in this work. A bulk lot of seed of this line was received from Dr. Putt at Morden. This variety also proved heterozygous for reaction to races 3 and 4, but the majority of the seedlings in early tests gave appropriate reactions. Later it was found that this variety also at times gave erratic reactions, especially to race 4.

Supplemental lines

M69-W59-11-MC62-13-r4). This line was derived from a segregating head of Morden 69 when sown at Macdonald College in 1962. The S_1 derivatives of this line at first appeared to be susceptible only to race 4, thus resembling Putt and Sackston's source 41, but when a larger number of progeny were tested this reaction was not consistent and subsequent generations became increasingly unpredictable. No single head was found to give consistently susceptible progeny.

M62-2672-2-r1 (=F_2 of S37-388 x 150-2-1). It is derived from a cross S37-388 x Wild Annual Sunflower; seeds of the wild sunflower were collected in Chicago by Dr. Sackston. The characteristic of these plants is that they are susceptible to race 1 and highly resistant to the other three races. Crosses were made at Morden by Dr. Putt and the F_2 seeds were sent to Dr. Sackston for further studies. In the spring of 1964 when the progeny seeds were sown in the greenhouse to test the seedling reactions to the four parental races most of the seeds appeared to have abortive embryos and only a few germinated. When planted for seed production, only two plants had a good seed set and they were heterozygous for rust reactions. The seeds of these two heads were used as one of the supplemental lines. Due to the limited quantity of seed they were used in only a few tests and they served as a useful marker in rust crosses.

M62-2685-14-I (=F_2 of S37-388 x 150-6-1-1-1). The history of the origin of this line is similar to the previous one except that the seedlings of this source are either highly resistant or immune to all four races. In this case a number of plants produced good quantities of seed. The seeds of two heads were used in this investigation and they were phenotypically homozygous for rust reaction to the parental races. Like the previous one, this line also served as a very useful marker in detecting successful rust crosses.

Environmental conditions

In studies of both host and pathogen genetics the seedlings were raised in the greenhouse under a 16-hour day length. The additional hours of light were supplied by artificial light at about 600 to 700 foot-candles. The temperature in the greenhouse during winter and spring was maintained

at 22°C during the day and at 20°C during the night. However, at times during a sunny day the temperature went higher than 27°C. Seeds were grown in 4-inch pots with 4 or 5 plants per pot, 3-inch pots with 2 or 3 plants per pot and 9 oz Dixie paper cups containing 2 or 3 plants per pot. The larger pots were difficult to accommodate in the space available, the paper cups did not last long and 3-inch pots were found to be most satisfactory. The soil used was a mixture of 3 parts soil to 1 part sand. Seedlings were inoculated with uredial inoculum at the age of 16 to 18 days when the first pair of true leaves was fully expanded, using one of the methods described later. The inoculated plants were maintained overnight in a moist chamber and removed on the following day and kept for 2 to 3 hours under low light for slow drying of the leaves before being placed either on a greenhouse bench or in a growth cabinet.

Temperature and light conditions in the greenhouse were approximately the same as those provided during the pre-inoculation stage of growth. The controlled environment chambers were maintained at 22°C ~~72°F~~ day temperature and 20°C ~~68°F~~ night temperature. The light period was 16 hours at an intensity of 800 to 1000 foot-candles. Two or three days after inoculation, the plants were given nutrient solution, made by dissolving 'Instant Vigoro', 19-28-14, at the rate of 2 level tablespoons per gallon of water, at the rate of approximately 150 ml. per 4-inch pot, and 100 ml per paper cup or per 3-inch pot.

Rust reactions

The rust reactions were generally recorded 13 to 14 days after inoculation but on some occasions they were recorded as early as 10 days or as late as 17 days after inoculation, depending upon the speed of rust development. In the spring maximum development of rust reactions was

frequently obtained within 10 days from the date of inoculation while in mid-winter it sometimes took up to 17 days. In the latter case the rust reactions were recorded twice, once at 13 to 14 days and again at a later date.

The expression of host-parasite interactions was recorded according to the system of classification proposed by Sackston (1962) as follows:-

"4" Very susceptible, producing large, vigorously sporulating uredia.

"3" Moderately susceptible, producing medium-sized, vigorously sporulating uredia.

"2" Moderately resistant, producing small, less strongly sporulating uredia.

"1" Very resistant, producing very small, very weakly sporulating uredia.

"0" Immune or no uredia produced. The hypersentive flecks which often occur are designated by a semicolon, 0;

However, in this work "0" was used to denote escape or doubtful infection and the immune reaction was noted by the letter "I". The presence or absence of chlorosis was indicated by "C" or "N" respectively. When the uredia were larger on the lower surface than on the upper surface, they were denoted by a stroke over the figure, e.g. $\overline{3}$. In this work although Sackston's system of classification was followed, it could not be adhered to closely, since in critical tests the seedling leaves were inoculated by the "multiple inoculation" method while in Sackston's work they were inoculated by the "routine" method of inoculation. The size of the pustules tended to be smaller in multiple inoculation than those

produced by routine inoculation. Furthermore, in this work the reaction types were found to be more variable than noted by Sackston, that is, at times even the susceptible varieties appeared nearly resistant and resistant varieties, susceptible.

In Sackston's work, restriction of uredia to the lower surface of the leaf was considered as an indication of resistance or of reduced susceptibility with increasing age of leaves when the plants are grown under greenhouse conditions. In this study, it was observed that sometimes during mid-winter in the greenhouse-grown plants the uredia tended to be restricted to the lower surface, or larger on the lower than on the upper surface of the leaf. These considerations were taken into account in classifying the seedlings as suceptible or resistant. When the development of rust reaction was poor even on the universal suscept the seedlings showing 2^{+} and 3^{-}, 3^{-}, 3 or 4, whether on the upper or lower surface, were classified as susceptible. In some cases seedlings with 2 to 2+ were distinguished by the descriptive terms, such as, intermediate types (S= or R=), nearly susceptible or nearly resistant and so on.

Uredial inoculation methods

The seedlings were inoculated by one of two methods:

1. Routine or mass inoculation method

This is a method commonly used by rust workers. The leaves were first sprayed with a fine mist of water and then they were inoculated by shaking a heavily rusted pot of plants over the experimental plants. In this operation care was taken that the inoculum laid on the leaves was of the right amount and well distributed, the plants were then inoculated overnight in the moist chambers as described before.

2. Multiple inoculation method

This method was developed in the course of this investigation when it was found that the varieties were heterozygous for rust reaction and that the routine method of inoculation might not be reliable in determining the inheritance of pathogenicity of the rust races. Prior to the development of this method, various ways were tried to inoculate individual leaves with a number of rust isolates. These included carefully placing small drops of spore suspensions on marked areas with a hypodermic syringe: positioning spores on the leaves with small blocks of water-agar, filter paper, toilet tissue, scotch tape, etc. and also the patch inoculation method of Geis, Futrell and Garrett (1958), utilizing pieces of filter paper soaked in a suspension of rust spores, attached to the leaves with strips of cellophane tape. None of the methods proved satisfactory; the size and shape of sunflower leaves made the "patch" technique impractical.

The multiple inoculation method was finally worked out in the following way:

Small bits ("plugs") of absorbent cotton were soaked in sterile water, touched to dry rust spores dispersed on a glass slide or to a suspension of spores in water, then pressed lightly with forceps against the upper surface of a sunflower leaf. Prior to inoculation, the leaf was marked off into sectors with India ink. Each area was labelled with the code designation of the rust isolates to be placed there. Each leaf was inoculated with four or five isolates and up to eight isolates have been placed on leaves about 6 cm long with good results. (Fig. 1).

The cotton plugs may be made very small to facilitate numerous inoculations on an individual leaf. A certain minimum distance, determined empirically, must be left between plugs to prevent coalescence of the films of moisture around each plug.

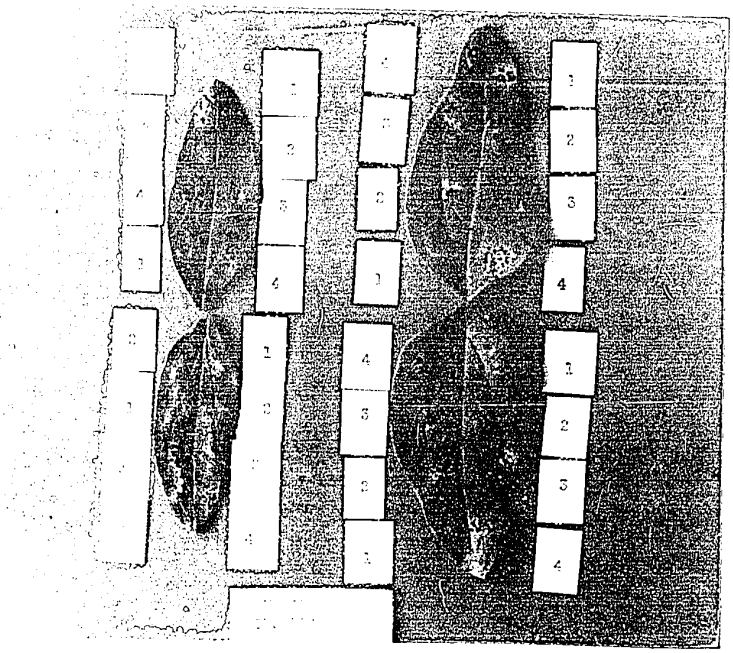

Fig. 1. Pairs of sunflower leaves showing result of inoculation with rust at eight locations per leaf by the multiple inoculation method.

To speed up the marking of hundreds of leaves inoculated in one experiment, a simple device was employed. A piece of sewing thread was stretched between two ends of a fork. The fork was made from a piece of copper wire (No. 14), approximately 25 cm long, by bending at the middle and then twisting once or twice. The thread was touched to an absorbent cotton soaked with India ink and then the ink-soaked thread was touched onto the leaf surface. The midrib served to divide the leaf longitudinally. The rust codes were written on the leaves with a drawing nib or with an ordinary nib not sharp enough to puncture the leaf.

The amount of inoculum applied on each plug may be standardized within rough limits. When dry spores are dusted onto slides or petri plates, the density of the spore deposit can be regulated, or the spores can be diluted with talc. When spores are suspended in water for inoculating, the concentration can be suitably adjusted.

Following inoculation, the plants were put in a saturated atmosphere in moist chambers overnight; galvanized metal garbage cans were used regularly, covered with wet, but not dripping, sheets of newspaper before putting the lids in place. This device was found to help keep the cotton plugs wet. On the following day the chambers were opened, and the plants and cotton plugs allowed to dry slowly in diffuse light before being transferred to the greenhouse or to the controlled environment chambers. The plugs were usually removed a day or two later. They adhere remarkably well when wet, and even after drying many remain on the leaf surface for days if not removed (Fig. 2).

To prevent contact between leaves and to keep the plants erect, the individual plants were supported by narrow strips of paper folded and stapled around bamboo sticks (Fig. 2). Recording of data is simplified by numbering all pots in an experiment in sequence, and numbering the

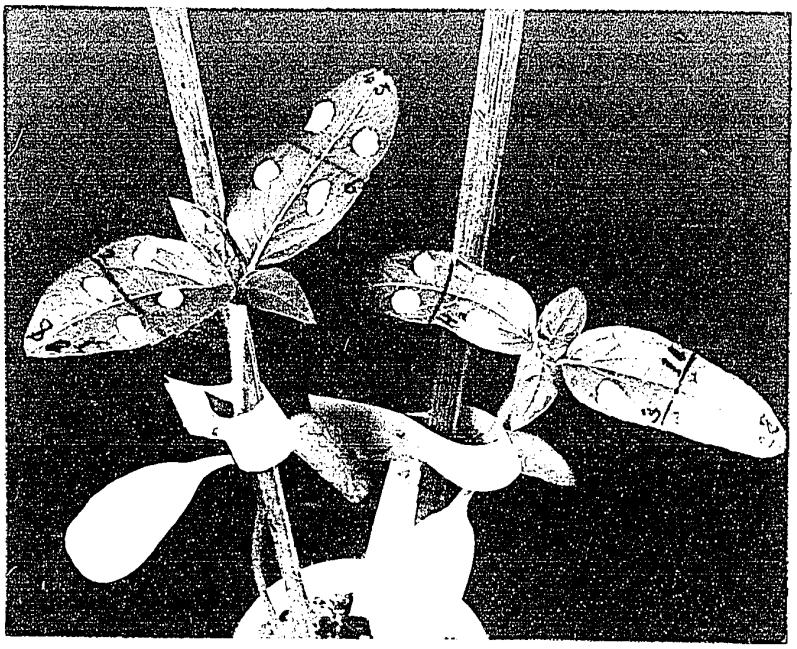

Fig. 2. Sunflower seedlings inoculated by the multiple inoculation method with cotton plugs in place on the first true leaves, with rust isolates identified by code numbers, and with seedlings held in position by paper loops.

individual plants if there are more than one per pot. Plant numbers are written on the paper bands supporting each plant.

The multiple inoculation method was found to work satisfactorily with stem rust of wheat (Puccinia graminis f. sp. tritici) (Fig. 3), and may prove useful for multiple inoculations of other plants with various leaf pathogens in the greenhouse and laboratory. With wheat leaves wet cotton plugs adhere well to both surfaces (Fig. 4) and remain attached for sometime even after drying out. Wheat leaves can be marked out into small areas and labelled with India ink with more rapidity than sunflowers. Wheat and other cereal seedlings do not require the mechanical support provided for sunflowers.

Telial production

Attempts were made to produce germinable telia under greenhouse conditions and in controlled environment chambers. For the production of telia of pure races, the cultures were purified by single pustule isolation and subsequent increase and then they were checked for purity on the differentials. The methods of the production and germination of telia and the amount of sporidial infection obtained, will be described in another section.

Methods of selfing and crossing

Two methods were employed in this study:

1. Reciprocal pairing method

This was used for some of the selfing, and all of the crossing. The pycnia which were to be used were labelled with india ink. Nectar was taken up with glass rods, camel hair brushes, or wire loops, or capillary glass tubing as used by various workers. The nectar from two pycnia was

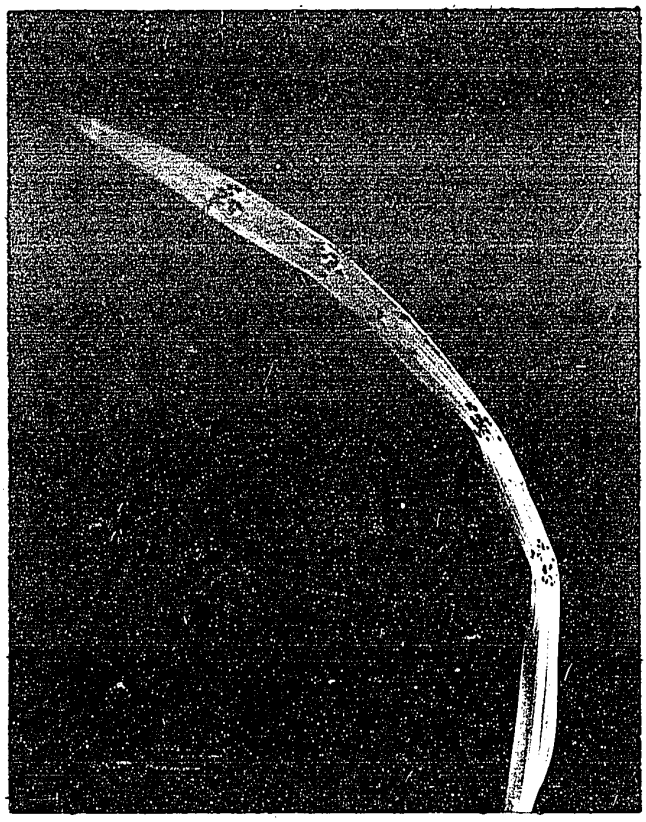

Fig. 3. Wheat leaf showing result of inoculation with stem rust at several locations by the multiple inoculation method.

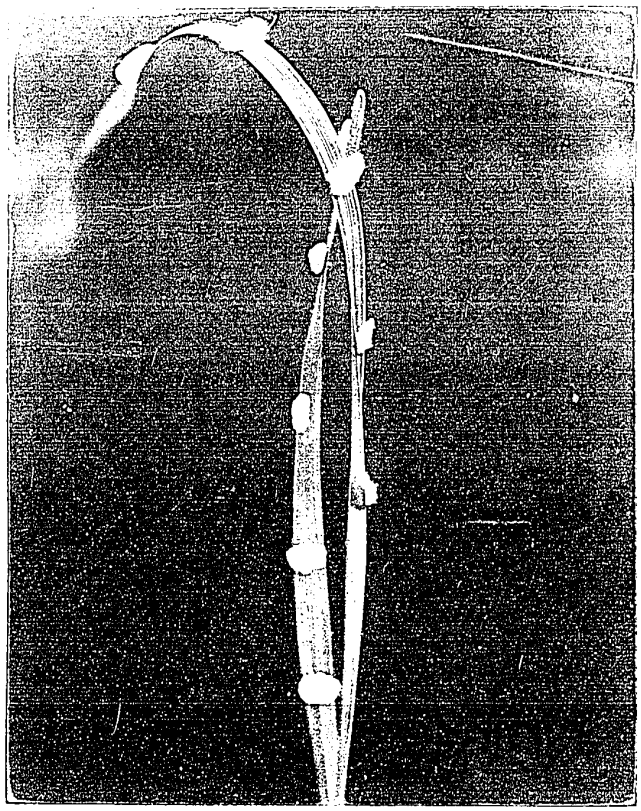

Fig. 4. Wheat leaves inoculated by the multiple inoculation method with cotton plugs in place.

transferred reciprocally. The paired pycnial method was used by Flor (1942), Vakili (1958), Flangas and Dickson (1961a), Loegering and Powers (1962), and Green (1965b, 1966). Not all of these workers made reciprocal pairings.

The proportion of successful fertilizations was very low. This may have been due either to chance pairing of pycnia of the same mating type (sex), or to faulty technique. Therefore, a different method was adopted for later selfing studies.

2. Pooled (mixed) nectar method

This is an old method, used by Johnson and his associates in the early 1930's (Johnson 1954), and has been employed extensively by other workers (Vakili, 1958; Zimmer, 1961; Samborski, 1963; Green, 1964, 1965b, 1966). The nectar from all available pycnia was taken up in glass capillary tubes, one for each pycnium. The nectar from all the tubes was thoroughly intermixed in a watch glass with a few drops of sterile water. The mixture was then applied to individual pycnia. The majority of the pycnial infections fertilized in this way gave rise to aecia.

Selfing was done 7 to 8 days after the date of appearance of the pycnia. This allowed time to detect aecial development as a result of accidental fertilization of closely adjacent or compound pycnia. Thereafter, when the aecia developed well, the aecial cups of a cluster were scraped off the leaf with a scalpel and crushed between two clean microscope slides to liberate and intermix the aeciospores which were then inoculated onto S37-388 seedlings.

Occasionally other differential varieties were inoculated as well as S37-388 plants. The inoculated seedlings were incubated overnight in

moist chambers and then, as with uredial inoculation, they were placed on a greenhouse bench or in controlled environment chambers. Plants inoculated with various cultures were isolated during the incubation and development of infection in plastic coverings. The uredial pustules appeared within 6 to 7 days. Before the pustules became loose, a few randomly selected uredia were isolated by cutting out small pieces of leaf supporting single pustules. They were stored temporarily in carefully labelled shell vials. These single uredial pustules were increased for 2 to 3 generations until sufficient inoculum was obtained.

One or two single uredial pustules were increased from each aecial infection. It was assumed that a single uredium would arise from a single aeciospore. Cultures obtained from both sides of a reciprocal paired transfer were considered as one progeny unless their pathogenicity proved to be different. When selfing was induced by pooled nectar, one or two uredial cultures were increased per aecial cluster. When two uredial cultures were tested from one aecial infection their behaviour was recorded separately.

EXPERIMENTAL RESULTS

Production and germination of telia

Great difficulties were encountered in obtaining germinative teliospores. Shortly after the start of this work in the winter of 1962-1963, unsuccessful attempts were made to germinate telial material which had germinated when collected in 1961 at Winnipeg, Manitoba.

In subsequent experiments, telia were produced under various environmental conditions in the greenhouse, in the field, and in controlled environment chambers, and tested for germination. Plants intended for telial production were inoculated lightly so that the leaves would remain alive until telia were mature.

Host varieties used. All three differentials were used for producing telia of race 4; S37-388 and Morden 29 were used for race 2; S37-388 and CM 90 RR were used for race 3; and S37-388 was used for race 1. The results were not encouraging in the first two years, so from 1964 on, telial production was confined to S37-388 in order to reduce the amount of space required for this work.

Age of host plants selected. In the initial period of this study, plants were inoculated at the first-leaf stage. In many of the later trials, the plants were inoculated at three growth stages: (1) with first pair of leaves fully expanded, (2) with first pair of leaves fully expanded and the second pair semi-expanded, (3) with first and second pair of leaves fully expanded and the third leaf one-fourth to half expanded. In the last few trials, plants of the third category were used. The plants inoculated for telial production were generally sown in 6" or 7"-inch pots.

Isolation of races. Until the winter of 1963-1964, plants inoculated with different races were kept isolated with polyethylene covers supported by a wooden frame. As the production of germinative telia was not satisfactory, it was thought that polyethylene covers might be unsatisfactory. Contamination by other races should occur only through secondary infections, when uredospores are released. As all material in a given greenhouse was inoculated at one date, by the time experimental telia were harvested the contaminants would still be in the uredial stage. Therefore, from the mid-winter of 1963-1964 plants inoculated with different races were left uncovered in the same rooms.

Seasonal effects. In these trials, owing to the problem of isolation and shortages of greenhouse space, pots were not arranged according to any experimental design. In some trials conducted without any specific environmental conditions in mind, the inoculated plants were kept in greenhouses, or controlled environment chambers or wherever space was available.

In the winter of 1961-1962 several sets of telia were produced. Among them the telia produced in one trial collected on March 8, germinated satisfactorily. The seedlings were raised prior to inoculation in an Agronomy Department greenhouse with artificial light provided from midnight to dawn. Plants inoculated with races 1 and 2 were kept in the same room; plants inoculated with races 3 and 4 were moved to a greenhouse without artificial light. The temperature in both rooms was controlled thermostatically between 20°C and 27°C.

In the summer of 1962, telia of races 1, 2 and 3 were produced on varieties S37-388, Morden 29 and Morden 69 respectively in isolated plots

in the field. The plants were inoculated in the middle of August; and in late September and early October, when the telia were forming or had already formed, the telial samples were collected and tested for germination. In both tests only race 2 gave some sporidial infection. The last telia were supposed to be collected after exposure to killing frost in the field. However, this could not be done with races 1 and 2, because the field plots were ploughed before frosts occurred. The telia of race 3, although collected after frost, did not germinate. The telial leaves were heavily infected with mildew, and were dead and brittle before frost.

From the winter of 1962-1963 onward a number of trials were conducted on the production of germinative telia. The seedlings were raised in the greenhouse under the conditions described in Methods and Materials.

Of telia produced from the winter of 1962-1963 onward, satisfactory germination was obtained from only three trials which will be discussed here. In a number of other trials a few scattered sporidial infections were obtained. In some of these cases, it was felt that telia produced under these conditions might germinate if suitably manipulated, but repeated experiments were not successful.

In the first trial in which satisfactory germination was obtained the plants were inoculated in the second week of October, 1963 and the infected plants kept for almost two months in greenhouse 4A without artificial light. Telia from this trial were collected on December 13 and germination tested a week after harvest.

In the second trial the plants were inoculated initially not for telia production but for the increase of single uredial pustules of races 1, 2, 3 and 4. The plants were inoculated on October 11, 1964 and were

then placed in controlled environment chambers to protect them from contamination. The same plants were reinoculated using the small amount of inoculum produced from the first inoculation. After the uredospores were collected on November 10 the plants were placed in greenhouse No. 4A. A few days after they were placed in the greenhouse, the leaves infected from the first and second inoculations died but the newly emerging leaves which became infected through secondary infections while the plants were in the growth cabinets, survived and formed telia. These germinated. Another set of plants, which was inoculated on November 10, failed to produce germinable telia in the same greenhouse. The telia of both sets of plants were collected on the same day. Those which germinated were slightly older than those which did not, because the plants were infected before they were transferred to the greenhouse.

The last trial in the series on telial production was conducted in the spring of 1965, in the Agronomy greenhouse. The seedlings were grown in greenhouse 1 in 7-inch pots with 12 to 15 seedlings per pot. The temperature was controlled between 18°C and 27°C. Day length was 17 hours with artificial light provided from midnight to dawn. The plants were inoculated about a month after sowing, when the third pair of leaves had partly emerged. After inoculation they were divided into three lots and placed in greenhouses 1, 2, and 3 respectively. In 1, pots were placed on shelves along the corner of the walls facing the south and the west, in 2 on a shelf along the wall facing the south, and in 3, on a central bench. The temperature in house 1 was kept between 18° and 27°C and day length at 17 hours. The temperature in house 2 ranged from 15° to 20°C and day length was 17 hours. However, the plants were shaded by oat plants which

were taller than the sunflower plants. In house 3, no artificial light was provided and the temperature was maintained between 7° and 10°C, too low for sunflowers. New leaves were pale green to yellow. In house 3 it took 16 to 18 days for uredia to appear compared to 7 to 8 days in the other two houses. By the time the uredial pustules became visible telial formation was also evident. The telial leaves from all three sets of plants were collected twice, on April 5 and on April 11, 33 and 39 days respectively after inoculation.

The telia collected from this trial were tested for germination within a week after harvest. The amount of sporidial infection was estimated quantitatively visually.

Telia harvested on April 11 gave more sporidial infection than those collected on April 5. Telia produced in houses 1 and 2 germinated quite well, but those collected from house 1 germinated better than those from house 2. The telia produced in house 3 (low temperature) gave relatively few sporidial infections.

Satisfactory telial germination occurred in four trials. In two of them, plants were inoculated about mid-October; in one, on February 8, and in the fourth, on March 3, of different years. Teliospores resulting from inoculations at other times gave rise to few or no pycnial infections.

Although the data are limited, it may be that environmental conditions in which plants were grown prior to inoculation, or conditions during uredial and telial formation, determined the germinability of the teliospores. The fact that telia produced under three different sets of conditions in April, 1965, all gave rise to pycnial infections suggests that the conditions prior to inoculation may have been the critical ones.

Liang (1966) working with races 1 and 3 obtained good sporidial

infection in one of her experiments in which the seedlings were grown in the greenhouse but were placed in controlled environment chambers after inoculation, for uredial and telial production. The telia which germinated were collected approximately three weeks before those which germinated in my experiments. She tried to repeat her experiment in May but without success. Since in both her experiments the seedlings were raised in the greenhouse and the telia were produced in the controlled environment chambers, the difference in results was probably attributable to a change in the environmental conditions in the greenhouse. Possibly during early to mid-spring conditions in the greenhouse were favourable for the production of host plants on which germinable telia could be formed.

Bailey (1923), working with sunflower rust, noted that greenhouse-produced telia collected on November 19, 1920, and stored at room temperature , in an icebox, or outdoors gave good sporidial infection when tested in the greenhouse on March 5.

An attempt was made to determine if conditions of storage affected germinability of teliospores. Teliospores produced in the greenhouse at various times were tested for germination. Non-germinating telia were stored for various periods at room temperature, in a cold room at 10°C, in a freezer at about -15°C, and in some tests in the winter, under snow out of doors. Samples were tested at intervals for three to six months. No germination was observed in any of the materials at any time.

It was impossible to tell from the results if the telia were dormant or dead. Teliospores of a lot which germinated well when collected in April 1965, did not germinate when tested 40 days later. In other experiments, some telia germinated well when collected and also after storage at

room temperature for a month, whereas others failed to germinate when tested after 3 weeks of storage.

With reference to the time of year in relation to the production of germinative telia the reports of two other findings with different rusts may be mentioned. Samborski (1963) working with a culture of wheat leaf rust, attempted to germinate teliospores by alternate wettings and dryings without preliminary cold treatment. He noted that the telia produced in the month of March germinated after fewer wettings and dryings than those produced in December. Flor (1942) working with flax rust experienced considerable difficulty in germinating teliospores produced in the greenhouse. He attempted to germinate telia by various treatments, such as freezing and thawing followed by alternate wettings and dryings. He noted that telia developed on greenhouse-grown plants and ripening in April, gave the best germination. Again these two reports suggest that certain periods of the year, particularly during early to mid-spring, may provide favourable conditions for the production of germinative telia not only with sunflower rust but with some other rusts also.

Johnson and his associates (Newton and Johnson, 1932; Johnson et al., 1934; Johnson, 1954) who have done extensive work on the inheritance of pathogenicity in cereal rusts reported that telia produced at low temperature (about 60°F) in the greenhouse germinated after freezing and thawing followed by alternate wettings and dryings. However, they did not mention the time of year the telia were produced, so it is not apparent whether germination was primarily due to the treatments they applied or due to seasonal effects.

From the results of this study it appears that at two seasons, one in the fall (September to November) and the second in early to mid-spring (March to mid-April), environmental conditions in the greenhouse may be favourable for the production of germinative telia. If this is found to be true this may explain what happens in nature. In nature telia are formed in the early fall with the approach of winter and it may be that this period is favourable for the formation of telia and the environmental conditions existing in the spring may be favourable for the germination of telia. The function of low winter temperature may be just to retain the viability of teliospores by preventing their germination prematurely.

The basic problem in inheritance studies of pathogenicity in rust fungi remains the production of germinative telia. Therefore it may be rewarding to investigate whether there is any correlation between time of year and the production of germinative telia as suggested in this study. If it is found to be true, it may be possible to determine the environmental conditions by measuring the daily macro- and micro-climates prevailing in this period and then it would be possible to produce germinative telia in the controlled environment chambers by reproducing these conditions.

Differences among four races. Race 2 gave more vigorous telial germination than the other three races. The telia of race 2, collected in April, 1965 produced so many pycnial infections that most of the pycnia produced aecia spontaneously and could not be used for experimental purposes.

Selfing studies with physiologic races

In selfing rust races other difficulties were encountered besides inducing teliospore germination.

Sunflower rust is autoecious, and uredospore infections were usually encountered simultaneously with sporidial infections. Due to the paucity of telial germination no method was devised to kill the urediospores without affecting the germination of teliospores. In germination tests teliospores were generally alternately wetted and dried twice. To reduce the probability of uredial infection, the alternate soakings and dryings were continued up to 5 times with some leaves. From the limited tests made, it appeared that repeated soakings and dryings drastically cut both sporidial as well as uredial infections. This may be ~~explainable~~ explained by Bailey's (1923) finding that teliospores floated on the surface of water, began to germinate within 2 hours. After 12 to 24 hours sporidia were formed and germinated soon after.

Both sporidial and uredial infections were visible almost simultaneously. Since the primary object of this study was to investigate the inheritance of pathogenicity in rust races, as soon as sporidial infections were visible the uredial infections were removed by cauterizing the leaf. If there were too many uredia on a leaf, ~~it~~ they were removed. Only those pycnia which were at least 1 cm or more away from uredia were used for experimental purposes. Both surfaces of every leaf were examined twice a day for at least 4 or 5 days to be sure that all uredial infections were removed.

The size of the pycnia varied from normal (4 to 5 mm diameter) to tiny (about 1 mm) on all varieties. Some of the smallest pycnia disappeared within 5 or 6 days after they were formed. A few of the

"tiny" pycnia were fertilized successfully when pooled nectar was applied to them. The number of aecial cups produced by them varied from 2 to 7 per infection compared with 50 to over 100 cups produced from the fertilization of a normal pycnium. However, at the S_1 generation the uredial cultures derived from the abnormally small pycnia were indistinguishable from those derived from the fertilization of normal pycnia.

A few normal pycnia produced a reddish brown granular substance resembling a spore-mass on their ostioles, and the pycnial nectar dried up within 2 or 3 days. Under the low power microscope they appeared to be sterile masses of minute urediospores. A few S37-388 seedlings were inoculated with these masses of spores but no visible symptoms of infection were observed.

Some normal pycnia were not fertilized successfully even when pooled nectar was applied. These pycnia enlarged greatly and continued to produce nectar for almost three weeks. A similar phenomenon was reported by Johnson and Newton (1938) in wheat stem rust and by Vakili (1958) in wheat leaf rust and Zimmer (1961) in crown rust of oats. Johnson and Newton suggested that genetic factors may be involved in governing the formation of aecia. With one race of wheat leaf rust, in spite of mass fertilization, Vakili (1958) found that among 144 pycnia, 101 produced neither spermatia nor aecia, 24 produced spermatia but did not produce aecia and only 19 produced spermatia and aecia. He concluded that spermatial and aecial formation were governed by genetic factor(s).

Another difficulty encountered was with infection by aeciospores. Aeciospores transferred from apparently normal and well developed aecia sometimes failed to infect seedlings of the variety S37-388. In those

aecial cultures which did infect the host seedlings, the number of uredia produced varied from 2 to over 100. Almost 50 per cent of the aecia gave very poor infection, producing only a few uredia. Similar difficulties were encountered by other workers in other rusts (Johnson and Newton, 1938; Vakili, 1958; Zimmer, 1961; Green, 1964). Zimmer, working with crown rust of oats, reported that some normal pycnia produced aecia but the aeciospores failed to infect varieties uniformly susceptible. He concluded that the aeciospores of these aecia were either inviable or incapable of infecting the susceptible varieties. These are heterothallic rusts which might normally be cross-fertilized. The failure of some aeciospores to infect the susceptible variety may be due to an injurious effect of self-fertilization as found in some cross-fertilizing species. Alternatively, it may be that S37-388 has some recessive factors for resistance which are inoperative against the parental races, but when the corresponding genes in the fungus were brought to homozygosity by selfing, the aeciospores possessing the homozygous genotypes failed to attack the variety.

Inheritance of pathogenicity in the selfed progeny of four rust races on the sunflower differentials

The results on inheritance of pathogenicity in rust races will be presented with the progenies obtained from the winter of 1962-1963 onward. In the winter of 1961-1962 some S_1 and F_1 progenies were obtained. At that time the peculiar behaviour of the rust races and of the differential varieties ~~were~~ was not recognized; some of the cultures were thought to arise from contaminants, therefore all were discarded.

The pathogenicity of the F_1 hybrid cultures was evaluated against that of the four parental races by inoculating them using the multiple

inoculation method. The pathogenicity of those S_1 cultures which were obtained before 1965 was determined in the same way as that of the F_1 cultures. The pathogenicity of those S_1 cultures obtained in 1965 was, for want of time, determined by mass inoculation. The number of seedlings inoculated with selfed cultures varied from 5 to 10 and with F_1 hybrids from 3 to 15. When tests were performed by multiple inoculation, the pathogenicity of the progeny was determined from the seedlings which gave appropriate reactions to the parent races. However, if the parent race was avirulent but its progeny were virulent, or vice-versa, the reactions on these seedlings were taken into consideration in determining the pathogenicity of the progeny.

The number of S_1 progeny obtained in different experiments and their pathogenicity are shown in Tables 1 to 4. A visual examination of the results shows that the ratios of dominant to recessive classes varied widely from experiment to experiment. There may be several reasons for this:

1. Pycnia were few in practically all experiments. Although they were fertilized successfully by using pooled nectar, there might be serious sampling errors because the populations were so small.

2. Difficulties were encountered in classifying some of the progenies as virulent or avirulent. Some cultures gave intermediate reactions. It was impossible to classify them by progeny tests because of difficulties in inducing telia to germinate.

3. Some data indicated the possibility of non-Mendelian inheritance of virulence in some cases.

Because of these difficulties, the data were not analyzed statistically. The use of X^2 tests would be questionable in any case because in

TABLE 1. REACTION OF SUNFLOWER DIFFERENTIAL VARIETIES TO S_1 UREDIOSPORE CULTURES OF RACE 1

Telial designation	Method of selfing	Pycnia produced on	Single pustule culture number.	Uredial inoculation method.	Varieties [1]							
					Morden 29		CM9ORR		M62-2672-2-r1		M62-2685-14-I	
					A[2]	V	A	V	A	V	A	V
63(1)	Reciprocal	S37-388	-	multiple	2[2]	0	2[3]	0	-	-	-	-
64(1)	Pooled nectar	"	1	"	17	1	15	3	3	12	-	-
			2	"	12	2	12	2	2	10	-	-
65(2)	"	"	1	routine	15	2	17	0	-	-	-	-
			2	"	14	0	13	1	-	-	14	0
	Total	S37-388			60	5	59	6	5	22	31	0
65(2)	Pooled nectar	CM9ORR	1	routine	5	0	0	5	-	-	4	1[4]
			2	"	2	0	0	2	-	-	1	1[4]
	Total	CM9ORR			7	0	0	7	-	-	5	2

1/ Parent race 1 is virulent on S37-388, M62-2672-2-r1, and avirulent on others. Results are based on reactions of 5 to 10 seedlings in each case.

2/ A, avirulent; V, virulent.

3/ One culture showing reciprocal differences in pathogenicity was not included in the table.

4/ Two cultures were virulent on more than 50% of the seedlings, and were classed as virulent.

TABLE 2. REACTION OF SUNFLOWER DIFFERENTIAL VARIETIES TO S_1 UREDIOSPORE CULTURES OF RACE 2

Telial designation	Method of selfing	Pycnia produced on	Single pustule culture number.	Uredial inoculation method.	Varieties[1]							
					Morden 29		CM90RR		M62-2672-2-rl		M62-2685-14-I	
					A[2]	V	A	V	A	V	A	V
63(1)	Reciprocal	S37-388		multiple	0	3	3	0	-	-	-	-
64(1)	Pooled nectar	"	1	"	3	28	29[3]	2[6]	27	3	31	0
			2	"	1	12	11[3]	2[6]	8	2	13	0
65(2)	"	"	1	routine	4	31	26[4]	9[7]	-	-	33	2
	Total	S37-388			8	74	69	13	35	5	77	2

TABLE 2 - continued

Telial designation	Method of selfing	Pycnia produced on	Single pustule culture number.	Uredial inoculation method.	Varieties[1]							
					Morden 29		CM9CRR		M62-2672-2-r1		M62-2685-14-I	
					A[2]	V	A	V	A	V	A	V
65(1)	Reciprocal	Morden 29		multiple	0	3	3	0	-	-	-	-
65(2)	Pooled nectar	"	1	routine	2	8	8[5]	2	-	-	10	0
,	Total	Morden 29			2	11	11	2	-	-	10	0

1/ Parent race 2 is virulent on S37-388, and Morden 29, and avirulent on others. Results are based on reactions of 5 to 10 seedlings in each case.

2/ A, avirulent; V, virulent.

3/ One culture was avirulent on more than 50% of the seedlings and was classed as avirulent.

4/ Four cultures were avirulent on more than 50% of the seedlings and were classed as avirulent.

5/ Three cultures were avirulent on more than 50% of the seedlings and were classed as avirulent.

6/ One culture was virulent on more than 50% of the seedlings and was classed as virulent.

7/ Three cultures were virulent on more than 50% of the seedlings and were classed as virulent.

TABLE 3. REACTION OF SUNFLOWER DIFFERENTIAL VARIETIES TO S_1 UREDIOSPORE CULTURES OF RACE 3

Telial designation	Method of selfing	Pycnia produced on	Single pustule culture number.	Uredial inoculation method.	Varieties[1]							
					Morden 29		CM9ORR		M62-2672-2-r1		M62-2685-14-I	
					A[2]	V	A	V	A	V	A	V
63(1)	Reciprocal	S37-388		multiple	3	0	0	3	-	-	-	-
64(1)	Pooled nectar	"	1	"	27	1	2	26[4]	19	0	19	0
			2	"	26	0	2[3]	24	18	0	18	0
65(2)	"	"	1	routine	2	1	1[3]	2	-	-	3	0
			2	"	2	0	0	2	-	-	2	0
	Total	S37-388			60	2	5	57	37	0	42	0

1/ Parent race 3 is virulent on S37-388, and CM9ORR and avirulent on others. Results are based on reactions of 5 to 10 seedlings in each case.

2/ A, avirulent; V, virulent.

3/ One culture was avirulent on more than 50% of the seedlings, and was classed as avirulent.

4/ One culture was virulent on more than 50% of the seedlings, and was classed as virulent.

TABLE 4. REACTION OF SUNFLOWER DIFFERENTIAL VARIETIES TO S_1 UREDIOSPORE CULTURES OF RACE 4

Telial designation	Method of selfing	Pycnia produced on	Single pustule culture number.	Uredial inoculation method.	Morden 29		CM9ORR		M69-W59-11-MC62-13-r4		M62-2672-2-r1		M62-2685-14-I	
					A[2]	V	A	V	A	V	A	V	A	V
63(1)	Reciprocal	S37-388		multiple	0	1	0	1	-	-	-	-	-	-
64(1)	Pooled nectar	"	1	"	0	6	1	5	1	5	6	0	6	0
			2	"	1	5	0	6	2	4	6	0	6	0
65(1)	"	"	1	"	0	6	0	4[4]	1	2	1	0	0	1
			2	"	1	4	2	3	1	2	0	1	1	1
65(2)	"	"	1	routine	0	15[3]	1	14	-	-	-	-	11	4
			2	"	1	13	2	12	-	-	-	-	12	2[5]
	Total	S37-388			3	49	7	42	5	13	13	1	36	8

1/ Parent race 4 is virulent on S37-388, Morden 29, CM9ORR and M69-W59-11MC62-13-r4 and avirulent on M62-2672-2-r1, and M62-2685-14-I. Results are based on reactions of 5 to 10 seedlings in each case.

2/ A, avirulent; V, virulent.

3/ One culture was virulent on more than 50% of the seedlings, and was classified as virulent.

4/ Four cultures were virulent on more than 50% of the seedlings, and were classified as virulent.

5/ One culture was virulent on more than 50% of the seedlings, and was classified as virulent.

most experiments the expected number of individuals in the recessive class would be less than 5 (Smith 1954, p.624, Srb et al.,1965, p.57).

The pathogenicity data of S_1 progeny show that all four races are heterozygous on varieties Morden 29 and CM9ORR, and races 1, 2 and 4 are also heterozygous on line M62-2672-2-r1 and M62-2685-14-I. Race 3 did not yield any segregants on M62-2672-14-I. However, the number of aecial clusters investigated and the number of progeny cultures tested per cluster in each experiment are not sufficient to conclude that race 3 is homozygous for virulence on this line.

Some S_1 progenies were virulent on some seedlings and avirulent on other seedlings of a line, while the parent races on the same seedlings gave consistent reactions. This was observed most frequently on CM9ORR (Tables 1, 2, 3) but also occurred on Morden 29 (Table 1). These cultures were arbitrarily classified as virulent or avirulent, according to the reaction on the majority of the seedlings. It was realized that if more seedlings had been tested, the classification of the cultures might have been different.

These "aberrant" reactions cannot be attributed to mixtures of genotypes in the rust, because all the seedlings concerned were inoculated with spores from the same source at the same time. The number of spores applied to individual leaves is in the thousands. The probability of only one pathogen genotype of a mixture being applied to a given leaf is therefore practically nil. The variation in reaction must therefore be attributed to differences in host genotypes not discernible with the parent races.

In one experiment three S_1 cultures of race 1 were obtained by reciprocal transfer of spermatia (Table 1). Cultures from one of these

pairings exhibited reciprocal differences on Morden 29 and CM9ORR. The culture from one side was avirulent like race 1 and that from the other side virulent like race 4. It is possible that the culture which behaved like race 4 might have originated from contamination or that the pathogenicity of these cultures may be governed by non-chromosomal factors. Vakili (1958) working with leaf rust of wheat found that in a few instances S_1 progeny secured by reciprocal fertilization displayed maternal inheritance. Similarly Green (1965b) working with oat stem rust race 6A found that some of the cultures obtained by reciprocal transfer of nectar showed reciprocal differences on variety Sevnothree; cultures from one side were avirulent and those from the other side were virulent.

Hybridization studies

Crosses were made between four parental races in different experiments and some F_1 hybrids were obtained from crosses made between pycnia on S37-388. The pathogenicity of most of these hybrid cultures was tested twice or thrice on differentials and the results are shown in Tables 5 to 9 and Appendix Tables I to XII. All of the F_1 hybrids were tested on S37-388, CM9ORR and Morden 29, but not all hybrids were tested on the supplemental lines, M69-W59-11-MC62-13-r4, M62-2672-2-r1, and M62-2685-14-I.

TABLE 5. PATHOGENICITY ON VARIETY CM9CRR OF F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN PARENTAL RACES IN ALL POSSIBLE COMBINATIONS[1/]

Cross	Number of crosses	Pathogenicity of F_1 hybrids
Race 1 x Race 2		
Reciprocal	5	Avirulent
	1	Virulent
One-sided race 2 maternal	2	Avirulent
Race 1 x Race 3		
Reciprocal	1	Virulent
	1	Virulent on some seedlings and resembled maternal parent on others.
	1	Variable, or virulent on some and avirulent on others
Race 1 x Race 4		
Reciprocal	2	Avirulent
	1	Virulent on some and avirulent on others
	1	Variable or virulent on some, avirulent on some and reciprocal differences on a few others (race 1 side virulent and race 4 side avirulent)
One-sided race 1 maternal	1	Virulent
"	1	Virulent on some and avirulent on others
race 4 maternal	1	Virulent

TABLE 5 - continued

Cross	Number of crosses	Pathogenicity of F_1 hybrids
Race 2 x Race 3		
Reciprocal	3	Virulent
	1	Virulent on some and avirulent on some others
	2	Virulent on some and resembled maternal parent on others
	1	Virulent on some, avirulent on some and displayed reciprocal differences on a few others (race 3 side avirulent and race 2 side virulent)
One-sided race 2 maternal	1	Virulent on some and avirulent on others
Race 2 x Race 4		
Reciprocal	1	Virulent
	1	Avirulent
	2	Variable or virulent on some and avirulent on others
One-sided race 2 maternal	1	Virulent
Race 3 x Race 4		
Reciprocal	2	Virulent
	2	Variable on virulent on some and avirulent on others

1/ The variety is susceptible to races 3 and 4, and resistant to races 1 and 2. Results are based on reactions of 10 to 15 seedlings in each case.

TABLE 6. PATHOGENICITY ON VARIETY MORDEN 29 OF F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN PARENT RACES IN ALL POSSIBLE COMBINATIONS[1/]

Cross	Number of crosses	Pathogenicity of F_1 hybrids
Race 1 x Race 2		
Reciprocal	6	Resembled maternal parent
One-sided race 2 maternal	2	Resembled maternal parent
Race 1 x Race 3		
Reciprocal	3	Avirulent like parent races
Race 1 x Race 4		
Reciprocal	3	Resembled maternal parent
	1	Avirulent like parent race 1
One-sided race 1 maternal	2	Resembled maternal parent
race 4 maternal	1	Resembled maternal parent
Race 2 x Race 3		
Reciprocal	6	Resembled maternal parent
	1	Virulent like parent race 2
One-sided race 2 maternal	1	Resembled maternal parent
Race 2 x Race 4		
Reciprocal	4	Virulent like parent races
One-sided race 2 maternal	1	Virulent like parent races
Race 3 x Race 4		
Reciprocal	4	Resembled maternal parent

1/ The variety Morden 29 is susceptible to races 2 and 4 and resistant to races 1 and 3. Results based on reactions of 10 to 15 seedlings in each case.

TABLE 7. PATHOGENICITY ON LINE M69-W59-11-MC62-13-r4 OF F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN PARENT RACES IN ALL POSSIBLE COMBINATIONS[1/]

Cross	Number of crosses	Pathogenicity of F_1 hybrids
Race 1 x Race 2		
Reciprocal	5	Resembled maternal parent
	1	Avirulent
Race 1 x Race 3		
Reciprocal	3	Avirulent
Race 1 x Race 4		
Reciprocal	2	Resembled maternal parent on some and avirulent on others
	1	Resembled maternal parent on some and virulent on others
	1	Avirulent
Race 2 x Race 3		
Reciprocal	3	Resembled maternal parent
	1	Resembled maternal parent on some and avirulent on others
	1	Virulent
Race 2 x Race 4		
Reciprocal	2	Virulent on some and avirulent on others
	1	Avirulent
	1	Virulent
Race 3 x Race 4		
Reciprocal	4	Resembled maternal parent

1/ Most of the seedlings on this line are resistant to races 1 and 3 and susceptible to races 2 and 4 or to race 4 only. Results based on reactions of 5 to 8 seedlings in each case.

TABLE 8. PATHOGENICITY ON LINE M62-2672-2-r1 OF F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN PARENTAL RACES IN ALL POSSIBLE COMBINATIONS[1/]

Cross	Number of crosses	Pathogenicity of F_1 hybrids
Race 1 x Race 2		
Reciprocal	3	Avirulent
Race 1 x Race 3		
Reciprocal	3	Avirulent
Race 1 x Race 4		
Reciprocal	2	Virulent on avirulent but the reactions were similar to parent race 1
	2	Virulent on seedlings susceptible as well as resistant to race 1
Race 2 x Race 3		
Reciprocal	5	Avirulent
Race 3 x Race 4		
Reciprocal	2	Avirulent
	2	Avirulent on some and reciprocal differences on others

1/ Most of the seedlings of this line are susceptible to race 1 and highly resistant to the other three races. Some are also resistant to race 1. Results are based on the reactions of 3 to 8 seedlings in each case.

TABLE 9. PATHOGENICITY ON LINE M62-2685-14-I OF F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN PARENT RACES IN ALL POSSIBLE COMBINATIONS[1/]

Cross	Number of crosses	Pathogenicity of F_1 hybrids
Race 1 x Race 2		
Reciprocal	5	Avirulent
Race 1 x Race 3		
Reciprocal	3	Avirulent
Race 1 x Race 4		
Reciprocal	4	Avirulent
Race 2 x Race 3		
Reciprocal	5	Avirulent
Race 2 x Race 4		
Reciprocal	4	Avirulent
Race 3 x Race 4		
Reciprocal	2	Avirulent
	2	Virulent

1/ The seedlings are highly resistant to all 4 parental races. Results are based on reactions of 6 to 10 seedlings in each case.

Inheritance of pathogenicity to variety CM9CRR in uredial cultures of F_1 hybrids of crosses in all possible combinations of races 1, 2, 3, and 4

Race 1 x Race 2

Hybrids were obtained from 6 reciprocal and 2 one-sided crosses, (Table 5). Of the 6 reciprocal crosses, the hybrids in 5 crosses were avirulent and in one cross virulent. In the two one-sided crosses the hybrids (race 2 maternal) were avirulent.

Race 1 x Race 3

Hybrids were obtained from three reciprocal crosses (Table 5, Appendix Tables II, VIII). Of these, the hybrids in one cross were virulent; in another cross the hybrids were virulent on some seedlings and resembled maternal parents on other seedlings. In the third cross, in the first test made by the mass inoculation method, the F_1 from race 1 side was avirulent and that from race 3 side was virulent on some seedlings and avirulent on other seedlings. In the second test (inoculated by the multiple method) the F_1's from both sides were avirulent. In all cases the parent races gave the appropriate reactions on the same seedlings.

Race 1 x Race 4

Of the 4 crosses, the receiprocal F_1 hybrids in two crosses were avirulent and in two other crosses the F_1's were virulent on some seedlings and avirulent on others (Table 5, Appendix Tables III, IX). The F_1's from one of these crosses were tested twice. Each time there were reciprocal reactions on one of the seedlings: the culture from race 1 side was virulent and that from race 4 side avirulent.

The F_1 cultures of two of the three one-sided crosses, (one from the race 1 side and one from race 4) were avirulent. In the other one-sided cross, the F_1, from the race 1 side, was virulent on some seedlings and avirulent on other seedlings.

Race 2 x Race 3

F_1 hybrids were obtained from 7 reciprocal crosses and one one-sided cross (Table 5, Appendix Tables IV, X), made at three different times. The F_1 hybrids of three reciprocal crosses were virulent like race 3. The F_1's of the four other crosses were variable or virulent on some seedlings and avirulent on others. In one of these reciprocal crosses, the pathogenicity of the F_1's resembled the maternal parents. In another cross, the F_1's exhibited dissimilar reactions on some seedlings: the F_1 from the race 3 side was avirulent and that from the race 2 side virulent.

The F_1 from the one-sided cross (race 2 maternal) was virulent on some seedlings and avirulent on others.

Race 2 x Race 4

Four reciprocal crosses were successful. The F_1 of one cross was virulent, and in another cross avirulent (Table 5, Appendix Tables V, XI). In the two other crosses, the F_1 was variable or virulent on some seedlings and avirulent on others. The F_1 obtained from one one-sided cross (race 2 maternal) was virulent.

Race 3 x Race 4

Of 4 reciprocal crosses, the F_1's in two were virulent and in two others were variable or virulent on some seedlings and avirulent on others (Table 5, Appendix Tables VI, XII).

Inheritance of pathogenicity to variety Morden 29 in uredial cultures of F_1 hybrids of crosses in all possible combinations of races 1, 2, 3, and 4

Data on the virulence of all possible combinations of four races of F_1 hybrids on variety Morden 29 are summarized in Table 6 and Appendix Tables I to XII. In most crosses the F_1 hybrids behaved like the maternal parent race.

The hybrids from one of four reciprocal crosses, between races 1 and 4 were similar and avirulent like the parent. The hybrids of one of seven reciprocal crosses between races 2 and 3 were similar and virulent like the parent race 2.

Inheritance of pathogenicity to M69-W59-11-Mc62-13-r4 in uredial cultures of F_1 hybrids of crosses in all possible combinations of races 1, 2, 3 and 4

The host line M69-W59-11-MC62-13-r4 is highly heterozygous for reaction to races 2 and 4, but fairly consistent to races 1 and 3. Seedlings of this line were always inoculated by the multiple method. The seedling reactions of this line to hybrid cultures were also highly variable. The data for pathogenicity of the F_1 hybrids are shown in Table 7 and Appendix Tables I to XII. The hybrids in the majority of the crosses resembled the maternal parent races.

Inheritance of pathogenicity to line M62-2672-2-rl in uredial cultures of F_1 hybrids of crosses in all possible combinations of races 1, 2, 3 and 4

The host line M62-2672-2-rl is heterozygous for reactions to all four races. The distinguishing characteristic of this line is that some seedlings are susceptible to race 1 but immune or highly resistant to the other three races. The seedlings' reaction to race 1 varies from completely resistant to completely susceptible; in some cases it is difficult to decide whether the seedlings should be considered resistant or susceptible. The reaction to the other three races is quite distinct, either highly susceptible or highly resistant, and inherited as a unit, i.e., either resistant or susceptible to all three races. In this study we are concerned with the pathogenicity of F_1 hybrids on the seedlings which are susceptible to race 1 but resistant to the other three races.

In crosses between races 1 and 2, the F_1 hybrids from three reciprocal crosses were avirulent like race 2 (Table 8 and Appendix Tables I to XII). Similarly in crosses between races 1 and 3 the F_1 hybrids of three reciprocal crosses were avirulent like race 3. In reciprocal crosses between races 1 and 4, the F_1 hybrids were virulent on some seedlings and avirulent on some other seedlings but the reactions were similar to race 1; F_1 hybrids in two other crosses, unlike either parent, were virulent on seedlings susceptible as well as on seedlings resistant to race 1. In crosses between races 2 and 3 the hybrids were avirulent like the parent races. In two crosses between races 3 and 4 the hybrids were avirulent like the parent races. The hybrids in two

other crosses were avirulent on some seedlings and displayed reciprocal differences on other seedlings. In one of these crosses the F_1 culture from the race 3 side was virulent and that from the race 4 side avirulent. In another cross the culture from race 4 was moderately virulent on one seedling and that from the race 3 side was avirulent.

Inheritance of pathogenicity to line M62-2685-14-I in uredial cultures of F_1 hybrids of crosses in all possible combinations of races 1, 2, 3 and 4

The host line M62-2685-14-I is immune or highly resistant to all races. In all crosses except those between races 3 and 4 the F_1 hybrids were avirulent like the parent races (Table 9 and Appendix Tables I to XII). In crosses between races 3 and 4, the F_1 hybrids in two crosses were avirulent like the parent races; those in two other crosses, unlike either parent, were virulent.

DISCUSSION

In any studies on selfing and crossing an autoecious rust, contamination of cultures with other races is an obvious danger. Every effort was made to avoid contamination or mixture of uredial cultures used in this work by appropriate isolation of inoculated plants. When uredial cultures were well established in isolation on plants intended for the production of telia, however, it was felt that the probability of producing telia of a "contaminant" race was so slight that the telia of various races were produced in the same greenhouse when necessary. By the time that the experimental telia were "harvested", any contaminant infections which might have occurred would probably still be in the uredial stage.

A second possible source of error is the "diploidization" of pycnia by adjacent uredial infections (Brown, 1932). Any pycnia with uredial cultures closer than 1 cm were discarded, as were also any "spontaneously" fertilized pycnia. Any uredia which developed were cut away, usually at the "flecking" stage. It was therefore felt that the probability of aberrant results arising from this type of "contamination" was also extremely remote.

Some of my first reciprocal crosses made in 1961-1962 yielded progenies which behaved like the maternal parent in some cases, or did not resemble either parent in other cases. As earlier work had indicated simple, non-allelic dominant factors controlling resistance in the host (Putt and Sackston, 1963), and had inferred similar uncomplicated control of pathogenicity in the rust (Sackston, 1962), these

"aberrant" results were attributed to contamination and rust progenies were discarded.

Similar results were obtained in later experiments when special precautions at every stage made it possible to exclude contamination as an explanation.

Reciprocal differences or distorted ratios in the progeny can be attributed to non-chromosomal factors, or to chromosomal factors such as maternal influence, preferential segregation during gametogenesis, gene conversion, paramutation, aneuploidy, and mitotic recombination (Jinks, 1964; Williams, 1964; Srb et al., 1965). To exclude these phenomena with reasonable certainty the F_1 hybrids should be studied beyond the F_1 generation. The difficulties encountered in inducing telial germination, emmerated earlier, made such studies of later generations impossible.

The F_1 hybrid progenies of reciprocal crosses between rust races behaved like the maternal parent on the differential variety Morden 29. On CM9ORR, however, most of the reciprocal hybrids behaved like one of the parents, indicating chromosomal control of pathogenicity. The discussion on the possible mechanism of chromosomal inheritance of pathogenicity will therefore be confined to the results on CM9ORR.

The F_1 hybrids between pairs of races were obtained at different times. For this discussion, because of the limited progeny size in each experiment, it will be assumed that the data can be treated as if all the F_1 hybrids of crosses between any two races were obtained in one experiment.

Cultures were classified as virulent or avirulent on the basis of reactions of limited numbers of seedlings. The variety CM9ORR appeared to be heterozygous to some of the F_1 hybrid races. If more seedlings could have been included in each test, it is possible that more of the F_1 cultures would have been shown to be virulent on some seedlings and avirulent on others.

Discussion of the apparent nature of inheritance of pathogenicity will be based on the assumption that pathogen genes conditioning virulence or avirulence to a host variety, correspond to host genes conditioning susceptibility or resistance to a race of the pathogen. This assumption will be examined critically in a later section of this thesis.

In crosses between races 1 and 2, both avirulent on CM9ORR, the hybrids of one out of a total of 8 crosses tested were virulent (Table 5). Although a progeny size comprising hybrids of 8 crosses is not enough to draw a definite conclusion, a segregation within this size of progeny would be expected if the avirulence of both the races is governed by the same factor and both are heterozygous, i.e., if both races possess the same genotype on CM9ORR.

In crosses between races 1 and 3, (Table 5), the F_1 hybrids of one cross were virulent. In another cross, the F_1 progeny was variable, or was virulent on some seedlings and avirulent on others. The F_1 cultures of a third cross were virulent on some seedlings, but resembled the maternal parent on others. The hybrids of the last two crosses did not behave like either parent nor like intermediates between the parents.

A progeny comprising hybrids of only three crosses is not large enough to determine whether avirulence of race 1 or virulence of race 3 is dominant. Since the S_1 progeny of both races segregated, however, the genes governing pathogenicity of the two races on CM9ORR cannot be allelic. If they were alleles, the race with the recessive character should not have segregated in the S_1 progeny.

However, some clue in this problem can be obtained if we consider the behaviour of host variety CM9ORR. This variety appears to be homozygous resistant to race 1. It may be considered heterozygous for susceptibility to race 3 because occasional seedlings are resistant to race 3. Now the fact that seedlings susceptible to race 3 are resistant to race 1, and that seedlings resistant to race 3 are also resistant to race 1 suggests that susceptibility of this variety to race 3 may be governed by two genes, one being epistatic to the other. For example, if the genotype of the parent plant from which the variety originated was $A_hA_hB_hB_h$ with A_h conditioning susceptibility to race 3, B_h conditioning resistance to both races 1 and 3, and with A_h epistatic to B_h; then, the genotypes of the population of CM9ORR could be A_h-B_hB_h and $a_ha_hB_hB_h$, with the host parasite interaction represented as follows:

Host	Race 1	Race 3
	$a_pa_pB_pb_p$	$A_pa_pB_pB_p$
A_h-B_hB_h	Resistant	Susceptible
$a_ha_hB_hB_h$	Resistant	Resistant

The phenomenon of seedlings being resistant to race 1 regardless of their susceptibility or resistance to race 3 can however be explained in other terms besides epistasis. This question will be discussed further under host genetics.

Assuming a gene-for-gene relationship between a host variety and a pathogen strain it may be that the virulence of race 3 is dominant over the avirulence of race 1 and that the gene, A_p, conditioning virulence of race 3 is epistatic to the dominant gene B_p, conditioning avirulence to both race 1 and race 3. If enough crosses were made between races 1 and 3, we would expect two or four kinds F_1 genotypes, as follows:

$A_p a_p B_p$ -(virulent) and $a_p a_p B_p$ -(avirulent),
or
$A_p a_p B_p$ -(virulent), $A_p a_p b_p b_p$ (virulent),
$a_p a_p B_p$ -(avirulent) and $a_p a_p b_p b_p$ (virulent)

depending upon whether the genotype of race 3 is $A_p a_p B_p B_p$ or $A_p a_p B_p b_p$, and assuming the genotype of race 1 to be $a_p a_p B_p b_p$ (since race 1 is heterozygous). The distribution of the crosses producing virulent and avirulent hybrids would be expected to be 1:1 or 5:3, depending upon whether the genotype of race 3 is $A_p a_p B_p B_p$ or $A_p a_p B_p b_p$.

In this context, again by assuming a gene-for-gene relationship, we may say something about the possible genotypes of the plants composing the variety CM9ORR in relation to their reactions to races 1 and 3. Since the variety is heterozygous to race 3, if the susceptibility of the variety to race 3 is governed by two genes A_h (susceptibility) and B_h (resistance), and A_h is epistatic to B_h, then it should include

genotypically two or four kinds of plants as follows:

$A_hA_hB_hB_h$ and $a_ha_hB_hB_h$,

or

$A_hA_hB_h-$, $A_hA_hb_hb_h$, $a_ha_hB_h-$ and $a_ha_hb_hb_h$

depending upon whether the genotype of the original parent plant from which the variety was derived was $A_hA_hB_hB_h$ (susceptible) or $A_hA_hB_hb_h$ (susceptible).

Selfed derivatives possessing the genotypes, $A_hA_hB_h-$, $A_hA_hb_hb_h$ and $a_ha_hb_hb_h$ should be susceptible to race 3 and those possessing the genotype $a_ha_hB_h-$ should be resistant. Derivatives with the genotypes $A_hA_hB_h-$ and $a_ha_hB_h-$ should be resistant to race 1 and those possessing the genotypes $A_hA_hb_hb_h$ and $a_ha_hb_hb_h$ should be susceptible.

Seedlings resistant to race 3 are occasionally encountered but seedlings susceptible to race 1 are rarely found. It may be that the seedlings which genotypically ought to be susceptible to race 1 are the ones which exhibited differences or variable reactions to the reciprocal F_1 hybrids of cross race 1 x race 3. That is, resistance of these seedlings may be governed by non-nuclear factors.

Seedlings of CM9ORR are rarely susceptible to race 1 or race 2. On two or three occasions a considerable number of seedlings gave susceptible or near susceptible reactions to races 1 and 2 in routine mass inoculations. The rust collected from these plants gave appropriate reactions in subsequent tests. It may be that the resistance of these plants was conferred by non-chromosomal factors and that under certain environmental conditions this resistance broke down, leaving the seedlings vulnerable to the attack of races 1 and 2.

There is a weakness in the hypothesis that the susceptibility to race 3 may be governed by two non-allelic genes, one of which is epistatic to the other. Under this hypothesis the resistance of the seedlings conditioned by the hypostatic dominant gene should be heritable. No selfing studies were made on CM9ORR. As described later in the section on host genetics, studies were conducted with selfed progeny of the differential line Morden 69, carrying the same resistance factor as that of CM9ORR (Dr. E.D. Putt, personal communication). No conclusive evidence was obtained whether the resistance of seedlings to race 3 is heritable or is an artefact of environment.

Studies on the pathogenicity of F_1 progenies of crosses between races 1 and 2 suggested that these races had the same genotype as determined on CM9ORR. The results of studies on host genetics, reported in a later section, show that the resistance of the host line Morden 69 to races 1 and 2 is inherited as a unit except in one S_2 line. The genetic relationship between races 2 and 3 should therefore be similar to the relationship between races 1 and 3.

The data available for such a comparison are limited; only three successful reciprocal crosses were made between races 1 and 3, and seven between races 2 and 3. The reaction patterns of F_1 progenies of race 1 x race 3 were observed also in the F_1 of race 2 x race 3. Probably because there were more F_1 progenies of the cross between races 2 and 3, an additional reaction pattern was observed in this cross: the cultures were virulent on some seedlings, avirulent on some, and gave reciprocal reactions on others (Table 5).

The relationships of race 4 with the other three races are not clear. The pathogenicity of race 4 on CM9ORR is variable. In reciprocal crosses between races 1 and 4, the hybrids in two crosses were avirulent like race 1; the hybrids in two other crosses, unlike either parent, were virulent on some plants and avirulent on some other plants, or variable. The hybrids in one of the last named crosses exhibited reciprocal differences on a few plants. In crosses between races 2 and 4 the hybrids in one cross were virulent like race 4, in one cross avirulent like race 2, and in two other crosses, unlike either parent, virulent on some seedlings and avirulent on others, or variable. In crosses between races 3 and 4, hybrids of two crosses were virulent like the parent races but in two other crosses, unlike either parent, the hybrids were either variable, or virulent on some seedlings and avirulent on others (Table 5).

The hybrids of most reciprocal crosses between rust races behaved like the maternal parent on variety Morden 29. In a few crosses the pathogenicity of the hybrids resembled one or other of the parents. Furthermore, the parent races have also shown segregation in their respective S_1 progenies. More or less similar results were obtained by Green (1965b) on variety Sevnothree in crosses between some oat stem rust races. Green found that in crosses between oat stem rust races the F_1 hybrids resembled the maternal races except with those involving races 6A and 13A with other races. In crosses between race 6A or race 13A with "other races", the F_1 hybrids from the "other races" side resembled maternal parents, while those from race 6A or race 13A side segregated.

In crosses between races 6A and 13A segregation was obtained from both sides of the crosses. Both these races also showed segregation in their respective S_1 progenies.

Cytoplasmic inheritance has been reported in some races of both wheat and oat stem rusts (Johnson and Newton, 1946; Johnson, 1949; Green, 1965a, 1965b). Their studies were facilitated by the use of urediospore colour as markers, and also by pathogenicity on a relatively large number of differential varieties. Unfortunately, no morphological characters, such as differences in urediospore colour, were available for use as markers in the studies on sunflower rust, and only two differential host varieties were available during most of this work.

In the last few months of this investigation, two additional host lines became available. A few viable seeds of derivatives from crosses with wild annual sunflowers collected in Chicago, provided by Dr. Putt, were sown in the field at Macdonald College in 1964. Selections M62-2672-2-r1 and M62-2685-1r-I proved useful in distinguishing F_1 hybrid progenies of crosses between races which on the standard differentials had been described as "selfs" or contamination respectively.

The nature of inheritance of pathogenicity in rust can only be determined from the behaviour of the rust on its host. Variability of rust reaction may be attributable to genetic make up of the rust, or of the host. Genetics of the sunflower host will be discussed in a later section. A brief discussion of possible sources of variability in the host is necessary here, however, to explain the behaviour of the rust.

Rust resistance in Canadian sunflower varieties was derived from natural crosses between cultivated Helianthus annuus and wild annual

sunflowers occurring near Renner, Texas (Putt and Sackston, 1957; Putt, 1964). The resistance factors in Morden 69 (and CM9ORR) and Morden 29 were reported to be single, dominant, non-allelic factors, designated R_1 and R_2 respectively (Putt and Sackston, 1963).

Seedlings of the differential varieties show great variability in vigor, growth habit, size and shape of leaves and seeds, and the occurrence of various leaf abnormalities as well as variability in reaction to various rust races. This variability is unpredictable and appears to be influenced by environmental changes which are difficult to control in the greenhouse.

The differential varieties originated from selections made in 1952 to 1954 (Putt and Sackston, 1957), and have been selfed repeatedly since then. The morphology and growth habit of these lines should be uniform after repeated selfing if these characters were governed by simple Mendelian factors.

The variability observed in selfed progenies of the sunflower differential varieties might be explained on the basis of chromosomal factors, non-chromosomal factors, or the interaction of both. Determination of the factors responsible would require extensive, intricate, and long-continuing genetic studies well beyond the limits of this investigation. My work was intended to be a study of the genetics of the rust pathogen, using available host materials which were thought to have well defined genetic patterns. Many of the irregularities arose late in the work, when time did not permit starting again to try to breed differentials with clearly determined genotypes.

Putt and Heiser (1966) found, in studies on male sterility in a number of sunflower lines, that meiosis was apparently normal in all cases, including the lines related to the differentials used in my work. If their results can be extrapolated, then perhaps chromosomal aberrations or abnormal chromosomal behaviour may not be responsible for the variable behaviour of the differentials.

According to Caspari (1948) reciprocal differences are observed especially in species crosses. They have also been found in crosses between inbred strains, and between local or geographic biotypes or varieties of the same species. The higher frequency of reciprocal differences in species hybrids is attributed to greater sensitivity of the hybrid genome. Cytoplasmic inheritance in crosses between species and varieties has been studied intensively in Epilobium, the willow herb (Bhan, 1964; Jinks, 1964; Williams, 1964; Whitehouse, 1965).

Various workers have reported that characters controlled by cytoplasmic factors or by the interaction of nuclear and cytoplasmic factors, such as pigmentation in Habrobracon, and male sterility and other characters in corn, sugar beet, and Epilobium, are particularly sensitive to environmental influence (Caspari, 1948; Hogaboam, 1957; Bhan, 1964; Williams, 1964; Duvick, 1965). The possibility that cytoplasmic factors, or a combination of nuclear and cytoplasmic factors, are responsible for the variability of the sunflower rust differentials, must not be excluded. The fact that rust resistance resulted from relatively "wide" crosses between cultivated and wild species of Helianthus may increase the probability of non-chromosomal inheritance of various characters.

If non-nuclear factors are involved in inheritance of rust resistance and other characters in sunflowers, then on the basis of complementary genetic mechanisms postulated for host plants and their obligate parasites, non-nuclear factors may also be involved in the inheritance of rust pathogenicity. The suggestion that non-nuclear factors are responsible for the reciprocal differences and irregular patterns of behaviour of selfed and hybrid rust progenies may therefore be supported by evidence even though not experimentally documented, for non-nuclear inheritance in the host and may strengthen the argument for this explanation of host genetics.

PART II

GENETICS OF RUST RESISTANCE IN SUNFLOWERS

Rust resistance in the two differential varieties, Morden 69, a selection of resistance source 22, and Morden 29, a selection of resistance source 88, has been reported to be controlled by two dominant non-allelic genes, R_1 and R_2 respectively (Putt and Sackston, 1963). However, at the start of the present study on the genetics of pathogenicity in rust races it was found that the differential varieties are heterozygous for rust reaction. Sunflower varieties supposedly possessing one of Putt and Sackston's genes for resistance, either R_1 or R_2, exhibited an array of reaction types suggesting that more than one gene was probably involved.

This study was proposed to investigate the mode of inheritance in the self-derivatives of sources 22 and 88 in the belief that the picture was actually more complex than Putt and Sackston reported it to be.

REVIEW OF LITERATURE

The rediscovery of Mendel's laws of inheritance in 1900 provided the foundation necessary for analysing the differences in disease reactions between varieties of crop plants. Biffen in 1905 was the first to apply Mendel's laws to the inheritance of disease resistance (Biffen, 1905, 1912). He reported that resistance to yellow rust (Puccinia glumarum) was governed by a single recessive gene in crosses between certain susceptible varieties and the resistant variety Rivet. Similar investigations conducted by Armstrong (1922) confirmed Biffen's work. Armstrong concluded that susceptibility and immunity behave as unit characters, depending primarily upon definite factors which are inherited according to the Mendelian laws of inheritance.

Following Biffen, numerous studies have been made on inheritance of resistance to disease. With respect to the development of rust resistant varieties of crop plants, considerable progress has been made in recent years and much information has accumulated regarding the nature of inheritance of rust reaction.

Studies on rust resistance in sunflowers

The history of sunflower and sunflower rust, and the historical development of rust resistant varieties of sunflowers have been reviewed by Putt and Sackston (1957), Putt (1963, 1964) and Sackston (1962, 1964).

The cultivated sunflower is a diploid species. Its wild ancestors originated in south central North America, but its development as an economic crop occurred in Russia and eastern Europe (Putt and Sackston 1957). Sunflower rust, an emautoecious species, was first described in 1822 from specimens collected from southeastern United States. Rust has been a major limiting factor in sunflower production throughout the sunflower growing areas of the world (Putt and Sackston, 1957).

The first source of resistance to sunflower rust in Canada was discovered in 1949 from a single plant of the progeny of a natural cross, Sunrise x Wild Annual from Texas. This source of resistance was termed source 88. The second source of resistance, designated source 22, was discovered in 1950 and it also was obtained from Texas Wild Annual from a single plant in the progeny of the natural cross, California Oilseed x Wild Annual. Source 22 is the main source of resistance in the Canadian breeding programme and it is also being used in breeding for resistance in Peru and Argentina (Putt, 1963, 1964). Several other resistance sources have been reported since, (Putt, 1963, 1964; Sackston, 1962, 1964; Sackston and Jabbar Miah, 1963). In Argentina, hybrids of the cultivated sunflower with the annual wild forms Helianthus cucumerifolius and H. argophyllus and in Russia, the wild sunflower from Texas and the hybrids of the cultivated forms with the perennial species designated as H. divaricatus, H. scaberrinus, H. strumosus and H. tuberosus have provided useful resistance in breeding programmes (Putt, 1963).

Physiologic specialization in sunflower rust was first investigated by Bailey (1923) and by Brown (1936). Sackston (1962) described 4 physiologic race groups of rust from the specimens collected from various parts of the world on cultivated H. annuus. The four physiologic races identified were based on three sources of resistance as summarized below:

Differentials	Physiologic races			
	1	2	3	4
Universal suscept (S37-388)	S	S	S	S
Source 88	R	S	R	S
Source 22	R	R	S	S
Source 41	R	R	R	S

R, resistant; S, susceptible

Putt and Rojas (1955) studied the inheritance of resistance of source 22 in the field. In crosses with susceptible varieties as female and source 22 as male, Putt and Rojas reported that the resistance of source 22 in the field is controlled by a single dominant gene. Putt and Sackston (1963) investigated the mode of inheritance of resistance of source 22 and source 88 and reported that resistance in each of these two sources is governed by a single dominant non-allelic gene, designated as R_1 and R_2 respectively.

MATERIALS AND METHODS

The sunflower lines studied were Morden 69, carrying resistance factor R_1, and Morden 29, carrying resistance factor R_2 (Putt and Sackston, 1963). The inheritance of resistance was studied systematically only in selfed progenies of Morden 69. Some lines of Morden 29 were increased by selfing for up to three generations. The inheritance of resistance in these lines, was inferred from the results of various inoculation experiments. All inoculations were made with the four races of rust described earlier.

The seedlings for all experiments were started in the greenhouse. In the early part of this work, inoculated plants were kept in the greenhouse. In later experiments they were transferred to growth cabinets after inoculation.

Seeds from 12 individual plants of Morden 69 selfed at Winnipeg in 1959 were used to grow differentials for my rust studies. One lot of seed was used up in routine rust identifications. Seedlings of the remaining 11 lines were inoculated with four rust races by the multiple method. After the rust reaction was recorded, (Table 10) the rusted leaves were removed and the seedlings were labelled and transplanted to the field. To prevent the spread of rust the plants in the field were sprayed periodically with a suspension of the fungicide Parzate[1/] in water (1.5 : 1000).

To prevent cross pollination the flowering heads were covered with bags just prior to the opening of the flowers. In 1962 paper bags were

1/ Zinc ethylene bisdithiocarbamate

TABLE 10. SEEDLING REACTIONS TO RACES 1, AND 2 OF S_1 PROGENIES DERIVED FROM MORDEN 69

Line number	Environment and date tested	Observed number R[1]	Observed number S[1]	Ratio	Expected Number R	Expected Number S	X^2	P
M69-W59-7	GH,[2] 30 May 1962	1	32					
M69-W59-8	GH,	31	7	3 : 1	28.50	9.50	0.877	0.30 - 0.50
	30 May 1962			13 : 3	30.87	7.13	0.003	0.90 - 0.95
	GH,	92	27	3 : 1	89.25	29.75	0.339	0.50 - 0.70
	2 June 1963			13 : 3	96.68	22.32	1.198	0.20 - 0.30
	Total	123	34	3 : 1	117.75	39.25	0.936	0.30 - 0.50
				13 : 3	127.56	29.44	0.896	0.30 - 0.50
M69-W59-9	GH,	36	6	3 : 1	31.50	10.50	2.572	0.10 - 0.20
	2 June 1963			13 : 3	34.12	7.88	0.554	0.30 - 0.50
M69-W59-10	GH,	27	8	3 : 1	26.25	8.75	0.085	0.70 - 0.80
	30 May 1963			13 : 3	28.44	6.56	0.389	0.50 - 0.70
	GH,	121	33	3 : 1	115.50	38.50	1.060	0.30 - 0.50
	1 June 1963			13 : 3	125.11	28.89	0.718	0.30 - 0.50
	Total	148	41	3 : 1	141.75	47.25	1.103	0.20 - 0.30
				13 : 3	153.57	35.43	1.078	0.20 - 0.30

TABLE 10 - continued

Line number	Environment and date tested	Observed number R	Observed number S	Ratio	Expected Number R	Expected Number S	X^2	P
M69-W59-11	GH, 30 May 1962	29	0					
	GH, 24 June 1963	21	0					
	Total	50	0					
M69-W59-15	GH,	11	4	3 : 1	11.25	3.75	0.023	0.80 - 0.90
	30 May 1962			13 : 3	12.19	2.81	0.632	0.30 - 0.50
	GC,[2/]	24	7	3 : 1	23.25	7.75	0.097	0.70 - 0.80
	7 June 1964			13 : 3	25.19	5.81	0.300	0.50 - 0.70
	Total	35	11	3 : 1	34.50	11.50	0.030	0.80 - 0.90
				13 : 3	37.37	8.63	0.801	0.30 - 0.50
M69-W59-38	GH,	19	10	3 : 1	21.75	7.25	1.388	0.20 - 0.30
	30 May 1962			13 : 3	23.56	5.44	4.705	0.02 - 0.05
	GH,	38	15	3 : 1	39.75	13.25	0.308	0.50 - 0.70
	1 June 1963			13 : 3	43.06	9.94	3.171	0.05 - 0.10
	Total	57	25	3 : 1	61.50	20.50	1.317	0.20 - 0.30
				13 : 3	66.62	15.38	7.406	< 0.01

TABLE 10 - continued

Line number	Environment and date tested	Observed number R	Observed number S	Ratio	Expected Number R	Expected Number S	X^2	P
M69-W59-40	GH, 30 May 1962	34	0					
	GH, 24 June 1963	11	0					
	Total	45	0					
M69-W59-52	GH,	34	3	3 : 1	27.75	9.25	5.630	0.01 - 0.02
	30 May 1962			13 : 3	30.06	6.94	2.753	0.05 - 0.10
	GH,	9	3	3 : 1	9.00	3.00	0.000	1.00
	24 June 1963			13 : 3	9.75	2.25	0.308	0.50 - 0.70
	Total	43	6	3 : 1	36.75	12.25	4.257	0.02 - 0.05
				13 : 3	39.81	9.19	1.363	0.20 - 0.30
M69-W59-54	GH,	23	2	3 : 1	18.75	6.25	3.853	0.02 - 0.05
	30 May 1962			13 : 3	20.31	4.69	1.899	0.10 - 0.20
	GH,	24	7	3 : 1	23.25	7.75	0.168	0.50 - 0.70
	24 June 1963			13 : 3	25.19	5.81	0.207	0.50 - 0.70
	GC,	58	10	3 : 1	51.00	17.00	3.781	0.05 - 0.10
	7 June 1964			13 : 3	55.25	12.75	0.856	0.30 - 0.50
	Total	105	19	3 : 1	93.00	31.00	6.193	0.01 - 0.02
				13 : 3	100.75	23.25	0.956	0.30 - 0.50

TABLE 10 - continued

Line number	Environment and date tested	Observed number R	Observed number S	Ratio	Expected Number R	Expected Number S	X^2	P
M69-W59-77	GH,	19	5	3 : 1	18.00	6.00	0.223	0.50 - 0.70
	30 May 1962			13 : 3	19.50	4.50	0.079	0.70 - 0.80
	GH,	19	6	3 : 1	18.75	6.25	0.031	0.80 - 0.90
	24 June 1963			13 : 3	20.31	4.69	1.310	0.20 - 0.30
	GC,	47	14	3 : 1	45.75	15.25	0.136	0.70 - 0.80
	4 June 1964			13 : 3	49.50	11.50	0.669	0.30 - 0.50
	Total	85	25	3 : 1	82.50	27.50	0.303	0.50 - 0.70
				13 : 3	89.37	20.63	1.140	0.20 - 0.30

1/ R, resistant; S, susceptible

2/ GH, greenhouse; GC, growth cabinet

used, but in 1963 and 1964, cotton bags of the kind described by Putt (1954) were used.

The heads, after ripening, were cut, wrapped individually in separate pieces of muslin cloth, and dried. After drying they were threshed individually and the seeds were cleaned with a small grain cleaner.

The seed-set was very satisfactory in 1962, but was poor in 1963 and not very satisfactory in 1964. In 1963 the plants were sprayed prior to transplanting with nickel chloride mixed with the fungicide Parzate in an effort to eradicate incipient rust infections. Presumably as a result of this spray, the apical leaves were almost killed and the plants were stunted. In 1964, the rust reaction of the seedlings was tested in the growth cabinets. Flower buds appeared only 10 to 15 days after the seedlings were transplanted into the field and the flowers opened much earlier than in previous years. Presumably the light and temperature conditions in the growth cabinets induced the abnormally early flowering.

EXPERIMENTAL RESULTS

The work which I started in 1961 was meant to be a study of the inheritance of pathogenicity in four physiologic races of sunflower rust on the differential varieties Morden 69 and Morden 29. Information on the genetics of resistance of these two differential varieties to rust was published by Putt and Sackston (1963) and Sackston (1962).

It is a prerequisite for studies of this nature that the differential varieties should be homozygous. This was assumed to be the case with the above mentioned varieties. However, in the course of routine rust work both differential varieties appeared to be heterozygous. It was therefore necessary to attempt to obtain homozygous lines. For this purpose, seedlings of the differential varieties were inoculated with the four physiologic races using the multiple method and their rust reactions were determined. This work was done in the spring of 1962 under greenhouse conditions. The resistant seedlings were then transplanted to the field for selfing.

The seedlings of the progeny lines produced in 1962 were subsequently tested in the spring of 1963 under greenhouse conditions. It was intended to keep only lines with desirable rust reactions. However, in these tests some unexpected types of rust reaction were observed. Morden 69 is supposed to be resistant to races 1 and 2 and susceptible to races 3 and 4. Some seedlings in the selfed progenies showed the expected Morden 69 reactions (Fig. 5), some were susceptible to all four races (Fig. 6); some seedlings were found to be resistant to all four races; some were found to be resistant to races 1, 2 and 3, and susceptible to race 4 (Fig. 7), some were resistant to races 1, 2 and 4, and susceptible

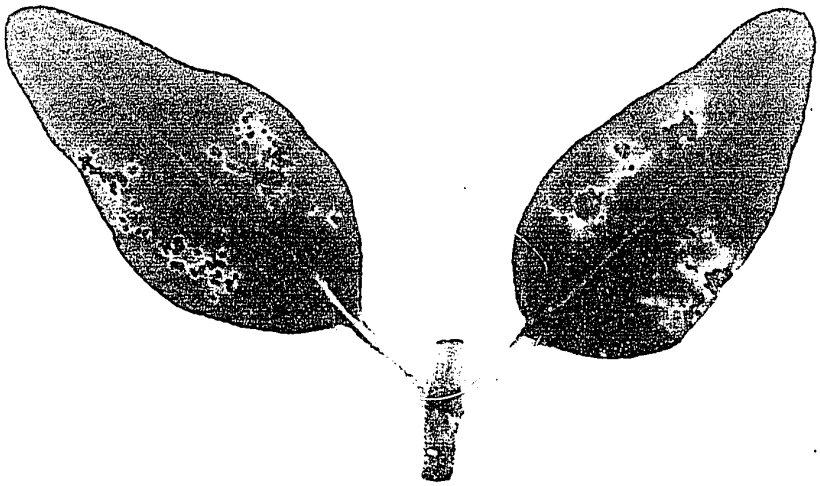

Fig. 5. Leaves of Morden 69 derivative seedling showing resistant reactions to rust races 1 and 2 and susceptible reactions to races 3 and 4.

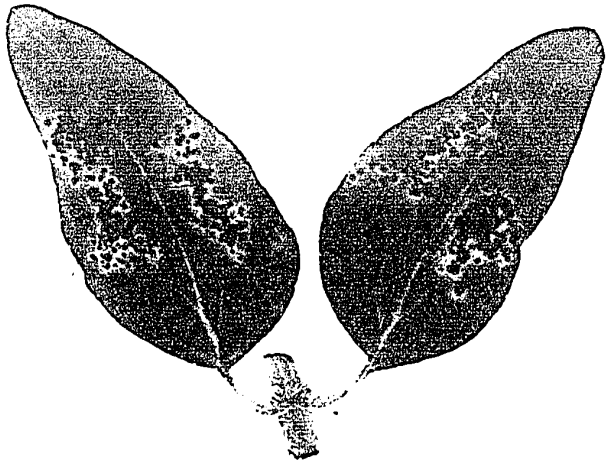

Fig. 6. Leaves of Morden 69 derivative seedling showing susceptible reactions to rust races 1, 2, 3, and 4.

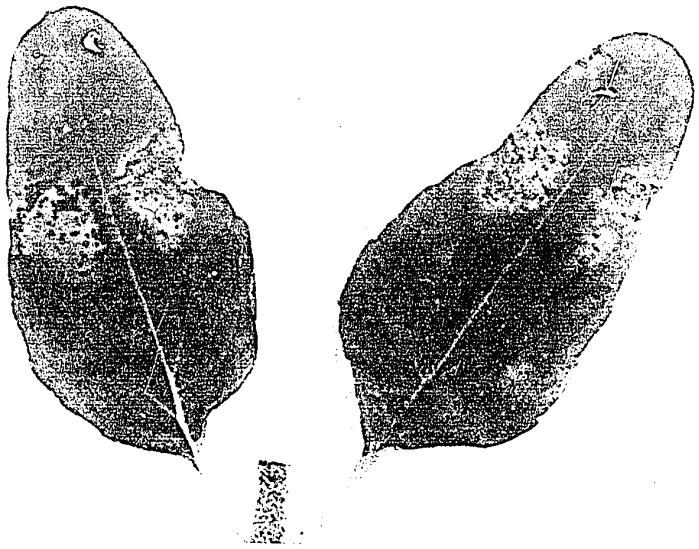

Fig. 7. Leaves of Morden 69 derivative seedling showing resistant reactions to rust races 1, 2, and 3 and susceptible reaction to race 4.

to race 3; and a few were found to be susceptible to races 1, 2, and 4 and resistant to race 3 (Fig. 8.) From these results it was apparent that the genetics of rust resistance was more complex than reported by Putt and Sackston (1963) and Sackston (1962).

Seed production of plants selfed in the field in 1962, after determining their seedling rust reaction in the greenhouse, was satisfactory. It was therefore possible to produce S_2 lines from the S_1 plants whose seedling reactions had been determined in the greenhouse. Data obtained by testing S_2 progenies could be incorporated in the analysis of the S_1 data by the method described by Smith (1937) (see also Mather, pp. 84-86, 1957), to determine if segregating characters showed incomplete manifestation.

Limitations of time and facilities made it impossible to embark on a large scale programme for studying genetics of host resistance. It was therefore necessary to confine work on the genetics of rust resistance in Morden 69 to a limited study of its selfed progeny. With this objective seedlings of some selected S_1 families were tested in late May and early June, 1963, for reaction to the four rust races by the multiple method and they were then transplanted to the field for selfing.

The progeny lines produced in 1962 were tested in the winter of 1963-1964 under greenhouse conditions. The results were found to be inconsistent. The seedlings of a number of resistant parents gave susceptible reactions. Since the seedling reaction of the parent plants was determined in the greenhouse in late May and June, it was thought that the resistant reactions observed at that time might be attributed to excessively high temperatures which might have interfered with normal rust development. To avoid this difficulty in 1964, seedlings from selected lines started in the greenhouse

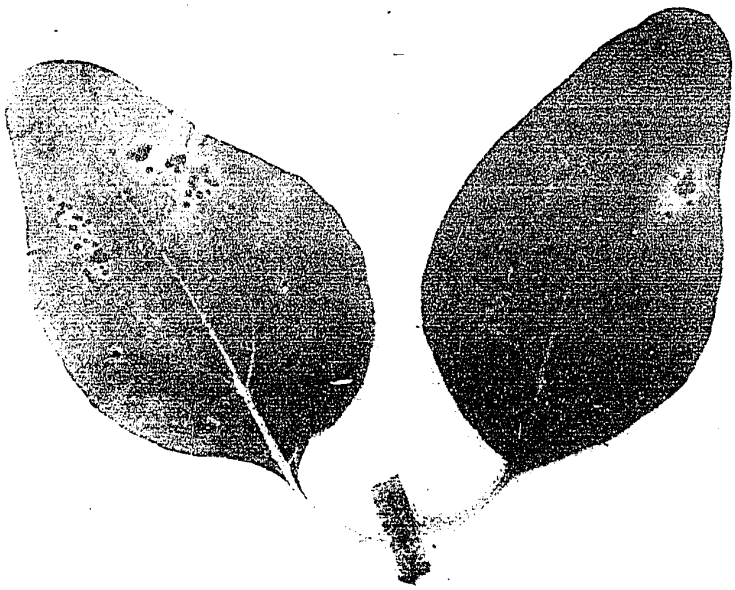

Fig. 8. Leaves of Morden 69 derivative ssedling showing resistant reactions to rust race 3 and susceptible reactions to races 1, 2, and 4.

were inoculated by the multiple method in late May and June and were then transferred to controlled conditions in growth cabinets for rust reactions to develop. They were then transplanted to the field for selfing.

The progeny lines produced in 1964 were tested in the winter of 1964-1965. The seedlings were started in the greenhouse; after inoculation by the multiple method they were transferred to growth cabinets. Due to poor seed germination the number of seedlings tested per S_2 line varied from 3 to 20. A total of 110 S_2 lines derived from S_1 plants resistant to races 1 and 2 were tested: 59 lines were uniformly resistant, and 51 segregated to races 1 and 2. On the basis of the 3 : 1 ratios observed in the S_1 (Table 10), the ratios of resistant to segregating S_2 progenies should have been 1 : 2.

Because the progeny sizes tested of many of the S_2 lines were too small to detect segregation even for a single factor hypothesis (Hanson, 1959), it is not legitimate to conclude that there was an excess of resistant lines and a deficiency of segregating lines.

In those experiments in which numerous progenies segregated, the ratio of resistant to segregating progenies tended to be 1 : 2. The ratio of resistant to susceptible plants in the segregating progenies tended to be 3 : 1. In the other experiments the resistant reactions might have been a phenotypic response to environmental conditions, as the control plants appeared less susceptible than expected.

Similar lack of agreement between S_1 and S_2 data was observed in late May and June of 1963. The observed ratios in all S_1 families were in good agreement with a 3 : 1 ratio (Table 10). The ratio of resistant

to susceptible plants observed among the S_2 lines derived from the S_1 line M69-W59-54, varied from 3 : 1 through 2 : 1, to almost 1 : 3 (Table 11).

One hundred and forty-six seedlings of the S_2 line M69-W59-11-MC62-13, derived from the S_1 M69-W59-11, were tested. The observed ratios showed a good fit to 15 : 1 or 61 : 3 for race 1, and 3 : 1 for race 2 (Table 12). The S_3 lines and selected S_4 lines were tested in the spring of 1964 and in the winter of 1964-1965 respectively. As in the progeny tests of the other S_1 families, the results were found to be inconsistent. In some experiments the progeny lines showed normal segregation while in others the seedlings of all or most lines tested appeared resistant. A few lines resistant to races 1, 2 and 3, but susceptible to 4, derived from the S_3 and S_4 generations, were employed as a supplemental differential under the code number M69-W59-11-MC62-13-r4 to test the pathogenicity of the F_1 hybrid rust progenies (Table 7).

Selfed progenies of Morden 69 were inoculated with races 3 and 4 as well as races 1 and 2. They were expected to be susceptible to races 3 and 4. Some seedlings however gave resistant reactions. The proportion of such resistant seedlings in any progeny varied from test to test (Table 13). The S_2 progenies derived from resistant S_1 seedlings were tested in the winter of 1963-1964 in the greenhouse and in 1964-1965 in growth cabinets. Most of the S_2 progenies tested in 1963-1964 were completely susceptible to races 3 and 4. Of the five tested in 1964-1965, two were susceptible to moderately susceptible; and four segregated (Table 13).

In the S_2 line M69-W59-11-MC62-13, the observed ratio showed a fair approximation to 3 : 1 for race 3 and 7 : 9 or 1 : 1 to race 4 (Table 12).

TABLE 11 SEEDLING REACTIONS TO RACES 1, AND 2 OF S_2 PROGENIES DERIVED FROM M69-W59-11 AND M69-W59-54

Line number	Environment and date tested	Observed number R[1]	Observed number S[1]	Ratio	Expected Number R	Expected Number S	X^2	P
S_2 lines of M69-W59-11:								
M69-W59-11-MC62-1	GH,[2] 11 May 1963	18	0					
-2	"	12	3	3 : 1	11.25	3.75	0.200	0.50 - 0.70
				13 : 3	12.19	2.81	0.016	0.80 - 0.90
	GH, 2 June 1963	70	23	3 : 1	70.00	23.00	0.000	1.00
	Total	82	26	3 : 1	81.00	27.00	0.049	0.80 - 0.90
				13 : 3	87.75	20.25	2.010	0.10 - 0.20
-6	GH, 11 May 1963	11	0					
	GH, 2 June 1963	37	1					
	Total	48	1					
-7	GH, 11 May 1963	14	0					
-9	"	13	0					
-10	"	16	0					
-12	"	11	0					
-13	see Table 12							

TABLE 11 - continued

Line number	Environment and date tested	Observed number R	Observed number S	Expected Ratio	Expected Number R	Expected Number S	X^2	P
S_2 lines of M69-W59-54:								
M69-W59-54-MC62-125	GH, 11 May 1963	17	1					
	GH, 5 June 1963	30	19	3 : 1	36.75	12.25	4.959	0.02 - 0.05
				2 : 1	32.67	16.33	0.655	0.30 - 0.50
				9 : 7	27.56	21.44	0.494	0.30 - 0.50
	Total	47	20	3 : 1	50.25	16.75	0.841	0.30 - 0.50
-141	GH,	12	24	1 : 2	12.00	24.00	0.000	1.00
	5 June 1963			1 : 3	9.00	27.00	1.333	0.20 - 0.30
-142	GH,	12	5	3 : 1	12.75	4.25	0.176	0.50 - 0.70
	8 June 1963			13 : 3	13.81	3.19	1.264	0.20 - 0.30
-143	"	7	0					
-144	"	9	7					

1/ R, resistant; S, susceptible

2/ GH, greenhouse

TABLE 12 SEEDLING REACTIONS TO RACES 1, 2, 3, AND 4 OF S_3 PROGENIES DERIVED FROM M69-W59-11-MC62-13 1/

Race	Observed number R 2/	Observed number S 2/	Expected Ratio	Expected Number R	Expected Number S	X^2	P
Race 1	138	8	15 : 1	136.87	9.13	0.149	0.50 - 0.70
			61 : 3	139.16	6.84	0.150	0.50 - 0.70
Race 2	105	41	3 : 1	109.50	36.50	0.740	0.30 - 0.50
			13 : 3	118.62	27.38	8.339	< 0.01
Race 3	108	38	3 : 1	109.50	36.5	0.185	0.50 - 0.70
			13 : 3	118.62	27.38	5.070	0.02 - 0.05
Race 4	72	74	7 : 9	63.87	82.13	1.840	0.10 - 0.20
			1 : 1	73.00	73.00	.026	0.80 - 0.90

1/ Tested in late May 1963 under greenhouse conditions

2/ R, resistant; S, susceptible

TABLE 13 SEEDLING REACTIONS TO RACES 3, AND 4 OF S_1 AND S_2 PROGENIES DERIVED FROM MORDEN 69

Line number	Environment and date tested	Observed number Race 3 R[1]	Race 3 S[1]	Race 4 R	Race 4 S
S_1 lines:					
M69-W59- 7	GH,[2] 30 May 1962	0	33	0	33
- 8	GH, 30 May 1962	0	38	0	38
	GH, 2 June 1963	0	116	13	106
	Total	0	154	13	144
- 9	GH, 2 June 1963	0	36	1	35
-10	GH, 30 May 1962	0	35	0	35
	GH, 1 June 1963	1	153	6	147
	Total	1	188	6	182
-11	GH, 30 May 1962	0	22	0	22
	GH, 24 June 1963	4	17	4	17
	Total	2	31	4	29
-15	GH, 30 May 1962	2	13	2	13
	GC,[2] 7 June 1964	2	29	2	29
	Total	4	41	4	41
-38	GH, 30 May 1962	1	28	4	25
	GH, 1 June 1963	2	51	18	35
	Total	3	79	22	60
-40	GH, 30 May 1962	1	33	3	31
	GH, 24 Jun3 1963	0	11	2	9
	Total	1	44	5	40
-52	GH, 30 May 1962	0	37	0	37
	GH, 24 June 1963	0	12	0	12
	Total	0	49	0	49

TABLE 13 - continued

Line number	Environment and date tested	Observed number Race 3 R	Race 3 S	Race 4 R	Race 4 S
S_1 lines:					
M69-W59-54	GH, 30 May 1962	1	24	1	24
	GH, 24 June 1963	5	26	8	23
	GC, 7 June 1964	2	66	33	35
	Total	8	116	42	82
-77	GH, 30 May 1962	1	23	2	22
	GH, 24 June 1963	2	23	7	18
	GC, 4 June 1964	3	58	4	57
	Total	6	104	13	97
S_2 lines:3/					
M69-W59-15-WC64-4	GC, 19 Feb 1965	0	13	0	13
M69-W59-54-WC64-12	GC, 25 Feb 1965	0	9	0	9
-41	GC, 5 Mar 1965	2	9	4	7
M69-W59-77-WC64-59	GC, 6 Mar 1965	2	10	11	1
-64	" "	2	9	7	4
-67	" "	3	9	8	4

1/ R, resistant; S, susceptible

2/ GH, greenhouse; GC, growth cabinet

3/ Progeny from S_1 plants resistant to races 3 and 4

The selfed derivatives of Morden 29 were also tested for reaction to four races, specifically to select lines with desirable rust reactions for use as differentials. They were generally resistant to races 1 and 3, but their reactions to races 2 and 4 were very variable. In some tests the seedlings of a given progeny line gave the normal susceptible reactions. In other tests their reactions varied from moderately susceptible to moderately resistant. Some of these lines were employed in testing the pathogenicity of F_1 hybrid rusts (Table 6).

The results of experiments with the various progeny lines derived from Morden 69 were studied in the spring of 1965. The seedling rust reactions of lines varied from test to test even in controlled environment cabinets. Because it appeared difficult or impossible to obtain useful information about the genetics of rust resistance of our differential varieties by this type of testing, the work was discontinued.

DISCUSSION

Rust reactions in selfed progenies of Morden 69, and also of the less intensively studied variety Morden 29, varied from experiment to experiment. As some experiments were conducted in mid-winter and some in spring, some under greenhouse conditions and some in controlled environment cabinets, the inconsistencies in results were atributed to variations in environmental conditions from experiment to experiment, or possibly to some unidentified seasonal factor.

Temperature sensitivity is well documented in the cereal rusts. Some varieties of wheat are resistant to specific races of stem rust at relatively low temperatures, 20°C or lower, but susceptible at high temperatures, about 30°C (Mohamed, 1960). It has also been found that a temperature sensitive variety grown at high temperature prior to inoculation may be susceptible even though maintained after inoculation at a lower temperature, at which it would normally give a resistant reaction (Mohamed, 1960).

The effect of varying light and temperature regimes on the reaction of sunflowers to rust was investigated at Macdonald College by Liang,(1966) She observed very little effect on reaction type of the universal suscept, S37-388, and of CM9ORR (resistant to races 1 and 2, susceptible to races 3 and 4) maintained at 15°, 20°, 25°, and 29°C after inoculation with races 1 and 3. She also found very little effect of light intensities of 500, 1200, 2000, and 2800 foot candles respectively on rust reaction.

Liang's results, and the variation in rust reaction types from one experiment to another in my work, even when the plants were grown in controlled environment cabinets after inoculation, indicate that some factor other than environmental differences after inoculation was responsible for the variability in my material. There is a possibility that great variations in light intensity from time to time in the greenhouses where the plants were grown prior to inoculation might have affected their responses. This has not been investigated.

The reported effect of high temperature on the rust reaction of temperature sensitive wheat varieties is to overcome the resistance observed at lower temperatures (Mohamed, 1960). The deviation from expected behaviour in my material is the occurrence of resistant reactions in seedlings I had expected to be susceptible. It is possible that short exposures to temperatures or light intensities which do not prevent rust development on most sunflower seedlings, may have such an inhibitory effect on certain host genotypes. There is no experimental evidence to support this hypothesis.

Putt and Sackston (1963) apparently did not encounter as much variability in reaction of the differential varieties as I observed. Their inoculations were made by the routine method, so they may have missed some of the aberrant reactions which could be recognized only by using the multiple method of inoculation.

They did, however, report variations in segregation ratios in two experiments, with Cross 67 and Cross 69 (lines deriving rust resistance from the same source as the line I designate Morden 69). An excess of susceptible seedlings in Cross 69 in one test was balanced by an excess of resistant individuals in another, giving totals which showed a good

fit to a 3 : 1 ratio. An excess of susceptible seedlings of Cross 67 in one experiment gave totals with a poor fit to 3 : 1. My re-analysis of their data for Cross 67 gave a good fit to 2 : 1 (x^2 = 1.339, P = 0.20 to 0.30).

The ratios of seedlings resistant or susceptible to races 1 and 2 in selfed progenies of Morden 69 in my experiments in most cases showed good fit to both 3 : 1 and 13 : 3, but some fitted only one or the other ratio. The progeny sizes were too small to make a critical distinction between the two ratios in the S_1. The results in S_2 progenies were too erratic to permit distinction between the two ratios. It is therefore impossible to conclude from my data whether resistance to these two races is governed by two non-allelic genes, one of which is epistatic to the other, or by a single dominant gene.

Morden 69 should be uniformly susceptible to races 3 and 4. Seedlings resistant to races 3 and 4 were observed in populations tested by the multiple method prior to transplanting and selfing in the field. Selfed progenies from these resistant plants were susceptible when tested in 1965 (Table 13). This reversion from resistance to susceptibility may be explained by incomplete penetrance of a gene governing susceptibility. This might be explained also if rust reactions were governed by an interaction between chromosomal and nonchromosomal factors. This might also explain the variability of ratios observed for reaction to races 1 and 2 by Putt and Sackston as well as by me.

Putt and Sackston (1963) concluded that resistance to races 1 and 2 in lines related to my Morden 69, was controlled by a single dominant factor. The F_2 progeny sizes they tested were too small to discriminate between the ratios 3 : 1 and 13 : 3, (requiring 800 to 900 seedlings per

family), 3 : 1 and 9 : 7 (95 seedlings), and 3 : 1 and 15 : 1 (50 seedlings), (Mather, 1957; Hanson, 1959). In addition, they used the X^2 test for goodness-of-fit between observed and expected ratios. This test is not appropriate when the smallest expected class contains less than five individuals, which was the case in many of their F_2 families[1/] (Smith 1954, p.624, Srb et al. 1965, p.57). In my experiments (Tables 10 and 11) the number of progeny in a few tests was inadequate for a valid X^2 and these data should perhaps be ignored. However, in the majority of cases the population size was adequate.

It is unfortunate that the difficulty of obtaining good seed sets in crossing and selfing experiments with sunflowers severely restricted the number of seedlings per family which they could test. My selfing studies were limited by the same difficulty. Because most of their genetic studies were secondary to the objective of producing rust resistant sunflower varieties, Putt and Sackston (1963) did not test enough F_3 progenies from susceptible F_2 plants to permit concluding whether susceptibility was conditioned by a single homozygous recessive gene, or if it was controlled by two non-allelic genes, one epistatic to the other.

In most of my studies I utilized CM9ORR, deriving its rust resistance from the same source as Morden 69, instead of Morden 69 because seed supplies were much larger. The data are not conclusive, but suggest that resistance in CM9ORR to races 1, 2, and 3 may be controlled by one dominant gene, and that susceptibility to race 3 is controlled by a dominant gene epistatic to the one for resistance.

1/ Family size was calculated from their Table 2 by dividing number of seedlings by the number of progenies in each case.

Selfing studies with race 3 indicated that it was heterozygous, and that virulence on CM9ORR was dominant.

Selfed progenies of race 3 segregated for virulence on seedlings of CM9ORR, which were uniformly resistant to races 1 and 2 and susceptible to parent race 3. Similar selfing studies with races 1 and 2 indicated that they were both heterozygous, and that the virulence of both on CM9ORR was recessive. Selfed progenies of races 1 and 2 segregated for virulence on seedlings of CM9ORR which were uniformly resistant to parent races 1 and 2, and susceptible to race 3.

If the gene for gene hypothesis holds for sunflowers and sunflower rust, a dominant gene for virulence in race 3 should be related to a dominant gene for susceptibility in CM9ORR. Similarly, recessive genes for virulence in races 1 and 2 should be related to a recessive gene for susceptibility in the host.

Occasional seedlings in populations of CM9ORR were observed to be resistant to race 3, as well as to races 1 and 2. One explanation of their occurrence, which cannot be ruled out completely, is that accidental outcrossing introduced resistance to race 3. This seems to have been the case in the S_2 progeny of Morden 69 which gave a good fit to a 15 : 1 ratio for reaction to race 1, and 3 : 1 to races 2 and 3 respectively (Table 12). The behaviour of the S_1 line from which it came, and of its S_3 and S_4 derivatives, indicated that it also possessed the factor for resistance of Morden 29. Another explanation is that the postulated dominant epistatic gene governing susceptibility to race 3 was not homozygous in the population. Occasional plants might be expected which are homozygous recessive for the epistatic gene, permitting expression of the

hypostatic gene controlling resistance to all three races.

It must be emphasized at this point that there is no evidence in the publications of Putt and Sackston for the occurrence in their material of a single gene conditioning resistance to races 1, 2, and 3. However, neither do they record the occurrence of seedlings resistant to race 3 in populations of Morden 69 or CM9ORR.

Crosses among races 1, 2, and 3 showed apparent maternal inheritance of virulence on a few seedlings of CM9ORR. On the assumption of a complementary relationship between pathogen genes and host genes, this indicates that rust reaction may be controlled by an interaction of chromosomal and extrachromosomal factors. Such an interaction would provide an additional explanation for the occurrence of occasional seedlings with unexpected reactions, such as Morden 69 or CM9ORR resistant to race 3.

Lines of Morden 29, selfed for use as differentials, appeared homozygous for resistance to races 1 and 3, but heterozygous for susceptibility to races 2 and 4. The F_1 hybrids of most reciprocal crosses between rust races behaved like the maternal parent (Table 6). On the assumption of a complementary relationship between host and pathogen genes this might indicate that rust reaction in the host is governed by nonchromosomal factors. Proof of maternal inheritance in the rust would require repeated backcrossing to the male parent. This it was impossible for me to do because of difficulties in inducing teliospore germination. Proof of nonchromosomal inheritance in the host would require crossing studies outside the possibilities of this investigation.

Putt and Sackston (1963) reported that rust reaction in "source 88", the source of resistance in Morden 29, was governed by a single dominant factor conferring resistance to races 1 and 3. Susceptibility to races 2 and 4 was reported to be recessive (Putt and Sackston 1963, Sackston 1962).

The discrepancy between their conclusions and mine might be explained if the reaction of this variety to races 1 and 3 was governed by the interaction of a dominant nuclear gene and cytoplasmic factors. My starting material may have been heterozygous for the dominant nuclear gene, and I may inadvertently have selected for selfing plants with the recessive allele of the resistance gene, with resistance governed only by cytoplasmic factors.

Seed is still available of many of the lines of Morden 29 produced by selfing at Macdonald College. If the F_1 hybrid rust cultures which behaved like the maternal parent in reciprocal crosses, should resemble only one of the parents on these untested host lines, it would indicate Mendelian inheritance of virulence in the rust. It would therefore presumably also indicate that rust reaction in the host is governed by nuclear genes. This would substantiate my suggestion that both nuclear and cytoplasmic factors govern rust reaction in this variety, and my explanation of the discrepancy between my results and those of Putt and Sackston.

Hoes and Putt (1962) reported the identification of 9 races of rust based on the reactions of 14 sunflower lines. Their tests were made in February and April, with different reactions on the two dates. These workers also appear to have encountered the extreme variability of rust

reaction which has so complicated my work. Whether their differences can be attributed to seasonal variations in environment, or any of the other explanations I have proposed, has not been determined.

PART III

CRITICAL EVALUATION OF GENETIC STUDIES OF PATHOGENICITY IN RUST FUNGI AND OF RESISTANCE IN THEIR HOSTS

INTRODUCTION

Flor (1942, 1946, 1947, 1955, 1956, 1959), working with flax and flax rust, conducted extensive studies on the genetics of host-pathogen interactions and postulated a gene-for-gene relationship in the flax-flax rust system. Later Person (1959) by a theoretical model showed that the relationship postulated by Flor should occur as a general rule in host-parasite systems. This stimulated the pursuit of similar studies in other host-parasite systems, and a large number of such studies have now been reported. However, an examination of the data on genetics of host-parasite relationships in rust fungi reveals that some of the studies, including those of Flor, are inadequate with respect to both the genetic tests carried out and the interpretation of the data presented. This paper presents a critical evaluation of these studies.

GENETIC STUDIES OF PATHOGENICITY IN FLAX RUST (MELAMPSORA LINI) AND OF RESISTANCE IN FLAX (LINUM USITATISSIMUM)

Flor's studies on genetics of pathogenicity in Melampsora lini

Selfing studies

Working with races 1, 6, 22 and 24 of M. lini, Flor (1942, 1946, 1960a) determined that pathogenicity was homozygous and proceeded to make crosses between races and studied inheritance in the F_1 and F_2 progeny. His investigations ranged from inheritance of pathogenicity (1946, 1955, 1956, 1959) and induced mutation rates (1958, 1960b) to somatic mutation (1960a, 1964). The number of S_1 progeny Flor used in these studies is shown below, condensed from his data (Flor 1946, 1960a).

	Races			
	1	6	22	24
	no.	no.	no.	no.
1946	-	9	9	15
1960	26	-	4	-
Total	26	9	13	15

The number of progeny tested by Flor is too small to detect segregation with certainty even for a single factor (Mather, 1957; Hanson, 1959). This being the case Flor's justification in claiming races to be homozygous seems doubtful. If the 26 S_1 cultures from race 1 were obtained in a single experiment they would be sufficient, but Flor does not say if this is so. Flor obtained a total of 13 cultures from race 22 in two experiments and claimed homozygosity of the race.

These 13 cultures are not enough to detect a single factor segregation. As the cultures were obtained in two experiments, the gametic populations in each of the experiments were inadequate. The different kinds of gametes in the population could be disproportionately represented because of sampling errors. The results thus obtained could be spurious and could result in a misleading conclusion.

Hybridization studies

Using crosses between races 22 x 24, 6 x 22 (Hybrid A), and 6 x 22 (Hybrid D) Flor (1946, 1955, 1959) postulated a 3 : 1 segregation of pathogenicity on a number of differential varieties. My recalculations of Flor's data confirm the 3:1 ratio in some cases. In some of the differential varieties, however, the data fit a 13:3 ratio as well, or in some cases better, than a 3:1 ratio. These selected data are given in Tables 14, 15, and 16.

The F_2 progeny from the cross race 22 x race 24 which was tested on Akmolinsk, Abyssinian and Leona showed pathogenicity to be inherited as a unit. By Flor's analysis (1946, 1959) segregation in these varieties gave a poor fit to a 3:1 ratio. My calculation shows a good fit to a 13:3 ratio (Table 14) suggesting that pathogenicity may be governed by two dominant non-allelic genes with the avirulent gene epistatic to the virulent gene.

On Williston Golden and Williston Brown the parent races of the cross race 22 x race 24 were virulent. In the F_2 progeny, 17 of the 133 cultures were avirulent. The observed ratio in Flor's analysis showed a poor fit to both a 3:1 and a 15:1 ratio. My re-analysis of the data shows that the observed ratio is in good agreement with a 13:3

TABLE 14. PATHOGENICITY OF F_2 CULTURES OF RACE 22 X RACE 24 ON SOME FLAX DIFFERENTIAL VARIETIES. RECALCULATION OF H.H. FLOR'S DATA (FLOR, 1946)

Variety	No. of F_2 cultures: Virulent	No. of F_2 cultures: Avirulent	Theoretical ratio		X^2	P
Williston Golden)) Williston Brown)	116	17	F, [1/]	3 : 1	10.59	< 0.01
			F,	15 : 1	9.69	< 0.01
				13 : 3	3.11	0.05 - 0.10
Akmolinsk)) Abyssinian)) Leona)	23	110	F,	1 : 3	4.21	0.02 - 0.05
				3 : 13	0.19	0.50 - 0.70
Ottawa 770B	32	101	F,	1 : 3	0.06	0.70 - 0.80
				3 : 13	2.46	0.10 - 0.20
Bombay	28	105	F,	1 : 3	1.07	0.30 - 0.50
				3 : 13	0.46	0.30 - 0.50
Bolley Golden	6	127	F,	1 : 15	0.69	0.30 - 0.50
				3 : 61	0.02	0.80 - 0.90
Italia Roma	9	124	F,	1 : 15	0.06	0.70 - 0.80
	19	114		1 : 3	8.14	< 0.01
				1 : 15	14.66	< 0.01
				3 : 13	1.74	0.10 - 0.20
Morye	3	130	F,	1 : 63	0.41	0.50 - 0.70
	10	123		1 : 15	0.365	0.50 - 0.70
				3 : 61	2.39	0.10 - 0.20
				1 : 63	30.68	< 0.01

1/ Ratios marked "F" were calculated by Flor. All other ratios were recalculated from data in Flor's Tables 1 and 2.

TABLE 15. PATHOGENICITY OF F_2 CULTURES OF RACE 6 X RACE 22 (HYBRID A) ON SOME FLAX DIFFERENTIAL VARIETIES. RECALCULATION OF H.H. FLOR'S DATA (FLOR, 1946).

Variety	No. of F_2 cultures: Virulent	No. of F_2 cultures: Avirulent	Theoretical ratio		X^2	P
Pale Blue Crimped	15	59	F, [1/]	1 : 3	0.88	0.30 - 0.50
				3 : 13	0.11	0.70 - 0.80
Kenya	15	59	F,	1 : 3	0.88	0.30 - 0.50
				3 : 13	0.11	0.70 - 0.80
Abyssinian	17	57	F,	1 : 3	0.16	0.50 - 0.70
				3 : 13	0.87	0.30 - 0.50
Leona	17	57	F,	1 : 3	0.16	0.50 - 0.70
				3 : 13	0.87	0.30 - 0.50
Ottawa 770B	10	64	F,	1 : 3	5.21	0.02 - 0.05
				3 : 13	1.33	0.20 - 0.30
Newland	17	57	F,	1 : 3	0.16	0.50 - 0.70
				3 : 13	0.87	0.30 - 0.50
Italia Roma	18	56	F,	1 : 3	0.02	0.80 - 0.90
				3 : 13	1.51	0.20 - 0.30

1/ Ratios marked "F" were calculated by Flor. All other ratios were recalculated from data in Flor's Table 4.

TABLE 16. PATHOGENICITY OF F_2 CULTURES OF RACE 6 X RACE 22 (HYBRID D) ON SOME FLAX DIFFERENTIAL VARIETIES. RECALCULATION OF H.H. FLOR'S DATA (FLOR, 1955).

Variety	No. of F_2 cultures: Virulent	No. of F_2 cultures: Avirulent	Theoretical ratio		X^2	P
Ottawa 770B	7	60	F_2 [1/]	1 : 3	7.56	< 0.01
			F_2	1 : 15	2.02	0.10 - 0.20
				3 : 13	3.03	0.05 - 0.01
Dakota	15	52	F_2	1 : 3	0.24	0.50 - 0.70
				3 : 13	0.58	0.30 - 0.50
Abyssinian, Koto, Leona Ward, Wells	14	53	F_2	1 : 3	0.60	0.30 - 0.50
				3 : 13	0.20	0.50 - 0.70
Bowman, Clay, Grant, Minnesota sel.	17	50	F_2	1 : 3	0.01	0.90 - 0.95
				3 : 13	1.92	0.10 - 0.20
Polk	17	50	F_2	1 : 3	0.01	0.90 - 0.95
				3 : 13	1.92	0.10 - 0.20
Birio	17	50	F_2	1 : 3	0.01	0.90 - 0.95
				3 : 13	1.92	0.10 - 0.20
Towner	16	51	F_2	1 : 3	0.04	0.80 - 0.90
				3 : 13	1.16	0.20 - 0.30
Argentine sel., Cortland, Lino 6899 M.A.	15	52	F_2	1 : 3	0.24	0.50 - 0.70
				3 : 13	0.58	0.30 - 0.50

1/ Ratios marked "F" were calculated by Flor. All other ratios were recalculated from data in Flor's Table 1.

ratio suggesting that pathogenicity on Williston Golden and Williston Brown may be governed by two dominant genes, with the virulence gene epistatic to the avirulence gene.

Flor (1946, 1955, 1959) studied the inheritance of pathogenicity on Ottawa 770B in crosses of race 22 x race 24, race 6 x race 22 (Hybrid A), and race 6 x race 22 (Hybrid D). By Flor's analysis, the observed F_2 ratio in the cross race 22 x race 24 showed good fit to a 3:1 ratio but those in the other two crosses a poor fit to the same ratio. In the cross race 6 x race 22 (Hybrid D) the observed ratio approached 15:1. A re-analysis of Flor's data shows that the observed ratios in all three crosses are in good agreement with a 13:3 ratio (Tables 14, 15, 16) suggesting that pathogenicity in Ottawa 770B may be governed by two non-allelic dominant genes with the avirulence gene epistatic to the virulence gene.

Flor (1946, 1959) postulated that pathogenicity on Italia Roma and Morye is governed by two and three genes respectively. An examination of Flor's data reveals that his classification of the F_2 progeny on these two varieties was inconsistent. Flor (1942, 1946, 1947, 1954) in all his studies considered moderately virulent cultures as virulent and moderately susceptible plants as susceptible and he himself stated that "a difference between an immune and a resistant or between a moderately susceptible and a susceptible host reaction is considered insufficient to differentiate races" (1946, p.341). However, in classifying the F_2 progeny in the cross race 22 x race 24 he placed the moderately virulent cultures on Italia Roma and Morye in the avirulent group and those on Buda in the virulent group (compare Flor's table 1 and 2, 1946). The reason seems to be that if the moderately virulent

cultures on Italia Roma are placed in the virulent group, the observed ratio fits neither a 3:1 nor a 15:1 ratio (my calculations show that it fits a 13:3 ratio) and the situation would be like that on Williston Golden and Williston Brown where Flor was unable to find an expected ratio. With Morye, if the moderately virulent cultures are placed in the virulent group the observed ratio fits only a 15:1 ratio and not a 63:1 ratio. This would be contrary to his a priori expectation that rust reaction in Morye is controlled by three genes.

Flor's studies on genetics of rust resistance in flax

In evaluating Flor's data on the genetics of rust resistance in flax a few selected examples will be described where Flor's interpretation appears to be inadequate.

Complementary genes

In the cross Victory C x Bombay, Flor analyzing the observed ratio for the four test races (races 4, 41, 148 and 227) combined for a 9:3:3:1 ratio obtained a highly significant X^2 but drew no conclusion (Flor, 1951). From the high X^2 value in Flor's analysis I suspected that either the segregation ratio was disturbed or the genes controlling the reactions to races 148 and 41 were linked. In this case the appropriate test for linkage is to calculate X^2, setting out the data in a 2 x 2 table (Mather, 1957) as follows:

		Race 148		
		R	S	Total
Race 41	R	56	42	98
	S	16	14	30
	Total	72	56	128

The X^2 value obtained in this way (0.025 for 1 d.f.) is very small suggesting that the genes controlling reactions to races 148 and 41 are inherited independently. I then analyzed Flor's data to

determine the expected ratios for individual races. The observed ratio for race 148 in my analysis showed a perfect fit to a 9:7 ratio but poor fit to a 3:1 ratio (Table 17). The population size was sufficient to differentiate between a 9:7 and a 3:1 ratio (Mather, 1957) and these figures suggest that resistance in Bombay to race 148 is governed by a pair of complementary genes.

The observed ratio for race 41 shows a good fit to a 3:1 ratio. Assuming a 9:7 ratio for race 148 and a 3:1 ratio for race 41, the observed ratio for races 4 and 227 (the reactions to these two were inherited as a unit) fits well to a 57:7 ratio suggesting that resistance in the cross Victory C x Bombay to races 4 and 227 is governed by three dominant genes of which two are complementary. Assuming a 9:7 ratio for race 148, a 3:1 for race 41 and a 57:7 ratio for races 4 and 227, the expected ratio for all test races combined would be 27:21:9:7 and the observed data for all test races combined agree with this hypothesis (Table 17).

Flor studied the inheritance of resistance in the cross Rio x Bombay to races 16, 41, and 19 (Flor, 1947). Analyzing the observed ratios for all these races combined he obtained an expected ratio of 44:16:3:1:0 but the X^2 was highly significant (Table 17). From this he did not draw any conclusion except to say that in the cross Rio x Bombay, in which four rust-conditioning genes are involved, an unsufficient number of plants was studied for a significant statistical test and that the deviation of the observed from the theoretical ratio suggests the Bombay factor for immunity to race 19 to be linked with one of the three Rio factors for immunity or resistance to race 16 (Flor, 1947).

TABLE 17. REACTIONS OF F_2 PROGENIES OF DIFFERENTIAL VARIETIES OF FLAX TO TEST RACES OF RUST. RECALCULATION OF H.H. FLOR'S DATA FOR SEVERAL SELECTED CROSSES

	Parental Reactions	F_2 progeny reactions				X^2	P
Victory C x Bombay (Flor, 1951, p.529)							
	Vi. Bo.						
Races 4 and 227	R R	R	R	R	S		
Race 148	S R	R	S	R	S		
Race 41	R S	R	R	S	S		
Observed		56	42	16	14		
Expected							
F_2 [1] 9:3:3:1		72	24	24	8	24.222	< 0.01
27:21:9:7		54	42	18	14	0.296	0.95 - 0.98
Races 4 and 227		R	S				
Observed		114	14				
Expected							
15:1		120	8			4.800	0.02 - 0.05
57:7		114	14			0.000	1.00
Race 148							
Observed		72	56				
Expected							
3:1		96	32			24.000	< 0.01
9:7		72	56			0.000	1.00
Race 41							
Observed		98	30				
Expected							
3:1		96	32			0.267	0.50 - 0.70

TABLE 17 - continued

	Parental Reactions	F_2 progeny reactions					X^2	P
Rio x Bombay (Flor, 1947, p.254)								
	Ri.Bo.							
Race 16	I I	I	I	I	I	S		
Race 41	I S	I	I	R to S-	S	S		
Race 19	S I	I	S	I	I	S		
Observed		101	77	11	3	0		
Expected F, 44:16:3:1:0		132	48	9	3	0	25.165	< 0.01
135:105:9:7		101.00	78.75	6.75	5.25	0	4.966	0.10 - 0.20
Race 4		I, R to S-		S				
Observed		189		3				
Expected 63:1		189		3			0.000	1.00
249:7		186.75		5.25			1.234	0.20 - 0.30
Race 19		I		S				
Observed		115		77				
Expected 3:1		144		48			23.361	< 0.01
9:7		108		84			1.037	0.30 - 0.50

TABLE 17 - continued

	Parental Reactions		F_2 progeny reactions					X^2	P
Italia Roma x Bombay (Flor, 1947, p.254)									
	It.	Bo.							
Race 6	I	I	I	I	I	R to S-	S		
Race 24	I	S	R to I	I	S	S- to S	S		
Race 105	S	I	I	S	I	S	S		
Observed			28	12	1	6	1		
Expected F,45:12:3:3:1			39	9	2	2	1	10.558	0.02 - 0.05
F,11:3:1:1:0			33	9	3	3	0	6.226	0.10 - 0.20
Race 105			I	S					
Observed			29	19					
Expected 3:1			36	12				5.444	0.01 - 0.02
9:7			27	21				0.338	0.50 - 0.70

1/ Ratios marked "F" were calculated by Flor. All other ratios were recalculated from data in Flor's tables.

Flor did not present any expected ratios for the individual races. I calculated these from his data and found the observed ratio for race 19 fits well to a 9:7 ratio but shows a poor fit to a 3:1 ratio with a population size sufficient to differentiate between a 9:7 and a 3:1 ratio (Table 17). This suggests that resistance in Bombay to race 19 is governed by a pair of complementary genes.

For race 41, I cannot imagine any expected ratio which can be combined with the 9:7 ratio for race 19 which will agree with Flor's data for both races combined. An explanation may be that the reaction to race 41 of the plants in the third group (see Table 17) varied from resistant to moderately susceptible making it difficult to classify them as "resistant" or "susceptible". However, if they are placed in the resistant group the observed ratio for this race fits a 249:7 which can be combined with a 9:7 ratio for race 19 to yield a 135:105:9:7 ratio for both races which agrees with Flor's combined data (Table 17).

In the cross Italia Roma x Bombay Flor (1947) analyzed the observed ratio for all test races combined for two expected ratios, namely for a 45:12:3:3:1 (three factors) and a 11:3:1:1:0 (two factors) ratio. The observed ratio gave a poor fit to the former but a satisfactory fit to the latter (Table 17). Therefore, he considered the number of F_2 plants insufficient for a statistically significant analysis and drew no conclusion from them.

Flor tested the F_2 plants of this cross against three races 6, 24 and 105 but did not present any expected ratios for individual races. I have calculated the expected ratio only for race 105 because the reaction to this is the only one which is clear. According to my calculation the observed ratio for race 105 shows a poor fit to a 3:1 ratio but fits a 9:7 ratio (Table 17).

According to Hanson (1959) 50 F_2 plants are required to test a 3:1 ratio when a 13:3 ratio can be ruled out. Flor tested 48 plants from this cross. Since there were too many susceptible plants to fit even a 3:1 ratio, a 13:3 ratio need not be considered.

Of the 48 plants tested 19 were susceptible. These data fit well to a 9:7 ratio (X^2, 0.338; P, 0.50 to 0.70) making it reasonable to conclude that resistance in Bombay to race 105 is governed by a pair of complementary genes.

I have described Flor's data from three crosses involving Bombay as resistant parent with three different susceptible parents. The results of all three crosses suggest that complementary genes are involved in controlling resistance in Bombay to some M. lini races. Flor, describing the results of the cross Victory C x Bombay, stated that "in the Victory C x Bombay progeny there were too many plants susceptible to race 148, the race attacking the Newland gene. This departure from the theoretical ratio has occasionally been encountered in hybrids of varieties having rust-conditioning genes in the Bombay linkage group . . . ." (Flor, 1951, p.528). This statement gives added support to the hypothesis that resistance in Bombay to some races is determined by complementary genes because with the suggested 9:7 ratio more susceptible plants would be expected than with a 3:1 ratio.

Epistasis

Flor's analysis of the F_2 data for all test races, from the cross Victory D x Bison for a 9:3:3:1 ratio showed a significant X^2 value (Flor, 1951). From the results of this cross and from those of the crosses involving Victory D with Ottawa 770B, Newland and Bombay Flor concluded

that resistance in Victory D is controlled by two dominant genes. However, my calculation of Flor's data (Table 18) from the cross Victory D x Bison shows that resistance in Victory D may be controlled by three dominant genes, two of which are epistatic to a third, as shown in Fig. 9 based on three assumptions:

(1) The resistance to race 210 is controlled by three dominant genes A, B and C; with A and B for resistance epistatic to C for susceptibility.

(2) The resistance to race 227 is controlled by two dominant genes A and C; with A for resistance epistatic to C for susceptibility. Gene B is considered to play no role in controlling the reaction to this race.

(3) The resistance to race 52 is controlled by a single dominant gene B. Genes A and C are considered to play no role in controlling reaction to this race.

According to Flor's hypothesis, the expected ratios for the test races 210, 227 and 52 should be 15:1, 3:1 and 3:1 respectively. In my analysis the observed ratio for race 227 fails to fit a 3:1 ratio but fits 13:3, with a population of 176 (Table 18). This suggests that resistance in this case is determined by two dominant genes, one controlling resistance being epistatic to another controlling susceptibility.

The observed ratio for race 210 fits both a 15:1 (according to Flor's expectation) and a 61:3 (my own analysis). Assuming a 13:3 ratio for race 227 the expected ratio for race 210 should be 61:3 which would suggest resistance in Victory D to race 210 to be governed by three dominant genes, ~~with~~ two of which are epistatic to a third.

TABLE 18. REACTIONS OF F_2 PROGENIES OF DIFFERENTIAL VARIETIES OF FLAX TO TEST RACES OF RUST. RECALCULATION OF H.H. FLOR'S DATA FOR SEVERAL SELECTED CROSSES

	Parental Reactions		F_2 progeny reactions				X^2	P
Victory D x Bison (Flor, 1951, p.530)								
	Vi.	Bi.						
Race 210	R	S	R	R	R	S		
Race 227	R	S	R	S	R	S		
Race 52	R	S	R	R	S	S		
Observed			115	20	34	7		
Expected								
F_2 9:3:3:1 [1]			99	33	33	11	9.191	0.02 - 0.05
39:9:13:3			107	25	36	8	1.834	0.50 - 0.70
Race 210			R	S				
Observed			169	7				
Expected								
15:1			165	11			1.555	0.20 - 0.30
61:3			168	8			0.131	0.70 - 0.80
Race 227								
Observed			149	27				
Expected								
3:1			132	44			8.757	< 0.01
13:3			143	33			1.343	0.20 - 0.30
Race 52								
Observed			135	41				
Expected								
3:1			132	44			0.273	0.50 - 0.70
13:3			143	33			2.388	0.10 - 0.20

TABLE 18 - continued

	Parental Reactions		F_2 progeny reactions				X^2	P
Victory B x Bombay (Flor, 1951, p.529)								
	Vi.	Bo.						
Race 1	R	R	R	R	R	S		
Race 73	R	S	R	S	S	S		
Race 41	R	S	R	R	S	S		
Observed			165	26	12	0		
Expected F, 12:3:1:0			152	38	13	0	4.978	0.05 - 0.10
52:9:3			165	28.5	9.5	0	0.877	0.50 - 0.70
Race 73			R	S				
Observed			165	38				
Expected 3:1			152	51			4.426	0.02 - 0.05
13:3			165	38			0.000	1.00
Race 41								
Observed			191	12				
Expected 15:1			190	13			0.088	0.70 - 0.80
61:3			193	10			0.421	0.50 - 0.70

TABLE 18 - continued

	Parental Reactions		F_2 progeny reactions				X^2	P
Leona x Ottawa 770B (Flor. 1947, p.247)								
	Le.	Ot.						
Race 24	R	I	I	R	I	S		
Race 68	R	S	R	R	S	S		
Race 52	S	I	I	S	I	S		
Observed			113	38	27	6		
Expected F, 9:3:3:1			104	34	34	12	5.487	0.10 - 0.20
39:13:9:3			112	37	26	9	1.074	0.70 - 0.80
Race 24			I,R	S				
Observed			178	6				
Expected 15:1			172.5	11.5			2.805	0.05 - 0.10
61:3			175	9			1.051	0.30 - 0.50
Race 68								
Observed			151	33				
Expected 3:1			138	46			4.889	0.02 - 0.05
13:3			149.5	34.5			0.080	0.70 - 0.80
Race 52								
Observed			140	44				
Expected 3:1			138	46			0.116	0.70 - 0.80
13:3			149.5	34.5			3.220	0.05 - 0.10

TABLE 18 - continued

	Parental Reactions		F_2 progeny reactions				X^2	P
Tammes Pale Blue x Punjab (Flor, 1947, p.253)								
	T.P.B.	Pu.						
Race 2	I	I	I	I	I	S		
Race 41	I	S	I	I	S	S		
Race 19	S	I	I	S	I	S		
Observed			114	48	30	0		
Expected F,2:1:1:0			96	48	48	0	10.125	< 0.01
9:4:3:0			108	48	36	0	1.231	0.50 - 0.70
Race 41			I	S				
Observed			162	30				
Expected 3:1			144	48			9.000	< 0.01
13:3			156	36			1.231	0.20 - 0.30
Race 19								
Observed			144	48				
Expected 3:1			144	48			0.000	1.00
13:3			156	36			4.923	0.02 - 0.05

TABLE 18 - continued

	Parental Reactions		F_2 progeny reactions				X^2	P
Kenya x Ottawa 770B (Flor, 1947, p.247)								
	Ke.	Ot.						
Race 7	R	I	I	I	I	S		
Race 68	R	S	R	R	S	S		
Race 79	S	I	I	S	I	S		
Observed			35	13	16	0		
Expected F,2:1:1:0			32	16	16	0	0.844	0.50 - 0.70
9:3:4:0			32	12	16	0	0.111	0.90 - 0.95
Race 68			R	S				
Observed			48	16				
Expected 3:1			48	16			0.000	m 1.00
Race 79			I	S				
Observed			51	13				
Expected 3:1			48	16			0.751	0.30 - 0.50
13:3			52	12			0.102	0.70 - 0.80

TABLE 18 - continued

	Parental Reactions		F_2 progeny reactions				X^2	P
Victory A x Newland (Flor, 1951, p.258)								
	Vi.	New.						
Race 4	R	R	R	R	R	S		
Race 227	S	R	R	S	R	S		
Race 148	R	S	R	R	S	S		
Observed			66	29	33	0		
Expected F,2:1:1:0			64	32	32	0	0.375	0.80 - 0.90
9:3:4:0			72	25	32	0	1.573	0.30 - 0.50
Race 227			R	S				
Observed			99	29				
Expected 3:1			96	32			0.375	0.50 - 0.70
13:3			104	24			1.281	0.20 - 0.30
Race 41								
Observed			95	33				
Expected 3:1			96	32			0.041	0.80 - 0.90

TABLE 18 - continued

	Parental Reactions		F_2 progeny reactions				X^2	P
Williston Brown x Newland (Flor, 1947, p.250)								
	W.B.	Ne.						
Race 7	R	I	I	I	R	S		
Race 47	R	S	R	S	R	S		
Race 16	S	I	I	I	S	S		
Observed			83	38	38	0		
Expected F, 2:1:1:0			79	40	40	0	0.308	0.80 - 0.90
9:3:4:0			89	40	30	0	2.637	0.20 - 0.30
Race 47			R	S				
Observed			121	38				
Expected 3:1			119	40			0.134	0.70 - 0.80
13:3			129	30			2.629	0.10 - 0.20
Race 16			I	S				
Observed			121	38				
Expected 3:1			119	40			0.134	0.70 - 0.80
13:3			129	30			2.629	0.10 - 0.20

1/ Ratios marked "F" were calculated by Flor. All other ratios were recalculated from data in Flor's tables.

	A-	A-	A-	aa	A-	aa	aa	aa
	B-	B-	bb	B-	bb	B-	bb	bb
	C-	cc	C-	C-	cc	cc	C-	cc
	27	9	9	9	3	3	3	1
Race 210 (61:3)	R	R	R	R	R	R	S	R
Race 227 (13:3)	R	R	R	S	R	R	S	R
Race 52 (3:1)	R	R	S	R	S	R	S	S
Expected ratio for all test races combined		39		9	13		3	
Race 210		R		R	R		S	
Race 227		R		S	R		S	
Race 52		R		R	S		S	
Observed number		115		20	34		7	
Expected number		107		25	36		8	

X^2 value, 1.834; P(3 d.f.), 0.50 - 0.70.

Fig. 9. Scheme showing genotypes which would fit ratios calculated from Flor's data for reactions of progeny of cross Victory D x Bison to flax rust races 210, 227, and 52.

The observed ratio for race 52 showed a better fit to a 3:1 ratio than to a 13:3 ratio. Assuming a 3:1 ratio for race 52, a 13:3 ratio for race 227 and a 61:3 for race 210, the expected ratio for all races combined would be 39:9:13:3 which is in agreement with the data (Table 18).

In the cross Victory B x Bombay the observed ratio was analyzed by Flor for a 12:3:1:0 and agreed with expectation (Flor, 1951). The results of this cross and from those of a few others involving Victory B with other varieties led Flor to conclude that resistance in Victory B is conditioned by two dominant genes. According to Flor's hypothesis the expected ratios for races 73 and 41 would be 3:1 and 15:1 respectively. With respect to race 73, the observed ratio in my calculation showed a poor fit to a 3:1, fitting instead a 13:3 ratio with a population of 203 plants suggesting that resistance in Victory B to race 73 is determined by two dominant genes, one of which is epistatic to the other (Table 18).

With race 41, the observed ratio agrees with both a 15:1 and a 61:3 ratio. Assuming a 13:3 ratio for race 73, the observed ratio for race 41 should be 61:3 which would indicate that resistance in Victory B to race 41 is controlled by three dominant genes, two of which are epistatic to a third. If race 73 yields a 13:3 ratio and race 41 a 61:3 then the expected ratio for both test races combined should be a 52:9:3 which is in agreement with the data (Table 18). The genotypic scheme for a 52:9:3 expected ratio is suggested in Fig. 10 based on the following assumptions:

	A-	A-	A-	aa	A-	aa	aa	aa
	B-	B-	bb	B-	bb	B-	bb	bb
	C-	cc	C-	C-	cc	cc	C-	cc
	27	9	9	9	3	3	3	1
Race 41 (61:3)	R	R	R	R	R	R	S	R
Race 73 (13:3)	R	R	R	S	R	R	S	R
Expected ratio for both test races combined		52		9			3	
Race 41		R		R			S	
Race 73		R		S			S	
Observed number		165		26			12	
Expected number		165		28.5			9.5	

X^2 value, 0.877; P (2 d.f.), 0.50 - 0.70

Fig. 10. Scheme showing genotypes which would fit ratios calculated from Flor's data for reactions of progeny of cross Victory B x Bombay to flax rust races 41 and 73.

(1) The resistance to race 41 is controlled by three dominant genes A, B and C; with A and B for resistance epistatic to C for susceptibility.

(2) The resistance to race 73 is controlled by two dominant genes A and C; with A for resistance epistatic to C for susceptibility. Gene B is considered to play no role in controlling the reaction to this race.

In the cross Leona x Ottawa 770B Flor (1947) postulated that resistance is controlled by two dominant genes. However, according to my calculation resistance in this cross to race 68 is determined by two dominant genes, one epistatic to the other, and resistance to race 24 by three dominant genes, two epistatic to the third (Table 18).

In the cross Tammes Pale Blue x Punjab, Flor (1947) tested his observed ratio from the combined data for races 41 and 19 for 2:1:1:0 which would indicate allelic genes. He concluded in spite of a highly significant X^2 (Table 18) that resistance in Tammes Pale Blue was allelic to that in Punjab and Bombay (see Flor's Table 7, 1947).

On this basis, considering races 41 and 19 separately, the expected ratio for each should be 3:1. The observed ratio for race 41 gives a poor fit ($P < 0.01$) to a 3:1 ratio but a good fit to a 13:3 ratio suggesting that resistance in Tammes Pale Blue to race 41 is in fact similar to that described in the other crosses above, being determined by two dominant genes, one for resistance epistatic to a second for susceptibility.

The data for race 19 fit a 3:1 ratio and the combined data for both races fit a 9:4:3 ratio (Table 18). The genotypic scheme for

a 9:4:3 ratio is suggested in Fig. 11, based on the assumption that dominant gene B imparts resistance to race 19 but susceptibility to race 41. Gene B is hypostatic to dominant gene A which is resistant to race 41 but plays no part in controlling reaction to race 19.

Allelic concept

Flor (1947, 1951, 1956, 1959) reported that rust resistance in flax is controlled by 25 genes occurring at 5 loci, 1 at the K, 11 at the L, 6 at the M, 3 at the N, and 4 at the P locus.

Flor's assumption that genes for rust resistance were allelic is based on the occurrence of ratios such as 1:0, 2:1:1:0 and 3:1:0 rather than 15:1, 9:3:3:1 and 12:3:1 respectively which occur when genes are independent and non-allelic. The ratios 1:0 and 3:1 indicate alleles by definition (Sinnot et al., 1958, Lee et al., 1963). The ratio 2:1:1:0 may not necessarily indicate the presence of allelic genes.

With a pair of allelic genes and complete dominance the phenotype of the heterozygote resembles the homozygote possessing the dominant allele. When dominance is incomplete the heterozygote is intermediate between the two homozygotes and when dominance is absent the heterozygote is exactly midway between two homozygotes (Sinnot et al., 1958).

However, there is another class of alleles known as co-dominant alleles. The M and N blood antigens of man are examples of co-dominant alleles. The genes involved are represented I^A, I^B and i. I^A and I^B are each dominant to i but neither is dominant to the other. In the heterozygote each produces its antigenes regardless of its partner (Hutt, 1964; Srb et al., 1965).

Flor's designation of genes as alleles because of F_2 data fitting a 2:1:1:0 ratio can be questioned. In the cross Ottawa 770B x J.W.S.

	A-B- 9	A-bb 3	aaB- 3	aabb 1
Race 41 (13:3)	R	R	S	R
Race 19 (3:1)	R	S	R	S
Expected ratio for both test races combined	9	4	3	
Race 41	R	R	S	
Race 19	R	S	R	
Observed number	114	48	30	
Expected number	108	48	36	

X^2 value, 1.231; P (2 d.f.), 0.50 - 0.70

Fig. 11. Scheme showing genotypes which would fit ratios calculated from Flor's data for reactions of progeny of cross Tammes Pale Blue x Punjab to flax rust races 41 and 19.

(Flor, 1947) the observed ratio showed a good fit to a 2:1:1:0 and the genes in Ottawa 770B and J.W.S. were designated as alleles at the L locus. On the basis of this hypothesis the genotype of the cross Ottawa 770B x J.W.S., the observed and expected ratios for individual and both test races combined are shown in Table 19.

In this cross one homozygote LL (Ottawa 770B) is immune to race 7 and completely susceptible to race 22, the other homozygote L^2L^2 (J.W.S.) is immune to race 22 and completely susceptible to race 7, but the heterozygote LL^2 is immune to both races. That is, the heterozygote neither resembles one of the homozygotes nor behaves like an intermediate between the two homozygotes. The alleles L and L^2, therefore, do not belong to the general class where one allele is dominant over the other or the heterozygotes are intermediate between homozygotes because of the incomplete dominance of either allele. The possibility that the genes L and L^2 are co-dominant alleles can be evaluated on the basis of the segregation ratio of the cross Ottawa 770B x J.W.S., to test races 7 and 22.

Ottawa 770B is resistant and J.W.S. susceptible to race 7. The F_2 ratio showed a good fit to a 3:1 ratio indicating that Ottawa 770B possesses the dominant allele for resistance and J.W.S. the recessive allele for susceptibility to race 7. This recessive allele in J.W.S. cannot be the L^2 gene because L^2 is a dominant gene. Let us represent the gene for susceptibility in J.W.S. by l as the recessive allele for the dominant gene L in Ottawa 770B for resistance. The genotype of J.W.S. thus becomes ll. According to Flor's designation the variety J.W.S. possesses the L^2L^2 pair of alleles. According to Flor's data on reaction to race 7 (Table 19) it must also possess the ll pair of

TABLE 19. REACTIONS OF F_2 PROGENY OF CROSS OTTAWA 770B X J.W.S. TO TEST RACES 7 AND 22. RECALCULATION OF H.H. FLOR'S DATA

	Parental Reactions		F_2 progeny reactions				X^2	P
	Ottawa 770B	J.W.S.						
	LL	L^2L^2	$2LL^2$	1LL	$1L^2L^2$			
Race 7	I	S	I	I	S	S		
Race 22	S	I	I	S	I	S		
Observed			95	45	48	0		
Expected F, 2:1:1:0 [1]			94	47	47	0	0.117	0.90 - 0.95
	Ottawa 770B	J.W.S.						
Race 7	I	S	I	S				
Observed			140	48				
Expected 3:1			141	47			0.028	0.80 - 0.90
	J.W.S.	Ottawa 770B						
Race 22	I	S						
Observed			143	45				
Expected 3:1			141	47			0.113	0.70 - 0.80

1/ The ratio marked "F" was calculated by Flor. The other two ratios were recalculated from data in Flor's Table 3 (Flor, 1947).

alleles which is not possible as the variety is homozygous and the crop is diploid. It follows that J.W.S. cannot possess the L^2 gene as an allele to L gene in Ottawa 770B.

Segregation of the F_2 of the cross Ottawa 770B x J.W.S. for reaction to race 22 is shown in Table 19. If the dominant gene for resistance in J.W.S. is represented by L^2 then by definition Ottawa 770B must possess the recessive allele l^2l^2 and not LL or ll.

Flor used two or more test races to test an F_2 family and on the basis of fitting the observed segregation a 2:1:1:0 ratio he designated the genes for resistance in the parent varieties as alleles. Genes in different varieties can be designated as alleles only on the basis of reaction to one race and not to two or more races. By definition allelic genes can affect only one character causing different degrees of expression (Hutt, 1964). Flor (1965, p.416) established his multiple allelic series on the basis of the observed segregation fitting a 2:1:1:0 ratio. According to my interpretation, as with the L and L^2 genes in Ottawa 770B and J.W.S. respectively (Table 19) all are situated at different loci.

Among the crosses where the F_2 data were shown by Flor to be in agreement with a 2:1:1:0 ratio, according to my calculation the observed ratios in some of them (e.g., in crosses Kenya x Ottawa 770B, Victory A x Newland and Williston Brown x Newland) also fit well a 9:3:4 ratio indicating epistasis (Table 18). As will be shown later, this is also the situation with a number of crosses in Kerr's study. In these instances without appropriate genetic tests it would be impossible to say which of the two ratios is the correct one.

In one cross, Tammes Pale Blue x Punjab (Table 18) the observed segregation did not agree at all with a 2:1:1:0 ratio yet Flor designated the Tammes Pale Blue factor as an allele of the Punjab and Bombay factor (see Flor's Table 7, 1947). However, this designation was not retained in his later publications (Flor, 1954, 1955) because apparently a 2:1:10 ratio could never be obtained from crosses between Tammes Pale Blue and Punjab or Bombay.

Kerr's studies on genetics of rust resistance in flax

Kerr (1960), working with Australian flax rust races, published extensive data on inheritance of rust resistance in flax varieties. An examination of his data reveals that like Flor, he apparently overlooked the possibility that rust resistance in some flax varieties may be governed by epistatic genes.

In the cross Argentine 462 (260) x Ottawa 770B (F_2 progeny) Kerr was unable to find an expected ratio for the two test races (races 2 and 7) combined and he drew no conclusion. My calculation of Kerr's data suggests that resistance in this cross may be governed by three dominant genes, with two genes for resistance epistatic to a third gene for susceptibility (Table 20).

In this cross Kerr analyzed the observed ratios for races 2 and 7 for a 15:1 and a 3:1 ratio respectively. The X^2 value for a 15:1 ratio for race 2 was nearly significant with a population of 305 and that for a 3:1 ratio for race 7 highly significant with a population of 230. According to my calculation, the observed ratio for race 7 shows a very good fit to a 13:3 ratio and that for race 2 a perfect fit to a 61:3 ratio. With this assumption, the expected ratio for both races combined should be 52:9:3 (see Fig. 10) which is in agreement with the data (Table 20).

TABLE 20. REACTIONS OF F_2 PROGENIES OF DIFFERENTIAL VARIETIES OF FLAX TO TEST RACES OF RUST. RECALCULATION OF H.B. KERR'S DATA FROM SEVERAL SELECTED CROSSES

	Parental Reactions		F_2 progeny reactions			X^2	P
Argentine 462 (260) x Ottawa 770B, F_2 (Kerr, 1960, p.289)							
	Ar.	Ot.					
Race 6	I	I	I	I	I		
Race 2	I	I	I	I	S		
Race 7	I	S	I	S	S		
Observed			192	28	10		
Expected K, No ratio given[1]							
52:9:3			187	32	11	0.725	0.50 - 0.70
Race 2			I	S			
Observed			294	11			
Expected K, 15:1			(286	19)[2]		-[3]	0.05 - 0.10
61:3			294	11		0.000	1.00
Race 7							
Observed			192	38			
Expected K, 3:1			(172.5	57.5)		-	0.01 - 0.001
13:3			187	43		0.715	0.30 - 0.50

TABLE 20 - continued

	Parental Reactions		F_2 progeny reactions				X^2	P
Bison (293) x Punjab and Punjab x Bison (284), F_2 (Kerr, 1960, p.294)								
	Bi.	Pu.						
Race 1	I	S	I	S	I	S		
Race 2	S	I	I	I	S	S		
Observed			101	17	32	5		
Expected K, 9:3:3:1			(87	29	29	10)[2/]	-	0.01 approx.
39:9:13:3			95	22	31	7	2.118	0.50 - 0.70
Race 1			I	S				
Observed			133	22				
Expected K, 3:1			(116.25	38.75)			-	Below 0.05
13:1			126	29			2.079	0.10 - 0.20
Race 2								
Observed			118	37				
Expected K, 3:1			(116.25	38.75)			-	0.70 - 0.80

TABLE 20 - continued

Parental Reactions			F_2 progeny reactions			X^2	P
Newland x Punjab and reciprocal, F_2 (Kerr, 1960, p.302)							
	Ne.	Pu.					
Race 1	I	S	I	S	I		
Race 2	I	I	I	I	S		
Observed			256	11	14		
Expected K, 14:1:1			(245	18	18)	-	0.10 - 0.20
58:3:3			255	13	13	0.389	0.50 - 0.70
57:3:4			250	13	18	1.341	0.50 - 0.70
Race 1			I	S			
Observed			270	11			
Expected K, 15:1			(263	18)		-	0.10 - 0.20
61:3			268	13		0.323	0.50 - 0.70
Race 2							
Observed			267	14			
Expected K, 15:1			(263	18)		-	0.30 - 0.50
61:3			268	13		0.081	0.70 - 0.80

TABLE 20 - continued

	Parental Reactions		F_2 progeny reactions			X^2	P
Abyssinian x Ottawa 770B and reciprocal, F2 (Kerr, 1960, p.282)							
	Ab.	Ot.					
Race 1	I	I	I	I	I		
Race 2	I	I	I	I	S		
Race 6	S	I	I	S	I		
Observed			163	10	9		
Expected K,14:1:1			(160	11	11)	-	0.50 - 0.70
57:4:3			162	11	9	0.097	0.95 - 0.98
Race 2			I	S			
Observed			173	9			
Expected K, 15:1			(171	11)		-	0.30 - 0.50
61:3			173	9		0.000	1.00
Race 6							
Observed			172	10			
Expected K, 15:1			(171	11)		-	0.50 - 0.70

TABLE 20 - continued

	Parental Reactions		F_2 progeny reactions			X^2	P
Abyssinian x Punjab, F_2 (Kerr, 1960, p.282)							
	Ab.	Pu.					
Race 1	I	S	I	S	I		
Race 6	S	I	I	I	S		
Race 2	I	I	I	I	I		
Observed			38	12	14		
Expected K, 2:1:1			(32	16	16)	-	0.30 - 0.50
9:3:4			36	12	16	0.361	0.80 - 0.90
Race 1			I	S			
Observed			52	12			
Expected K, 3:1			(48	16)		-	0.20 - 0.30
13:3			52	12		0.000	1.00
Race 2							
Observed			50	14			
Expected K, 3: 1			(48	16)		-	0.50 - 0.70

1/ Ratios marked "K" were calculated by Kerr. All other ratios were recalculated from data in Kerr's tables.

2/ Expected numbers were calculated personally, not given by Kerr.

3/ X^2 values not given by Kerr.

In the cross Bison (293) x Punjab and Punjab x Bison (248) (F_2 progeny) Kerr (1960) analyzing the observed ratio for two test races 1 and 2 combined for fit to 9:3:3:1 obtained a significant X^2 value (Table 20). He did not make any comment on this cross but from the results of a few other crosses involving Bison with other varieties he concluded that resistance in Bison to race 1 is controlled by a gene allelic to one of the Ottawa 770B genes. In the cross Bison (293) x Punjab and Punjab x Bison, according to Kerr's hypothesis the expected ratios for each of races 1 and 2 should be 3:1. In my analysis the observed ratio for race 1 fails to fit a 3:1 ratio (X^2, 9.653; $P < 0.01$) but fits a 13:3 with a population of 155. The observed ratio for race 2 fits a 3:1 ratio. Assuming a 13:3 ratio for race 1 and a 3:1 ratio for race 2 the expected ratio for both test races combined should be 39:9:13:3 which is in agreement with the data (Table 20).

Kerr, following Flor, postulated a 2:1:1 or a 14:1:1 ratio for the observed segregations of a number of crosses, (A 14:1:1 ratio, according to Flor and Kerr, is produced by the segregation of three dominant genes of which two are allelic and one non-allelic). A re-analysis of Kerr's data show that almost all the observed 2:1:1 and 14:1:1 ratios are also in good agreement with some other ratios which are produced by epistatic genes (Table 20).

DISCUSSION

The review of Flor's data presented suggests that the inheritance of rust resistance in his flax differentials may be more complex than reported (Flor 1947, 1951). According to my analysis, the data suggest that rust resistance in some varieties to some races may be governed by complementary genes and in some by epistatic genes. These possibilities were apparently overlooked by Flor.

In some crosses between flax varieties Flor was unable to find expected ratios for all test races combined although according to my calculations the segregation ratios in these crosses are in good agreement with some ratios which might be expected for complementary or epistatic genes. In some other crosses, although Flor's postulated ratios agreed with the observed segregation ratios for all test races combined, according to my calculation the observed ratios for individual test races do not agree with what should be the expected ratios for individual test races according to Flor's hypothesis. An explanation may be that Flor apparently determined the expected ratio for a cross for reaction to all test races combined, empirically, without taking into consideration the expected ratios for individual test races. Perhaps for this reason Flor did not point out that his data indicated the possibility of rust resistance in some flax varieties being governed by complementary genes. Flor was unable to find an expected ratio for the observed segregation in the rust cross race 22 x race 24 on the varieties Williston Golden and Williston Brown. He apparently did not consider that resistance in some host varieties and pathogenicity of rust races on some host varieties may be governed by epistatic genes.

Ratios indicating epistasis can be easily detected by F_3 progeny tests especially from the progeny of recessive F_2 individuals. Although Flor did not present F_3 data on inheritance of rust resistance in flax varieties he did say that his hypotheses were supported by the F_3 progeny tests (Flor, 1951). Either he may not have tested enough F_3 progeny to detect recessive genes for resistance or, if he found resistant plants in the progeny of susceptible F_2 individuals, he may have ignored them, considering that they were the result of outcrossing.

According to my analysis, Flor's and Kerr's data suggest that rust resistance in some flax varieties and pathogenicity in some flax rust races can be governed by epistatic or complementary genes. This may not necessarily be correct. The deviation of observed from the expected ratios in these cases might be due to chance alone, without invalidating their hypotheses. The point is that in genetic experiments it is necessary to differentiate between limiting ratios and neither Flor nor Kerr mentioned this.

Mayo (1956) and Person (1959) examined Flor's data on host and pathogen genetics but did not mention the points which I have emphasized in this review. Person (1959) analyzing the gene-for-gene relationship in the flax and flax rust system by a theoretical model found that most of Flor's "single gene" differential varieties possess two or more genes for resistance and that the resistance genes in these varieties need not fall into allelic or closely linked groups. According to my analysis, Flor's data suggest more or less the same thing except that the genes designated by Flor as multiple alleles could be either closely linked in repulsion or epistatic.

GENETIC STUDIES OF PATHOGENICITY IN OTHER RUST FUNGI

Green

Green (1964) studied the inheritance of colour and pathogenicity in the S_1 uredial progeny of race 15B-1L(Can.) and a hybrid (white x normal) race 11 of Puccinia graminis Pers. A greyish-brown mutant was recessive to normal coloured (Wild) type. With respect to pathogenicity on varieties Arnautka and Mindum virulence was dominant among both the normal and greyish-brown S_1 cultures of race 15B-1L; in the selfed progeny of race 11 virulence was dominant among normal coloured cultures but recessive in greyish-brown ones. Green explained this "apparent reversal of dominance" in race 11 by suggesting linkage between the loci for colour and virulence.

On the varieties Vernal and Lee, the parent race 15B-1L was virulent. All the S_1 progeny with greyish-brown spores were also virulent but most of the normal coloured type were avirulent. The observed ratio of avirulent to virulent cultures among normal coloured progeny approximated 15:1 indicating two recessive genes for virulence. This was, however, unacceptable to Green because normal colour is dominant over greyish-brown and the parent race 15B-1L is virulent on Vernal and Lee. Green explained this result as with the result in Arnautka and Mindum by linkage between virulence and colour loci.

If we ignore the uredial colour and simply study the inheritance of pathogenicity of race 15B-1L on varieties Vernal and Lee we should get a reasonable ratio. Unless virulence is governed by three or more complementary genes, we would expect more virulent cultures than avirulent ones.

In my opinion, Green's results were affected by sampling errors. Green selfed the pycnial population by applying pooled (mixed) nectar. As will be discussed later, with this method of selfing errors can occur at three points, namely, (1) the number of pycnia providing the nectar, (2) the number of aecial clusters to be investigated, (3) the number of progeny cultures established from each aecial cluster. Green did not mention whether this factor was taken into consideration.

According to Green race 11 is heterozygous for virulence on Arnautka, and its selfed progeny should segregate in a ratio of 3 virulent to 1 avirulent. Half of the aecial clusters should be segregating and half non-segregating. Green, however, obtained only one segregating out of 23 aecial clusters investigated.

Similarly on Marquis-Sr9a only three out of 23 aecial clusters segregated for pathogenicity. Green felt that apparently the pooled nectar method of selfing was ineffective and commented that " the reasons for the unexpected results were not clear, but apparently there was an obstruction to multiple fertilization, or the samples from each aecial infection (up to 10 cultures) were too small."

The segregation ratio suggested by Green on Arnautka, 3 virulent: 1 avirulent, should be differentiated from the three limiting ratios 13:3, 9:7, and 15:1. To distinguish between single factor and duplicate factor ratios (3:1 and 15:1 in the diploid stage, but 1:1 and 3:1 respectively in the haploid stage), at least 54 pycnia must be used in mixing the nectar[1/] to ensure proportionate representation of the gametes. Green did not specify the number of pycnia from which he obtained nectar. Similarly, to distinguish between 1:1 and 3:1 ratios for segregating and non-segregating aecial clusters, at least 54 clusters

1/ Calculated by using Mather"s formula (Mather, 1957)

should be sampled. As Green sampled only 23 clusters, the segregation ratios he obtained may be attributable to sampling too small a population. Similarly, the populations he sampled were too small to distinguish 3:1 from 9:7 and 13:3 ratios.

Green (1966) conducted selfing studies on four cultures of wheat stem rust race 11 and one culture of race 10 and determined the inheritance of pathogenicity on the differentials Arnautka, Mindum, Marquis-Sr9a and Marquis-Sr11. The data were presented to show a gene-for-gene relationship between wheat and wheat stem rust.

An examination of Green's data reveals, however, that the total number of S_1 progeny tested from each culture varied between 20 and 52 and these S_1 progeny were obtained over 9 years. The S_1 progeny of each culture were obtained in several experiments and the gametic population in each experiment was therefore not large enough to eliminate sampling errors.

In criticizing Green's results the point has been made that without large numbers of progeny reliable data cannot be obtained. This does not mean that his conclusions are wrong, indeed a gene-for-gene system seems to provide one of the more plausible explanations of the genetic control of pathogenicity and resistance.

Green (1965a, 1965b) studied the inheritance of pathogenicity in several races of oat stem rust on variety Sevnothree. In crosses between certain races the F_1 hybrids resembled the maternal parent except in crosses involving races 6A and 13A where the F_1 progeny segregated. Segregation was apparent in reciprocal crosses between races 6A and 13A and also when either was used as the maternal parent.

When 6A and 13A were the paternal parents, the pathogenicity of the F_1 hybrids invariably resembled the maternal parent regardless with which other race they were crossed. From this Green (1965a, 1965b) suggested cytoplasmic inheritance of pathogenicity except in races 6A and 13A where the pathogenicity was considered to be governed by the interaction of chromosomal and non-chromosomal factors.

Selfing of race 6A produced segregating progeny. The observed S_1 ratio of this race was explained by postulating a theoretical ratio of 10 virulent : 6 avirulent, which was interpreted as meaning that pathogenicity of race 6A is controlled by two genes and maternal cytoplasm. Green postulated a dominant gene (V_E) for virulence and a dominant gene (D) acting with the maternal cytoplasm to make the recessive gene (V_E) for avirulence dominant when both D and V_E occur in the monokaryotic infection.

Green demonstrated his hypothesis of a 10 virulent : 6 avirulent ratio by a checkerboard (Fig. 12). In Fig. 12 sixteen theoretical crosses were proposed by reciprocal transference of spermatia between pycnia in randomly selected pairs. A ratio of 8 pairs of virulent cultures from both sides : 4 pairs of avirulent from both sides : 4 pairs producing virulent from one side and avirulent from the other side would be expected. That is, a 2:1:1 ratio.

In support of this hypothesis Green presented the following data:

(1) In one experiment 37 S_1 progeny of which 24 were virulent and 13 avirulent were obtained by transferring nectar from one pycnium to another. According to Green this observed ratio approximated a 10:6 expected ratio.

	$V_E D$	$V_E d$	$v_E D$	$v_E d$
$V_E D$	1 $V_E V_E DD$	2 $V_E V_E Dd$	3 $V_E v_E DD$	4 $V_E v_E Dd$
$V_E d$	5 $V_E V_E Dd$	6 $V_E V_E dd$	7 $V_E v_E Dd$	8 $V_E v_E dd$
$v_E D$	9 $V_E v_E DD$	10 $V_E v_E Dd$	11 $v_E v_E DD$	12 $v_E v_E Dd$
$v_E d$	13 $V_E v_E Dd$	14 $V_E v_E dd$	15 $V_E v_E Dd$	16 $v_E v_E dd$

Fig. 12. Diagram from Green (1965b, Fig. 1) illustrating the derivation of a 10 virulent : 6 avirulent ratio. V_E is a dominant gene for virulence; D is a dominant gene which, acting with the maternal cytoplasm, makes the recessive gene v_E (for avirulence) dominant when both D and v_E occur in the same monokaryotic infection. Reciprocal S_1 progeny in cell Nos. 1, 2, 4, 5, 6, 8, 13, and 14 are virulent from both sides, those in cell Nos. 11, 12, 15, and 16 are avirulent from both sides, and those in cell Nos. 3, 7, 9, and 10 are virulent from one side and avirulent from the other.

(2) In another experiment reciprocal progeny were obtained from 15 crosses of which 6 pairs produced virulent cultures from both sides, 3 pairs avirulent cultures from both sides and 6 pairs virulent from one side and avirulent from the other side. The observed ratio was shown to agree with a 2:1:1 expected ratio.

(3) In the third experiment pycnia were selfed by mass nectar. After the development of aecia the cultures were established from 18 aecial clusters of which 9 produced only virulent cultures, 5 only avirulent cultures and 4 both virulent and avirulent cultures. The observed ratio also was shown to fit a 2:1:1 ratio.

(4) In the fourth experiment a second culture of race 6A was selfed by transferring nectar from one pycnium to another. A total of 32 cultures were obtained of which 23 were avirulent and 10 avirulent, approximating a 2: 1:1 ratio.

Green explained the observed segregation by a Mendelian ratio. The fact that the reciprocal F_1 hybrids involving race 6A with other races, and the reciprocal S_1 progeny of race 6A differed in pathogenicity is suggestive of non-Mendelian inheritance. However, Green's data may not be sufficient to determine the kind of inheritance involved. There are well-documented cases where one side of the crosses shows segregation, Mendelian or non-Mendelian and the other side no segregation (e.g. the male sterility in flax or the CO_2 sensitivity character in a strain of Drosophila melanogaster respectively) or one side shows Mendelian segregation and the other side non-Mendelian segregation (e.g. the ijap character in Zea mays) (Caspari, 1948; Jinks, 1964; Whitehouse, 1965). The lines of investigation to be followed in the situation encountered by

Green have been prescribed or reviewed by various workers such as Caspari (1948), Bhan (1964), Jinks (1964), Srb et al. (1965) and Whitehouse (1965).

Green's genetic model explaining a 10 virulent : 6 avirulent expected ratio, in my opinion, is not genetically sound. On the basis of this hypothesis the character concerned should be controlled not by the genotype of the fertilization product but by the genotype of the female gamete and the maternal cytoplasm. In Fig. 12 although the cultures in cell Nos. 4, 7, 10 and 13 would possess the same genotype, those in cell Nos. 4 and 13 should be virulent from both sides and those in cell Nos. 7 and 10 virulent from one side and avirulent from the other. Of the two dominant genes, V_E and D, the gene V_E exercises its control when present in either the male or female gamete, acting as a simple Mendelian dominant, while D interacts with the maternal cytoplasm to render the recessive gene v_E for avirulence dominant over its normal dominant allele (V_E) for virulence. In other words virulence is normally dominant over avirulence, but when gene D is present in the maternal gamete avirulence is dominant. It is of interest that Green cited only literature dealing with inheritance of resistance and of pathogenicity. He gave no indication that he had considered other explanations for his observations based on concepts in other fields of genetics.

Johnson

Johnson's (1949) studies of pathogenicity in crosses between oat stem rust races 7 and 11 on oat varieties White Tartar and Richland led him to conclude that avirulence in each variety was governed by a

different pair of complementary genes. According to Green (1965b) however, pathogenicity on these varieties is controlled by single genes. Green also cited the work of some other workers which suggested that rust resistance in these varieties is governed by single genes.

If resistance is governed by a single gene then on the basis of a gene-for-gene relationship pathogenicity also should be governed by a single gene. Johnson tested 85 F_2 cultures from one F_1 of which 34 were virulent on White Tartar and 32 on Richland. To differentiate between a 3:1 and a 9:7 ratio 95 individuals are required, of which 32 or fewer (r[1/] = 31.8) recessive will decide in favour of a 3:1 ratio while 33 or more will indicate a 9:7 ratio (Bailey, 1961). In Johnson's experiment there were enough recessive individuals (34 recessives) on White Tartar to conclude that pathogenicity on this variety is governed by a pair of complementary genes. On Richland, with a population of 85 the observed ratio showed a poor fit (X^2 = 7.25) to a 3:1 ratio but good fit (X^2 = 1.29) to a 9:7 ratio. It is thus reasonable to conclude that pathogenicity on Richland is also governed by a pair of complementary genes.

If resistance is determined by a single gene (host genetics were not under consideration in Johnson's investigations) then assuming the existence of a gene-for-gene system monogenic control of pathogenicity as suggested by Green seems to be likely. If this is so then Johnson's ratios must be considered erroneous and attributed to sampling error.

Johnson selfed the F_1 cultures by applying mass nectar but he did not mention whether he had taken into consideration the minimum progeny size required at the three critical stages mentioned above. The progeny

1/ r is the number of recessives occurring in a family of size n which will leave both 3:1 and 9:7 hypotheses equally likely (Mather, 1957).

ratios obtained by Johnson may have been spurious and caused by inadequate sample size.

The possibility that Johnson's results were affected by sampling error is strengthened by his data on inheritance of urediospore colour which were obtained from the same population as those on the inheritance of pathogenicity on White Tartar and Richland. Johnson obtained two sets of F_2 data and one set of F_3 data on urediospore colour. The first comprised 85 F_2 cultures which were the same as those studied for inheritance of pathogenicity. The second included 270 F_2 uredial cultures which originated from the counts of red and orange uredia on oat seedlings inoculated by mass collections of the F_2 aeciospores. In both sets of data, although the population sizes were adequate to test for a 3:1 ratio, the observed ratios showed poor fits to a 3:1 ratio (see Table 2 of Johnson, 1949). The third set of data comprising 68 aecia was obtained from selfing one F_2 culture and it showed good agreement with a 3:1 ratio.

Loegering and Powers, and Williams et al.

Loegering and Powers (1962) studied the inheritance of pathogenicity in a cross between wheat stem rust races 111 (culture 111-55A) and 36 (culture 36-55A) on several differentials. This discussion will be confined to the inheritance of pathogenicity on two varieties Marquis and Reliance. Race 111 is avirulent on these varieties and race 36 virulent. Results from tests of 108 F_2 cultures led Loegering and Powers to conclude the avirulence of race 111 on varieties Marquis and Reliance to be controlled by two (12:3:1 ratio) and three (48:12:3:1 ratio) dominant independent genes respectively.

Loegering and Powers made crosses between races 111 and 36 but did not mention the homozygosity of the parent races. In a previous study Wilcoxson and Paharia (1958) showed a culture of race 111 to be heterozygous for pathogenicity on the same test varieties. Loegering and Powers' culture could be different and homozygous but such an assumption would not be legitimate unless it was shown experimentally. This being a cross-fertilizing species there is every possibility that a culture, especially an avirulent one, would be heterozygous.

According to Loegering and Powers the 108 F_2 cultures were obtained "over a period of several months", i.e. in several experiments. The gametic population in each experiment was therefore inadequate to avoid sampling errors. As stated before in reference to Green's (1966) study, although Loegering and Powers' conclusion may be right, theoretically it would be inappropriate to draw any conclusion based on tests with small samples.

Furthermore, a progeny size of 108 F_2 cultures is not enough to test a three-factor hypothesis because it requires 235 F_2 to differentiate between a two- and a three-factor hypothesis.[1/] Loegering and Powers analyzed the data for a three-factor hypothesis by using the X^2 test for goodness-of-fit. The use of this test here is questionable because the theoretical frequency in the smaller class was less than 5 (Smith, 1954, p.624; Srb et al., 1965, p.57).

Williams, Gough, and Rondon (1966) reinvestigated the inheritance of pathogenicity in the cross race 111 (111-55A) x race 36 (36-55A) using the same F_2 cultures as studied by Loegering and Powers (1962).

1/ Calculated by using the formula given by Mather (1957)

Their study was initiated because of an apparent disagreement between the results reported by Loegering and Powers on inheritance of avirulence of culture 111-55A on Marquis and Reliance and those reported by Rondon et al. and Berg et al. (see Williams et al., 1966) on inheritance of resistance in Marquis and Reliance to culture 111-SS2 of the same race. Loegering and Powers (1962), on the basis of the inheritance of pathogenicity in culture 111-55A and assuming a gene-for-gene relationship in wheat and wheat stem rust, postulated that resistance in Marquis and Reliance should be governed by two and three dominant genes respectively. However, in later studies Rondon et al. and Berg et al. (see Williams et al., 1966) found that Marquis and Reliance both possess three dominant genes for resistance to culture 111-SS2.

Williams et al. (1966) tested 103 of the 108 F_2 cultures studied by Loegering and Powers (1962). These cultures were tested on Marquis and Reliance and on six monogenic lines. Three of the six monogenic lines, Mq-A, Mq-B, and Mq-C, each carrying a different gene for resistance to culture 111-SS2, were derived from Marquis and three, RI-A, RI-B, and RI-C, each carrying a different gene for resistance to the same culture, from Reliance. From this study Williams et al. reported that their results were in agreement with the findings of Loegering and Powers. They also stated that the disagreement between the results of Loegering and Powers on the inheritance of avirulence in culture 111-55A on Marquis and those of Rondon et al. and Berg et al. on the inheritance of resistance in Marquis to culture 111-SS2 could be explained by the fact that, of the three genes conditioning resistance in Marquis to culture 111-SS2, one was not effective against culture 111-55A.

Williams et al. reported that with respect to culture 111-55A the number of dominant genes for avirulence on Marquis and Reliance did not correspond with that on the monogenic lines, supposedly carrying the same genes for resistance as in Marquis and Reliance. On Marquis and Reliance the avirulence of culture 111-55A is controlled by two and three dominant genes respectively but on the monogenic lines Mq-C and RI-C the avirulence was conditioned by a single recessive gene and the locus involved was designated Arl-3. That is, the gene for avirulence at the Arl-3 locus behaved like a dominant gene in tests with Marquis and Reliance but recessive in tests with the monogenic lines Mq-C and RI-C. Williams et al. explained these results by saying that since both Marquis and Reliance possess additional genes for resistance, the genotype of the host appeared to affect the dominance at the Arl-3 locus.

In my opinion, since avirulence on Mq-C and RI-C was conditioned by a recessive gene, a recessive gene should also be present in the genotype of Marquis and Reliance. The discrepancy of these results may be explained by the fact that intermediate infection types in some F_2 cultures were apparently misclassified by both Loegering and Powers, and Williams et al. This is evident from a comparison of the pathogenicity data of Loegering and Powers and Williams et al. For example, on Marquis, with a population of 108 F_2 cultures Loegering and Powers found 7 virulent cultures whereas with 103 of the same 108 cultures Williams et al. reported 11 virulent cultures. On Reliance Loegering and Powers found two virulent cultures whereas Williams et al. found one virulent culture. This misclassification may invalidate the ratios 12:3:1 and 48:12:3:1 postulated on Marquis and Reliance respectively by Loegering

and Powers and Williams et al. If the cultures had been correctly classified the ratios on Marquis and Reliance might have been 13:3 and 61:3 respectively indicating avirulence on Marquis and Reliance to be conditioned by two avirulence genes, one dominant and one recessive, and by three avirulence genes, two dominant and one recessive respectively.

Since avirulence on the monogenic lines Mq-C and RI-C was recessive, assuming a complementary interaction of host and pathogen genes the resistance in the monogenic lines should be controlled by recessive genes for resistance. If this is so, the discrepancy in the results could be explained by assuming that either the recessive genes for resistance in Marquis and Reliance were not effective against culture 111-SS2, or that the studies of Rondon et al. and Berg et al. failed to detect the presence of recessive genes for resistance.

Luig and Watson

Luig and Watson (1961) conducted investigations on the inheritance of pathogenicity by selfing strain 21 Anz. 2 of Puccinia graminis var. tritici. A total of 285 S_1 cultures were tested on 23 differentials. All but one culture were virulent on three varieties and all were avirulent on two. On the remaining varieties the progeny segregated. From this study Luig and Watson concluded that on some differentials virulence seemed to be inherited in a Mendelian fashion but on others the mode of inheritance was difficult to explain. They suggested that their results indicated a gene-for-gene relationship on some varieties but not on others.

Luig and Watson grouped the 285 S_1 cultures into 37 strains based on pathogenicity on 23 differentials and used these 37 strains as 37 S_1

progeny to calculate genetic ratios. They did not justify this method of analysis, which may be invalid. In genetic analysis one must use actual numbers of progeny and not the number of strains. The number of progeny obtained in any experiment is a definite figure, not subject to manipulation. The number of strains can be increased or decreased by merely adding or subtracting some varieties from a set of differentials. Their whole population of 285 cultures might just as easily have been grouped into two strains if they had used one differential, four strains on two differentials, etc., numbers obviously inadequate for working out ratios.

It is possible that had they used the actual number of S_1 progeny they might have obtained different ratios. They selfed the pycnial population by applying mass nectar but did not mention the sample sizes they used at the three critical stages of this method of selfing. Their results therefore could also have been affected by sampling errors.

Samborski

Samborski (1963) investigated the inheritance of pathogenicity in a culture of Puccinia recondita f. sp. tritici on variety Transfer. This culture, 46-60, originated from a single uredial pustule classed as infection type 1^+ on Transfer. The pycnia were selfed by applying mass nectar. Samborski's results obtained from tests of the S_1 cultures are shown below:

Culture	No. of cultures	Infection types
Parent, 46-60		1^+
S_1 progeny	25	0;
"	51	1^- to 1^+
"	25	4

The parent and two of the S_1 cultures, one avirulent (infection type 0; on Transfer) and one virulent (infection type 4 on Transfer) were tested further on Aegilops umbellulata, the source of resistance in Transfer, and other varieties carrying the resistance of Transfer. From these studies Samborski concluded that the parent culture was heterozygous for pathogenicity with virulence incompletely dominant, and that it originated by spontaneous mutation. He designated the genotypes of the parent and the two S_1 cultures tested on various lines carrying the resistance of Transfer as follows:

Parent, 46-60	Pp
Virulent, 46-60-20	PP
Avirulent, 46-60-2	pp

Samborski's interpretation seems to be contrary to his results and to the concept of a spontaneous mutation. According to the system of classifying host-parasite interactions in rust fungi established by Stakman et al. (1944, 1962) for wheat stem rust and Johnston and Levine (1955) for wheat leaf rust the 102 S_1 cultures on Transfer should have been classified as follows: 76 cultures avirulent (25 cultures infection type 0; and 51 infection type 1^- to 1^+) and 25 cultures virulent (infection type 4). This would approximate a ratio of 3 avirulent to 1 virulent indicating avirulence to be controlled by a single dominant gene. On this basis the genotypes of virulent, 46-60-20, and avirulent, 46-60-2, would be the opposite to that assigned by Samborski, i.e. the virulent, 46-60-20, and the avirulent, 46-60-2 cultures should be labelled as pp and PP respectively. This interpretation would then also agree with the concept that spontaneous

mutations, as a rule, are recessive (Müntzing, 1961).

Flangas and Dickson

Flangas and Dickson (1961a, 1961b) conducted a genetic analysis of pathogenicity and antigen specificity of four heterozygous uredial clones of Puccinia sorghi on six maize lines. This discussion is concerned only with the inheritance of pathogenicity in P. sorghi. Flangas and Dickson tested a total of 285 subcultures derived from three generations of inbreeding of four original clones and 92 hybrids derived from crosses between lines of selected pathogenic types of sibling and non-sibling pedigree. From this study they concluded that both the selfed and the hybrid progeny showed a range of pathogenicity indicating an indeterminate nature of inheritance with a continuous potential for variation and commented as follows (Flangas and Dickson, 1961b, p.254):

"The data on inheritance of pathogenicity . . . in the dicaryon of P. sorghi indicate a complex genic mechanism not resoluble by the application of simple Mendelian concepts alone. . . . The use of the genetic linkage map of the pathogenic genotype is impractical in these dicaryotic, obligate parasites; therefore, immunochemical and biochemical methods must be developed to explore the magnitude, structure, and function of the pathogenic locus in P. sorghi and the Rp locus in the host."

According to Flangas and Dickson, their results cannot be interpreted in simple Mendelian terms and they looked for an explanation from the work on biochemical mutants where a complex genetic mechanism has been postulated. That concept, as far as the genetic analysis is concerned, is based on the size of a segregating population that can be analyzed for detection of recombinants. With certain microorganisms selective techniques are available which permit the screening of many millions of offspring with ease (Sager and Ryan, 1961). According to

Pontecorvo and Roper (1956) with some of these techniques it is feasible to analyze the number of products of meiosis in the order of 10^8 in one experiment. Similar studies are not practicable with higher organisms because of the difficulty of handling the population size required (Nelson, 1959). Flangas and Dickson's data show that the average number of progeny tested per mother culture in each generation of selfing varied from 5 to 49 in the first, 5 to 16 in the second and 8 to 9 in the third (see column C's of Table 21). Such population sizes are not sufficient to test even a single-factor hypothesis, let alone a more complex genetic system.

Flangas and Dickson's results seem to be explainable by a simple and well-documented genetic concept which they appear to have overlooked. From the description of their results (see Flangas and Dickson, 1961a) the gene controlling pathogenicity in the clones of P. sorghi seems to show incomplete penetrance. The nature of gene penetrance and expressivity has been described by Srb and Owen (1955, p.303) as follows:

> "A characteristic that seems to depend fairly regularly on a simple dominant gene, so that it is transmitted from one individual to another, generation after generation, may occasionally "skip" a generation, and be transmitted by an individual who carries the gene but does not evidence it phenotypically. Or, phenotypic expression may be so variable, from one individual to another, as not to look like a single characteristic at all. . . . Probably variations in penetrance and expressivity result from modifying genes, varying from one group and from one individual to another, and from fluctuations in the external and internal environment. It is usually difficult to identify these sources of variation concretely in any particular instance."

The description given by Flangas and Dickson (1961a) of the changes in pathogenicity of the selfed and hybrid progeny of P. sorghi seems to agree with the description of Srb and Owen of a character controlled by a gene which has reduced penetrance.

TABLE 21. NUMBER OF SELFED CULTURES TESTED IN THREE SUCCESSIVE GENERATIONS

Original culture	First generation			Second generation			Third generation		
	A	B	C	A	B	C	A	B	C
1-20 1/		49	49	5	43	9	4	35	9
2-22 2/		27	27	1	11	11	4	31	8
3-32 3/		15	15	3	48	16	-	-	-
4-1 4/		5	5	1	5	5	2	16	8

1/, 2/, 3/, 4/, Condensed from tables 3, 4, 5, 6 respectively, Flangas and Dickson, 1961a.

A, number of mother cultures selfed.

B, total number of selfed progeny tested in a generation.

C, average number of selfed progeny tested in a generation from each mother culture. The numbers were obtained by dividing the numbers shown in Column B by the corresponding ones shown in Column A.

PART IV.

METHODS OF SELFING F_1 CULTURES AND INTERPRETING GENETIC DATA IN RUST FUNGI

INTRODUCTION

Progress in genetic studies of pathogenicity in rust fungi is hampered by two main problems. The first is the uncertain and erratic germination of teliospores, so that the sexual stage needed for selfing and crossing often cannot be obtained. The second main problem concerns differences in procedures of selfing employed by various workers.

Essentially, two methods of selfing have been employed which have already been mentioned under methods and materials in Part I (Rust genetics). One may be termed the reciprocal or pairing method: Spermatia from one pycnium are transferred to another. Union of male and female gametes will take place provided they are of opposite mating type. This method was used by Flor (1942, 1946, 1955, 1956) and subsequently by several other workers.

The second, or pooled nectar method, involves bulking spermatia from several pycnia and applying the aggregate to the pycnia from which it was collected. The progeny cultures are established either from randomly selected individual aecial cups or randomly selected individual uredial pustules arising from inoculation with the mass aeciospores of a cluster. In the latter it is assumed that a single uredium arises from the increase of a single aeciospore. This method was followed by Johnson and his associates (Newton and Johnson, 1932;

Johnson, 1954), and several other workers, including Vakili (1958), Luig and Watson (1961), Zimmer (1961), Samborski (1963) and Green (1964, 1965b, 1966). Vakili employed only one progeny culture per aecial cluster represented by a single uredial pustule. Samborski obtained five single pustule uredial cultures per aecial cluster. Green (1964) established "up to 10" progeny cultures per aecial cluster. From this it appears that there is no uniformity in the number of cultures taken from each cluster.

It is reasonable to expect that differences in procedures of selfing and crossing, and the effects of sampling error introduced by the use of small samples will affect the results and may give rise to misleading conclusions. This has been emphasized by Dr. Clayton Person (Personal Communication). The ~~elative~~ relative effectiveness of the pairing and pooled nectar methods may be examined for points at which sampling error may occur.

The problem will be discussed and illustrated by some simple genetical situations, assuming that no linkage exists between mating type and the genes for pathogenicity.

Reciprocal or pairing method

The reciprocal or pairing method of selfing is comparable to that used in higher plants except that the union of the gametes (pycnia) is arranged through experimental manipulation. It is therefore necessary to include a population large enough to ensure a representative sample of gametes and to produce an adequate number of F_2 cultures to permit differentiation between critical ratios. The progeny size can be considered only in terms of the number of successful pairings. Because

there are two mating types, theoretically 50% of the matings will be unsuccessful, and some may fail due to various other reasons, such as faulty manipulation techniques. If the number of gametes is large enough to ignore the effects of sampling error, then the formulae and the procedures for calculating various family sizes given by Mather (1957) for two-class segregation ratios (see also Hanson, 1959 and Bailey, 1961) and by Seyffert (1962) for two or more than two-class segregation ratios may be used.

The question of gametic numbers and sampling error cannot be directly answered. In a given genetical situation, over a range of gamete numbers, the probability distributions for the progeny genotypes could be calculated. Comparisons among distributions might then indicate the point at which sampling error, introduced by the finite number of gametes, could be effectively ignored. At this point normal statistical procedures which assume an infinitely large number of male gametes, could be applied.

Let us assume for the moment that sampling error due to small gamete number may be ignored. Values to distinguish between a few simple two-class segregation ratios, as calculated by Mather's (1957) formula, are given in Table 22. Some values for two-class segregation ratios can be found in Hanson's paper (1959).

The pooled nectar method

Genetic ratios can be calculated in two ways: (i) By establishing a certain number of progeny cultures from each of a number of aecial clusters and adding them together. This would give an F_2 ratio. (ii) By determining the proportions of segregating and non-segregating

TABLE 22. MINIMUM FAMILY SIZE REQUIRED TO DIFFERENTIATE BETWEEN TWO LIMITING SEGREGATION RATIOS.

Probability	Ratios to be differentiated			
	3:1/13:3	3:1/9:7	3:1/15:1	15:1/63:1
.05	470	67	35	165
.025	667	95	50	235
.02	949	133	71	330
.01	1152	163	86	405

Probability	Ratios to be differentiated		
	1:1/3:1	3:1/7:1	7:1/15:1
.05	38	101	226
.025	54	144	323
.02	76	203	455
.01	106	248	558

aecial clusters and the proportions of segregations within individual clusters. The ratio obtained would be comparable to backcross data in genetic studies with higher plants. The first will be termed "the determination of F_2 ratios from the pooled nectar method" and the second "the determination of 'backcross' ratios from the pooled nectar method."

Determination of F_2 ratios from the pooled nectar method

As mentioned before, the data obtained by this method would be comparable to F_2 data obtained with the reciprocal method. However, unlike the reciprocal method where sampling error can occur only at one point, with the pooled nectar method sampling error can occur at several points, namely, (i) the number of pycnia to be utilized in intermixing the nectar, (ii) if the pooled nectar is not thoroughly intermixed, (iii) the number of aecial clusters to be investigated, (iv) the selection of the basic unit constituting a progeny culture: a single aecial cup (or a single aeciospore per aecial cup); or a randomly selected single uredial pustule (or a single urediospore per uredium) representing a single randomly selected aeciospore (derived from the inoculation of plants with mass aeciospores of a cluster).

For the above reasons, when a genetic hypothesis is to be tested by F_2 data obtained from the pooled nectar method and when the sample sizes per aecial cluster are limited, it may be necessary to obtain several F_2 ratios from the same aecial cluster population (by drawing several samples per aecial cluster and treating each sample as belonging to a separate F_2 family) and to measure the consistency of the F_2 ratios by the heterogenicity X^2. If the heterogenicity X^2 is significant

then the whole experiment and its technique is suspect (Mather, 1957). Alternatively, assuming that the protoaecial nuclei (female gametes) have been fertilized by a large sample of spermatia (male gametes) randomly, a large number of cultures may be taken from each member of an adequate aecial cluster population and they may be added together to determine an F_2 ratio. This may involve handling an impractically large number of cultures.

Determination of "backcross" ratios from the pooled nectar method

The basis of determining a "backcross" ratio from segregation ratios between and within clusters is shown diagramatically in Fig. 13 for four F_2 ratios, 3:1, 13:3, 9:7 and 15:1. The expected ratios of segregating to non-segregating clusters i.e. the expected backcross ratios corresponding to the F_2 ratios 3:1, 13:3, 9:7 and 15:1 are 1:1, 1:1, 3:1 and 1:3. Thus three of the four F_2 ratios, 3:1, 9:7, 15:1, can be easily distinguished. The F_2 ratios 3:1 and 13:3 can be differentiated by the fact that when a 3:1 ratio occurs expected segregation within a cluster would be 1:1. With a 13:3 F_2 ratio, half the segregating clusters would yield a 1:1 ratio and half a 3:1 (haploid) ratio. If one segregating cluster can be shown to yield a 3:1 (haploid) ratio, this immediately separates a 13:3 F_2 ratio from a 3:1 F_2 ratio.

The number of aecial clusters to be investigated may be calculated by using Mather's formula as before, provided that the aecial clusters developed from a representative sample of pycnia and the protoaecial nuclei (female gametes) were fertilized by a large number of spermatia. Values to distinguish between a few simple critical ratios are shown in Table 22.

F_2 ratio	Recipient pycnia		Genotypes of bulked spermatia				Proportion of segregation between aecial clusters	Proportion of segregation within aecial clusters
	Proportion	Genotype	Genotypes of aeciospores					
			A[1]	a				
	1/2	A[1]	AA	Aa			1/2 Non-seg.[5]	Non-seg.
3:1	1/2	a	Aa	aa			1/2 Seg.[5]	1 avi.[6] : 1 vi.[6]
			AB[2]	Ab	aB	ab		
	1/4	AB[2]	AABB	AABb	AaBB	AaBb	1/4 Non-seg.	Non-seg.
	1/4	Ab	AABb	AAbb	AaBb	Aabb	1/4 Non-seg.	Non-seg.
13:3	1/4	aB	AaBB	AaBb	aaBB	aaBb	1/4 Seg.	1 avi. : 1 vi.
	1/4	ab	AaBb	Aabb	aaBb	aabb	1/4 Seg.	3 avi. : 1 vi.
			AB[3]	Ab	aB	ab		
	1/4	AB[3]	AABB	AABb	AaBB	AaBb	1/4 Non-seg.	Non-seg.
	1/4	Ab	AABb	AAbb	AaBb	Aabb	1/4 Seg.	1 avi. : 1 vi.
9:7	1/4	aB	AaBB	AaBb	aaBB	aaBb	1/4 Seg.	1 avi. : 1 vi.
	1/4	ab	AaBb	Aabb	aaBb	aabb	1/4 Seg.	1 avi. : 3 vi.

Fig. 13 - continued on page 161

F_2 ratio	Recipient pycnia		Genotypes of bulked spermatia				Proportion of segregation between aecial clusters	Proportion of segregation within aecial clusters
	Proportion	Genotype	Genotypes of aeciospores					
			AB[4/]	Ab	aB	ab		
15:1	1/4	AB[4/]	AABB	AABb	AaBB	AaBb	1/4 Non-seg.	Non-seg.
	1/4	Ab	AABb	AAbb	AaBb	Aabb	1/4 Non-seg.	Non-seg.
	1/4	aB	AaBB	AaBb	aaBB	aaBb	1/4 Non-seg.	Non-seg.
	1/4	ab	AaBb	Aabb	aaBb	aabb	1/4 Seg.	3 avi. : 1 vi.

1/ A, a dominant gene for avirulence.

2/ A and B, dominant genes for avirulence and virulence respectively; A epistatic to B.

3/ A and B, a pair of complementary genes for avirulence.

4/ A and B, duplicate genes for avirulence.

5/ Non-seg., non-segregating; Seg., segregating.

6/ avi., avirulent; vi., virulent.

Fig. 13. Theoretical expectation of segregation between and within aecial clusters.

To determine the number of progeny cultures to be established per aecial cluster in order to detect segregation or to determine segregation ratios within individual aecial clusters, it is necessary first to consider what should constitute a basic unit of a progeny culture - a randomly selected aecial cup or a randomly selected aeciospore from the aecial cluster. [Various workers used either one or both units (Johnson, 1954; Green, 1964). With the latter method, a randomly selected uredial pustule arising from inoculation with mass aeciospores of a cluster was generally considered a basic unit on the assumption that a single uredium originates from a single aeciospore (Murphy, 1935; Brown and Johnson, 1949; Vakili, 1958; Zimmer, 1961; Zimmer, Schafer, and Patterson, 1965; and elsewhere)]. Each aecial cup contains numerous aeciospores presumably identical and supposedly originating from a common parent (Zimmer, 1961; Zimmer et al., 1965). There is a possibility that sampling error may be greater if randomly selected aeciospores rather than aecial cups are used as the basic unit of a progeny culture. As a refined technique, a progeny culture may be established from a single aeciospore per aecial cup. If a randomly selected aecial cup (or an aeciospore per aecial cup) can be considered as the basic unit, then, assuming the union of male and female gametes to be random, Table 3 of Mather (1957) or Table 1 of Hanson (1959) may be used to determine minimum progeny size required to detect segregation within individual clusters. The sample size required to determine segregation ratios within individual clusters would be the same as that enumerated earlier for the determination of segregation ratios between clusters.

Detection of segregation within individual clusters would involve handling a large number of cultures. However, it may be possible to reduce the work considerably if a somewhat different procedure were adopted. This would include selecting aecial cups and establishing mono-aecial cup cultures from a cluster, and using massed aeciospores of the remaining cups in the cluster to inoculate differential varieties. (If the quantity of mass aeciospores is not sufficient to inoculate all differential varieties probably the first generation uredial culture derived from the inoculation of plants with mass aeciospores could be used). From the pathogenicity of the mass culture it would be possible to determine whether a cluster is segregating or non-segregating by the relative frequency of avirulent and virulent pustules on a resistant host. In this way it may be possible to determine segregation ratios between clusters and permit a distinction between most of the critical ratios, for example, the F_2 ratios 3:1, 15:1, 9:7 (see Fig. 13). Further studies may be carried out with the cultures established from individual aecial cups.

To distinguish between a 3:1 and a 13:3 F_2 ratio we need to determine the proportional segregations within individual clusters (Fig. 13). For this we need to sample 6 to 7 segregating clusters to ensure inclusion of at least one segregating cluster which would yield a 3:1 (haploid) ratio (see Fig. 13 and then Hanson's Table 1, 1959). A rough estimation of segregation ratios within individual clusters may also be obtained simply by counting the proportions of resistant and susceptible infection types on an appropriate resistant host.

DISCUSSION

Two methods of selfing in rust fungi have been described, of which one, i.e., the reciprocal method, would give a straightforward F_2 ratio. The other, i.e. the pooled nectar method, would yield an F_2 or a "backcross" ratio depending on whether a ratio is determined from pooled progeny of an aecial cluster population or from segregation between and within clusters.

If a genetic hypothesis is to be tested simply from an F_2 ratio it may be advisable to employ the reciprocal method of selfing rather than the pooled nectar method. With the reciprocal method sampling error can occur only at one point, i.e., the number of pycnia included in the experiment. A number of pycnia sufficient to permit the establishment of an adequate number of F_2 cultures, would allow the reciprocal method to be safely used. Flor employed this method and his data show that in most cases the observed ratio approximated to a ratio which would be expected on theoretical grounds.

F_2 ratios calculated from the pooled nectar method of selfing may not be reliable because, as enumerated earlier, sampling error can occur at several points. This may be avoided by drawing a large number of cultures from each cluster of an aecial cluster population which may be impracticable from the point of view of time and labour. Johnson, (1949), Luig and Watson (1961) and Green (1964) determined F_2 ratios using the pooled nectar method of selfing. This may be the main reason for their questionable results.

If a genetic hypothesis is to be tested with reasonable accuracy it may be preferable to calculate ratio by the "backcross" method rather

than by the reciprocal method. Because the reciprocal method would give only F_2 data, F_2 individuals are often difficult to classify precisely because of intermediate infection types. (This problem was pointed out earlier when discussing the results of Loegering and Powers (1962) and those of Williams et al. (1966) who tested the pathogenicity of the same F_2 cultures). In such cases it becomes necessary to identify the genotypes of the F_2's by growing F_3 families. Due to the difficulty of inducing telial germination in some rusts, such tests may be difficult or impossible. In these cases the use of the "backcross" method may be extremely useful, because this method not only yields a "backcross" ratio (from the proportion of segregation between aecial clusters) but serves the purpose of a progeny test (from the segregation within individual clusters). It generally requires a large F_2 population to differentiate between some critical ratios, such as a 3:1 and a 13:3 F_2 but this may be done more economically by using the "backcross" method.

If a pycnial population can be fertilized by a sufficient number of spermatia it may be permissible to ignore the effects of sampling error for male gametes, and the "backcross" method would be satisfactory. The over-all number of cultures required for a test may be within the limits of feasibility if the procedure outlined above can be followed.

PART V

DISCUSSION ON THE GENE-FOR-GENE HYPOTHESIS IN HOST-PARASITE SYSTEMS

Since the gene-for-gene hypothesis was proposed by Flor (1955) and elaborated by Person (1959) it has become the subject of a continuing but stimulating debate. It is reasonable to assume, as suggested by Flor (1955), that such obligate parasites as rust, and powdery mildew and the nearly obligate smut fungi have evolved in association with their hosts. The host-parasite system is an integrated unit; the phenotypic expression of both host and parasite results from the same biochemical reaction and they are measured by the same criterion, i.e. by infection type. In this situation it seems that a gene-for-gene system would not only provide one of the more plausible explanations but it should be a necessary condition for the genetic control of resistance and of pathogenicity.

Laubscher (1963) questioned the validity of extending the gene-for-gene hypothesis to wheat and wheat stem rust. His arguments were based on the following points: (1) According to the gene-for-gene theory there should be as many genes for resistance in the wheat plant as there are genes for pathogenicity in the rust fungus. However, the number of identified genes for stem rust resistance is relatively small compared to the number of physiologic races known. (2) Since a resistant plant can be predisposed to become susceptible, this "undermines the theory's basic tenet of a 'complementarity of fit' as an essential to successful parasitism." He suggested that a more

satisfactcry genetic theory would be that resistance genes may be regarded as regulator genes which repress the biosynthesis of some metabilites essential to particular biotypes of the parasite. The induction of susceptibility by high temperature previous to infection may be due to the inactivation of repressor protein by the inductor metabolites which are formed in excess in the host during exposure to high temperatures.

The gene-for-gene hypothesis is based on classical genetic tests and it seems that it should remain so unless it can be shown that such tests are no longer valid. The concept of regulator genes has been developed from work on bacteria. According to Fincham and Day (1965) if it is true for bacteria it may also be of some relevance for other organisms but there is very little evidence as yet for its applicability to fungi.

The fact that resistant plants can be predisposed to become susceptible in the absence of a pathogen does not necessarily contradict the gene-for-gene theory. The phenotype of an organism is the consequence of an interaction between the genotype and environment, both of which are absolutely necessary (Sinnot et al., 1958). For example, in sun red Zea mays, which has the necessary genotype for anthocyanin pigment, the pigment is formed only in those parts of the plants which are exposed to sunlight (Whitehouse, 1965). Furthermore, the phenotype of an organism at a given moment is not only determined by the environment that prevails at that particular moment but also by the whole succession of environments which the organism has experienced during its lifetime (Sinnot et al., 1958).

The genetic data presented on sunflower and sunflower rust are insufficient to make any comment on the existance of a gene-for-gene relationship in this host-parasite system. However, in some instances, the results have been explained by invoking this relationship assuming that if it is true with other host-parasite systems it should be true with this system also. On Morden 29 the F_1 hybrids of most reciprocal crosses resembled the maternal parent but due to the difficulty of inducing telial germination it was not possible to prove by appropriate further crosses that these differences in the pathogenicity of the F_1 hybrid were due to cytoplasmic factors. With the host some circumstantial evidence has been presented to show that the reaction in Morden 29 to the parent races may possibly be governed by cytoplasmic factors. Such studies are easier to conduct with the host than with the rust. If the reaction in Morden 29 to the parent races is found to be governed by the cytoplasmic factors in studies on host genetics, and if telial germination can be improved, it may be possible to investigate the non-Mendelian aspect of the complementary relationship between host and parasite.

Questions about some details in the gene-for-gene concept.

Person (1965, 1966) indicated that genes for resistance in the host may be allelic or linked but that genes for virulence in the pathogen appear in general to be non-allelic and unlinked. This concept, like the gene-for-gene concept itself, is based largely on Flor's work. Flor (1955, 1956) reported that genes for resistance in flax occur as multiple alleles at five loci and some are linked but that genes for virulence in flax rust appear to be non-allelic and unlinked. Moseman and co-workers (Moseman, 1966) reported similar findings with barley

and barley powdery mildew, Erysiphe graminis hordei. However, I have not examined their data critically and therefore cannot comment on them.

As indicated earlier in this thesis, I believe that some of the host genes which Flor called alleles may be situated at different loci and closely linked in repulsion. It is also possible that some may be epistatic to others rather than allelic.

Flor used two appropriate races of rust to test the reactions of F_2 host families. If the observed segregation fitted a 2:1:1:0 ratio the resistance genes in the parent host varieties were assumed to be allelic. I believe that proof of non-allelism in the pathogen would require crossing of the two test races, and testing the rust progeny on the two parent varieties used in crosses to domonstrate allelism in the host. Non-allelism in the pathogen could be assumed if segregation in the F_2 rust cultures did not fit a 2:1:1:0 ratio. I have not seen any such evidence in Flor's work.

Since all genes in an allelic series occupy the same locus, they should show the same linkage relations with other genes on the chromosome (Hutt, 1964). A corollary of this, in my opinion, should be that if one of the genes in an allelic series acts against a specific locus of a pathogen then all other genes in the series should also act against the same locus in the pathogen. According to Person (1959, 1966) a gene-for-gene correlation is in fact a locus to locus relationship. If the resistance genes in flax are alleles and if there is a gene-for-gene relationship in the flax and flax rust system, then in my opinion the pathogenicity genes in the pathogen should also be allelic. Although Mode and Person both accepted Flor's statements on allelism, neither indicated if Flor's use of the term fits the classical definition of

allelism. Any disagreement about the validity of the allelism in the host and non-allelism in the rust observed by Flor may have a semantic rather than genetic basis.

Mode (1958) used Flor's findings to develop a mathematical model showing that there may exist dual systems of balanced polymorphism, one in the host population, the other in the pathogen population, and that these might have provided the mechanism for co-evolution of obligate parasites and their hosts. Person (1966) developed this concept further. He considered the interdependence of an R (resistance) gene in the host and the related V (virulence) gene in the pathogen. When an R gene enters a host population initially it has a high selective value because the frequency of the related V gene in the parasite is rare. The V gene however has a correspondingly high selective value. As its frequency in the parasite increases, the selective value of the R gene in the host population is reduced progressively, approaching zero when the V gene is present in all parasitic individuals. The R gene now becomes useless and is no longer kept at a high frequency through selection in the host population. As its frequency declines, the value of the related V gene is also reduced. Based on this idea, Person postulated a self-regulating system to explain the mechanisms of interactions between related genes in the host and parasite which would lead to the maintenance of balanced polymorphisms in the two interacting populations.

The underlying principle of the concept of balanced polymorphism is the superiority of the heterozygote over both homozygotes so that a population will always be composed of both heterozygous and homozygous

individuals (Sinnot et al., 1958). The question is, does balanced polymorphism exist in the self-fertilizing species such as wheat, barley, and flax? Mode assumed that these crops have evolved from open-pollinated encestors and therefore it is possible that the genetic structure of these species has evolved for the most part under a system of random mating. Person (1966) pointed out that in most inbreeding systems a certain amount of outcrossing does occur which, although low, introduces the possibility of maintaining an R gene polymorphism.

The next question is if there is any evidence that when a resistance gene is introduced in a self-pollinated crop it can spread in the population through superior fitness of the heterozygote. I cannot find any data to support this, but the data from some other sources indicate that there may be evidence to the contrary.

In one study Suneson (1949) grew a mixture of four similarly adapted barley varieties for 16 years and found that two of the component varieties became practically extinct. When grown in a pure stand one of these varieties had a significantly better yield and leaf disease record than the others, while the variety which dominated the mixture had the poorest leaf disease record and a mean yield below the average for the population. In another study Suneson and Stevens (1953) studied six composite crosses of barley for 6 to 24 generations. From this they commented that "the common conception that disease-susceptible plants are readily eliminated from mixtures through operation of the principle of survival of the fittest was not confirmed by these experiments." If this is so, then Person's and Mode's hypotheses of the

existence of a self-regulating mechanism and balanced polymorphisms in the interacting populations of host and pathogen may not approximate what actually occurs in the field.

An important assumption basic to their hypotheses is that the resistance genes in the host are allelic and the genes for pathogenicity in the parasite are non-allelic. I believe that at least some of the host resistance genes Flor says are allelic could be closely linked in repulsion. It could be argued that genes closely linked in repulsion may simulate a single locus system. I believe also that Flor has not proved conclusively that virulence genes in the rust are not allelic.

The discussion above is not intended as a refutation of the hypotheses of Mode and Person. It is meant to emphasize that, although considerable work has been done on the genetics of host resistance, there have been too few critical genetic studies on the inheritance of virulence in the pathogen. The mechanical difficulties I have encountered in my own studies, such as inability to induce germination of rust teliospores, are typical of the difficulties which plague other investigators. These will have to be overcome if the inheritance of pathogenicity is to have a sound genetic basis.

Allelism and linkage between some genes seem to be universal phenomena. They have been found in higher organisms, in bacteria, and even in obligately parasitic bacterial viruses (Whitehouse, 1965). Thus, even though Flor and Moseman may be correct in concluding that there is no allelism or linkage between pathogenicity genes in flax rust and in barley powdery mildew, will genetic studies with other pathogens eventually prove that these cases are exceptions rather than indicating a general rule?

SUMMARY

An investigation was made into the inheritance of pathogenicity in sunflower rust, Puccinia helianthi, and resistance in sunflowers, Helianthus annuus. During the course of this study a new method of inoculating with urediospores, the multiple inoculation method, was developed which enables several rust isolates to be inoculated onto a single leaf. This makes it possible to compare reactions of "unknown" isolates with the four test races on a single host genotype, thus overcoming the variability inherent in the host and making studies on the genetics of host-parasite relationships more precise than was possible with the routine method of inoculation. The technique was also found to work satisfactorily with stem rust of wheat and it may prove useful for a wide variety of plants and leaf pathogens in the greenhouse and laboratory.

Attempts were made to produce germinative telia under greenhouse conditions and in controlled environment chambers. A number of trials were conducted between the winter of 1961-1962 and the spring of 1965. Of these, only four trials gave satisfactory telial germination. The results were not conclusive but indicated that under greenhouse conditions the time of year when plants are grown and inoculated may be critical. Two seasons, one in the fall (September to November) and the second in mid-spring (March to mid-April), seemed most favourable for the production of germinative telia in the greenhouse.

Selfing and crossing studies conducted with the four Canadian physiologic races of sunflower rust, 1, 2, 3, and 4, indicated all to be heterozygous on differential varieties CM9ORR and Morden 29.

Races 1, 2, and 4 also appeared heterozygous on the supplemental lines M62-2672-2-r_1 and M62-2685-14-1.

The S_1 data were not analyzed statistically because of the high degree of sampling error attributable to the utilization of inadequate numbers of pycnia in the fertilizations. For ~~other~~ reasons, other than sample size, tests to distinguish Mendelian ratios were considered inappropriate in this instance.

Genetic control of pathogenicity appeared to have a somewhat different basis according to the host variety used. On CM9ORR most reciprocal crosses indicated chromosomal control but a few hybrids derived from races 1, 2 and 3 showed maternal inheritance on a small number of plants. From this it may be assumed that inheritance of pathogenicity is influenced by an interaction of chromosomal and non-chromosomal factors. On Morden 29 evidence of extra-chromosomal influence was stronger and the products of most reciprocal matings behaved like the maternal parent. The lack of telial germination made it impossible to make the appropriate genetic studies required to provide conclusive evidence of the kind of inheritance involved.

The F_1 data were inconclusive but the inference was drawn that on CM9ORR avirulence of races 1 and 2 may be governed by a single dominant gene. It was inferred that virulence of race 3 is governed by two non-allelic dominant genes, one epistatic to the other. There is, however, a weakness in the hypothesis of control by two non-allelic dominant genes through an epistatic-hypostatic relation, which is discussed under host genetics.

The inheritance of resistance in the selfed progeny of Morden 69, and to a lesser extent in the selfed progeny of Morden 29, was studied as an adjunct to investigation on the inheritance of pathogenicity.

Reactions of the selfed progeny of Morden 69 varied from experiment to experiment and this was attributed to sensitivity to environmental influence.

The ratios of resistant : susceptible seedlings with regard to races 1 and 2 in the selfed progeny of Morden 69 showed a good fit to both 3:1 and 13:3 ratios in most experiments but in some would fit only one or the other. The progeny sizes tested were inadequate for a critical distinction to be made between the two ratios. The results were also too erratic for much significance to be attached to them. It was impossible to assess satisfactorily whether resistance to these two races is controlled by two non-allelic genes, one epistatic to the other, or by a single dominant gene.

On the basis of earlier experiments Morden 69 should be susceptible to races 3 and 4, but some seedlings gave resistant reactions to these races. The proportions of these resistant seedlings varied from test to test. Tests of the S_2 progeny derived from S_1 resistant seedlings were inconclusive. Progeny of some resistant S_1 plants gave susceptible reactions while others segregated. Two possible explanations were advanced to explain these results. The gene governing susceptibility may have reduced penetrance, or the reaction may be governed by an interaction between chromosomal and non-chromosomal factors.

Selfed derivatives of Morden 29 were tested for reaction to the four races. They were generally resistant to races 1 and 3 but their

reactions to races 2 and 4 were variable. Circumstantial evidence suggests that this variability may be attributable to non-chromosomal factors governing reaction in the host. Lines showing uniform rust reactions were selected for use as differentials.

The literature on genetic studies of host-parasite relationships in rust infections has been critically evaluated. Some of the genetic tests used appear to me to be inappropriate for the interpretations based on them, and in some other cases the data appear to have been misinterpreted.

Flor tested the pathogenicity of S_1 progeny of several flax rust races to determine homozygosity, then used them in crosses to study the inheritance of pathogenicity. The number of progeny tested per race was in most cases inadequate to detect segregation even for a single factor hypothesis., His assumption that the parent races were homozygous is therefore open to question.

In crosses between several flax rust races Flor postulated pathogenicity on a number of varieties to be under monogenic control. A reanalysis of his data suggested that pathogenicity on some varieties may be governed by two dominant genes through an epistatic-hypostatic relationship.

In the cross race 22 x race 24 Flor postulated that pathogenicity on Italia Roma and Morye is governed by two and three dominant avirulence genes respectively. My calculation of Flor's data shows that pathogenicity on Italia Roma may be controlled by one dominant avirulence gene and one dominant virulence gene, with avirulence epistatic to virulence. On Morye there appear to be two dominant avirulence genes.

Flor was unable to find plausible expected ratios for the observed segregation in the cross race 22 x race 24 on varieties Akmolinsk, Abyssinian, Leona, Williston Golden and Williston Brown. A reanalysis of his data suggested that pathogenicity on these varieties may also be governed by two dominant genes through epistatic-hypostatic relationships.

Reanalysis of Flor's data on the inheritance of rust resistance in flax varieties suggested that resistance may be more complex than he reported. According to Flor, rust resistance in Bombay is governed by a single gene. According to my analysis of the data, resistance in Bombay to some races may be governed by complementary genes.

Resistance in several flax varieties is reported by Flor to be controlled by single or duplicate genes. My reanalysis of his data suggests that these varieties may possess more resistance genes, some of which may be epistatic.

Flor's use of the term allelism may not conform with the classical definition. Flor assumed two resistance genes to be allelic if the observed F_2 segregation fitted a 2:1:1:0 ratio. On this basis he placed various resistance genes in an allelic series. In my view they appear to be situated at different loci and may be closely linked in repulsion.

Kerr, following Flor, postulated a number of allelic genes by fitting observed segregations to a 2:1:1:0 or a 14:1:1:0 ratio. According to my analysis, the resistance in these varieties could also be controlled by several genes through an epistatic-hypostatic relationship which cannot be differentiated without appropriate tests.

Using the pooled nectar method of selfing Green studied the inheritance of colour and pathogenicity in S_1 uredial progeny of races 11 and 15B-1L (Can.) of Puccinia graminis.

In his study, the normal uredial colour was dominant over greyish-brown. The parent races 15B-1L and 11 were virulent on varieties Arnautka and Mindum. In the S_1 progeny of race 15B-1L virulence was dominant among both the normal and greyish-brown progenies; in the S_1 progeny of race 11 virulence was dominant among the normal coloured cultures but recessive in greyish-brown ones. The "apparent reversal of dominance" in race 11 was explained by linkage between the loci for colour and virulence.

On varieties Vernal and Lee, the parent race 15B-1L was virulent. All the S_1 progeny with greyish-brown spores were virulent but most of the normal coloured types were avirulent. The segregation ratio of avirulent to virulent cultures among the normal coloured progeny indicated two recessive genes for virulence. Green rejected this on theoretical grounds, and explained his results on the basis of linkage between loci for colour and virulence.

Green's data indicate that the number of aecial clusters sampled was inadequate to test even a single factor hypothesis. He did not report the number of pycnia from which he collected nectar for mixing. His results may be attributable to sampling error because of inadequate population sizes, rather than to linkage between colour and virulence loci.

In another study Green selfed progeny from four cultures of wheat stem rust race 11 and one culture of race 10 on several wheat differentials. The total number of cultures tested varied between 20 and 52 and

and were obtained over 9 years, i.e. in several experiments. These results were presented to show a gene-for-gene relationship between wheat and wheat stem rust.

Since the S_1 progeny were obtained in distinct experiments the gametic population in each was subject to sampling error. Although Green's conclusions are probably correct, I believe that he bases them on inadequate data.

In 1965 Green conducted crossing and selfing studies of several oat stem rust races on variety Sevnothree, employing race 6A and a number of other races. Segregation was apparent when race 6A was used as the maternal parent. When race 6A was the paternal parent, the pathogenicity of the F_1 hybrids resembled the maternal parent. Segregation was also obtained in the selfed progeny of race 6A.

Green explained the virulence of race 6A by postulating it to be controlled by a dominant gene for virulence, and a dominant gene that acts with the maternal cytoplasm to make the recessive gene for avirulence dominant when both occur in the same monokaryotic infection.

In my opinion Green's tests were not appropriate to determine whether inheritance of virulence was Mendelian or not. I believe also that the model proposed by Green is not genetically sound.

In crosses between oat stem rust races 7 and 11 Johnson concluded that avirulence on each of two oat varieties, White Tartar and Richland, is governed by complementary genes. Green in a later study reported pathogenicity on each of the two varieties to be controlled by single genes. Green cited the work of other workers which also suggested rust resistance in these two varieties to be governed by single genes.

If resistance is controlled by a single gene, then assuming the existence of a gene-for-gene relationship, monogenic control of pathogenicity as suggested by Green seems to be more plausible than by complementary genes. Johnson obtained the data using the pooled nectar method of selfing. As he made no mention of the numbers of pycnia he used, the number of aecial clusters he sampled, and the number of cultures established from each aecial cluster, his ratios may have been spurious because of inadequate sample size.

In a cross between wheat stem rust race 111 (culture 111-55A) and 36 (culture 36-55A) Loegering and Powers concluded on the basis of segregation ratios that the avirulence of race 111 on varieties Marquis (12:3:1) and Reliance (48:12:3:1) is governed by two and three dominant genes respectively. On the basis of gene-for-gene relationships, they postulated two and three dominant genes for resistance in Marquis and Reliance respectively.

Loegering and Powers obtained F_2 progeny in the course of several experiments. In my opinion the gametic population in each experiment was theoretically not sufficient to avoid sampling error. Although their conclusions may be correct, they are not justified on theoretical grounds.

Williams et al. tested the pathogenicity of 103 of the 108 F_2 cultures studied by Loegering and Powers on Marquis, Reliance, and on single gene lines supposedly carrying the same resistance genes as Marquis and Reliance. Their results on Marquis and Reliance agreed with those of Loegering and Powers. On two monogenic lines, however, they found that avirulence of race 111 was controlled not by a dominant gene but by a single recessive gene. They explained these results by the effect on dominance at a given locus in the rust, of the additional resistance genes

present in Marquis and Reliance.

In my opinion, since avirulence on the single gene lines derived from Marquis and Reliance is conditioned by a recessive gene, the same recessive gene should also be present in Marquis and Reliance. The discrepancy in results of the two groups of workers might well be attributed to the way each of them classified intermediate infection types induced by some F_2 cultures. Differences in classification of just a few cultures, indicated in their data, could lead to quite different ratios and different interpretations.

Luig and Watson tested 285 cultures derived from selfing of Strain 21 Anz. 2 of Puccinia graminis var. tritici on 23 wheat differentials. They grouped the whole population into 37 strains. These 37 strains were used as 37 S_1 progeny to calculate genetic ratios. From this study they concluded that virulence on some differentials seemed to follow Mendelian heredity but on others the mode of inheritance was difficult to explain. They also stated that their results indicated a gene-for-gene relationship on some varieties but not on others.

As the number of strains they obtained was determined by the number of differentials employed, it was an artificial population and should not have been used to calculate Mendelian ratios. They obtained the progenies by the pooled nectar method of selfing. As they did not mention the numbers of pycnia, aecial clusters, or number of cultures they obtained from each cluster, their results might have been affected by sampling error if the numbers involved were inadequate.

Samborski investigated the nature of the inheritance of pathogenicity in the S_1 progeny of a culture of wheat leaf rust on the variety Transfer.

He concluded that the parent culture was heterozygous for pathogenicity with virulence being incompletely dominant as the result of a spontaneous mutation. According to my analysis, his data indicate that the virulence was recessive. Thus it is much more likely to have arisen by spontaneous mutation than if it were dominant.

Flangas and Dickson tested the pathogenicity of 285 subcultures derived from three generations of inbreeding of four heterozygous clones of Puccinia sorghi. They also tested 92 hybrid cultures derived from crosses between lines of selected pathogenic types of sibling and non-sibling pedigree on six maize lines. They concluded that their results could not be explained in simple Mendelian terms. They looked for explanations from the work on biochemical genetics of microorganisms, where complex genetic mechanisms, based on tests with many millions of offspring, have been postulated.

The data presented by Flangas and Dickson, however, show that the population sizes they used are not sufficient to test even a single factor hypothesis, let alone more complex genetic systems. From the results described by Flangas and Dickson it appears that the genes controlling pathogenicity in the clones of P. sorghi may have reduced penetrance.

Workers have employed two methods of selfing (the reciprocal and the pooled nectar method) in rust fungi and postulated genetic hypotheses by calculating F_2 ratios. These two methods have not been standardized and the results obtained may be unreliable.

With the pooled nectar method, sampling errors may occur if the number of pycnia used for pooling the nectar is inadequate; if the nectar is not thoroughly mixed; if the number of aecial clusters sampled is too small; and if insufficient cultures are derived from each cluster. F_2 ratios calculated for progenies derived by this method may be spurious, as it is rarely convenient to handle the sample sizes theoretically required.

The reciprocal method of pairing may give rise to errors if the number of pycnia used in an experiment is insufficient. As there is only one point at which sampling error is likely to occur, this method is preferable to the pooled nectar method for deriving genetic hypotheses from F_2 data. Because of the difficulty of working with F_3, most rust workers base their conclusions on F_2.

I have described a method of calculating genetic ratios when the pooled nectar method is used, based on the theoretical expectation of segregation between and within aecial clusters. This method should give results comparable to the backcross method. It should also permit differentiation between such critical ratios as 3:1 and 13:3, and should serve the purpose of a progeny test. It may therefore be preferable to test genetic hypotheses by this method, rather than on the basis of F_2 data.

A gene-for-gene system seems to provide a rational explanation of the genetic control of resistance and of pathogenicity.

The genetic data presented in this thesis on sunflowers and sunflower rust are not sufficient to prove the existence of a gene-for-gene relationship in this host-parasite system.

Mode and Person have both proposed models to explain the self-regulating mechanisms and balanced polymorphisms in interlocking populations of host and pathogen in crops, most of which are self-pollinating. Although the kind of balance they postulate may occur in nature between wild plants and their pathogens, it may be less probable in cultivated crops such as cereals and flax. Experimental data provided by Suneson for mixed populations of barley varieties differing in reaction to leaf pathogens, do not seem to support their hypotheses.

One of the assumptions common to Mode's and Person's models is that virulence genes in the pathogen are not allelic. This may be the case, but I believe that Flor's work has not ruled out altogether allelism of virulence in flax rust. Allelism seems to be almost a universal phenomenon, found in obligately parasitic bacterial viruses as well as in higher organisms. When the mechanical difficulties that interfere with genetic studies on parasitic fungi are overcome, I believe that allelism may also be demonstrated for virulence genes.

CLAIM OF ORIGINAL WORK AND CONTRIBUTIONS TO KNOWLEDGE

1. I have developed a method for making multiple inoculations with rust on individual leaves. This method makes possible much greater precision in working with variable host genotypes and in identifying unknown races or genotypes in the pathogen, than was available with previous methods.

2. I have made an investigation into the inheritance of pathogenicity of the four Canadian sunflower rust races. My data suggest that pathogenicity of the races used may possibly be controlled by non-chromosomal or an interaction between chromosomal and non-chromosomal factors. An implication of these results is that resistance in sunflower differentials to rust may be more complex than reported by previous workers.

3. I have presented a critical review of the published work on genetic studies on pathogenicity in rust fungi and resistance in their hosts. I have pointed out that the results obtained in some of these studies may not be based on sound genetic tests and that in some other studies the interpretation of the genetic data presented is open to question.

4. I have pointed out that the two methods of selfing F_1 rust hybrids used by various workers have not been standardized. The data obtained by these two methods may be different and this may lead to misleading conclusions. I have examined the two methods critically

with regard to the stages at which sampling errors can occur and have suggested how to choose the more reliable method under various circumstances.

5. I have suggested an improved method of calculating genetic ratios for inheritance studies with rust fungi. I believe it will be more useful than the methods of analysis now in use and will permit more critical testing of genetic hypotheses.

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Explanatory note for Appendix Tables I to VI

Morden 29 is susceptible to races 2 and 4, and resistant to races 1 and 3. Results are based on reactions of 10 to 15 seedlings in each case.

CM9ORR is susceptible to races 3 and 4, and resistant to races 1 and 2. Results are based on reactions of 10 to 15 seedlings in each case.

M69-W59-11-MC62-13-r4 - Most of the seedlings of this line are resistant to races 1 and 3, and susceptible to races 2 and 4 or to race 4 only. Results are based on reactions of 5 to 8 seedlings in each case.

M62-2672-2-r1 - Most of the seedlings of this line are susceptible to race 1 and highly resistant to the other three races. Some are also resistant to race 1. Results are based on reactions of 3 to 8 seedlings in each case.

M62-2685-14-I is highly resistant to all four parental races. Results are based on reactions of 6 to 10 seedlings in each case.

APPENDIX TABLE I. PATHOGENICITY ON DIFFERENTIAL VARIETIES OF F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 1 AND 2.

Cross No.	Race x Race ♀ ♂	Varieties[1] Morden 29	CM9CRR	M69-W59-11-MC62-13-r4	M62-2672-2-r1	M62-2685-14-I
Reciprocal Cross						
Cr63-10A	2 x 1	V[2]	A	A	A	A
-10B	1 x 2	A	A	A	A	A
Cr63-11A	2 x 1	V	A	A, A=, V=	-	A
-11B	1 x 2	A	A	A, A, A	-	A
Cr63-14A	2 x 1	V	A	A, A=, V=	-	A
-14B	1 x 2	A	A	A, A, A	-	A
Cr65-20 -2A-1	1 x 2	A	A	A, A	A	A
-2B-1	2 x 1	V	A	A, V	A	A
Cr65-20 -3A-1	1 x 2	A	A	A, A	A	A
-3B-1	2 x 1	V	A	A, V	A	A
Cr65-2-1A	1 x 2	A	V-	-	-	-
-2-1B	2 x 1	V	V-	-	-	-
One-sided Cross						
Cr65-20 -1B-1	2 x 1	V	A	V	A	A
Cr65-20 -4B-1	2 x 1	V	A	A	A	A

1/ See note on page A-1

2/ A, avirulent; V, Virulent; -, not tested on the line

↕ Reactions of reciprocal F_1 cultures on the same seedlings

APPENDIX TABLE II. PATHOGENICITY ON DIFFERENTIAL VARIETIES OF F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 1 AND 3

Cross No.	Race x Race ♀ ♂	Varieties[1] Morden 29	CM9ORR	M69-W59-11-MC62-13-r4	M62-2672-2-r1	M62-2685-14-I
Reciprocal Cross						
Cr63-4A	3 x 1	A[2]	V, V[1]	A, A=, V=	A	A
-4B	1 x 3	A	V, A	A, A=, V=	A	A
Cr65-33-1A-3	1 x 3	A	V	A-, A=	A	A
-1B-1	3 x 1	A	V	A-, A=	A	A
Cr65-33-5A-1	1 x 3	A	A	A, A=	A, A-	A
-5B-1	3 x 1	A	A, V	A	A, A-	A

[1] See note on page A-1

[2] A, avirulent; V, virulent;

↕ Reactions of reciprocal F_1 cultures on the same seedlings

APPENDIX TABLE III. PATHOGENICITY ON DIFFERENTIAL VARIETIES OF F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 1 AND 4

Cross No.	Race x Race ♀ ♂	Varieties[1] Morden 29	CM9ORR	M69-W59-11-MC62-13-r4	M62-2672-2-r1	M62-2685-14-I
Reciprocal Cross						
Cr63-1A	4 x 1	V[2]	A	A, A-, V-	like race 1	A
-1B	1 x 4	A	A	A, A, A-	like race 1	A
Cr63-2A	4 x 1	V	V, A, V=, A	V=, V=	like race 1	A
-2B	1 x 4	A	V, V-, V=, A	V=, A-	like race 1	A
Cr65-25 -7A-1	4 x 1	A	V-, A	A	V, V-	A
-7B3 (7B-3)	1 x 4	A	V-, A	A	V, V-	A
Cr65-25 -10A-1	4 x 1	V	A	A, V=, V	V	A
-10B-1	1 x 4	A	A-	A, A, A	V	A
One sided Cross						
Cr65-25 -6B-1	1 x 4	A	V	A, A-	A	A
Cr65-25 -8B-1	1 x 4	A	A, V-	A	A, V-	A
Cr65-25 -9A-1	4 x 1	V	V	V	V	A

1/ See note on page A-1

2/ A, avirulent; V, virulent;

↕ Reactions of reciprocal F_1 cultures on the same seedlings

APPENDIX TABLE IV. PATHOGENICITY ON DIFFERENTIAL VARIETIES OF F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 2 AND 3

Cross No.	Race x Race ♀ ♂	Varieties[1] Morden 29	CM9ORR	M69-W59-11-MC62-13-r4	M62-2672-2-r1	M62-2685-14-I
Reciprocal Cross						
Cr63-15A	3 x 2	A[2]	V, V-	A	A	A
-15B	2 x 3	V	V, V-	V	A	A
Cr63-16A	3 x 2	A	A, V	A, A	A	A
-16B	2 x 3	V	A, A	V=, A	A	A
Cr63-17A	3 x 2	A	V, V=	-	-	-
-17B	2 x 3	V	V, A	-	-	-
Cr63-18A	3 x 2	A	A, V, A	-	-	-
-18B	2 x 3	V	A, V, V	-	-	-
Cr65-4-1A	2 x 3	V	V, A-	V=	A	A
-4-1B	3 x 2	V	V, V	V=	A	A
Cr65-24-4A-1	3 x 2	A	V	A	A	A
-4B-1	2 x 3	V	V	V	A	A
Cr65-24-5A-1	3 x 2	A	V	A	A	A
-5B-1	2 x 3	V	V	V	A	A
One-sided Cross						
Cr65-24-1B-1	2 x 3	V	A, V	A, V		

1/ See note on page A-1

2/ A, avirulent; V, virulent; -, not tested on the line

↕ Reactions of reciprocal F_1 cultures on the same seedlings

APPENDIX TABLE V. PATHOGENICITY ON DIFFERENTIAL VARIETIES OF F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 2 AND 4

Cross No.	Race x Race ♀ ♂	Varieties[1] Morden 29	CM9ORR	M69-W59-11-MC62-13-r4	M62-2672 -2-r1	M62-2685 -14-I
Reciprocal Cross						
Cr65-22						
-1A-1	2 x 4	V[2]	V, A	A-, V	A	A
-1B-1	4 x 2	V	A, A	A, V	A	A
Cr65-22						
-2A-1	2 x 4	V	A, A-	A, V	A	A
-2B-1	4 x 2	V	A, A-	A, V=	A	A
Cr65-22						
-3A-1	2 x 4	V	A, V	A	A	A
-3B-1	4 x 2	V	A, V	A	A	A
Cr65-25						
-2A-1	4 x 2	V	V	V	A	A
-2B-1	2 x 4	V	V	V	A	A
One-sided Cross						
Cr65-25						
-1B-1	2 x 4	A	V	A	A	A

1/ See note on page A-1

2/ A, avirulent; V, virulent

↕ Reactions of reciprocal F_1 cultures on the same seedlings

APPENDIX TABLE VI. PATHOGENICITY ON DIFFERENTIAL VARIETIES OF F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 3 AND 4

Cross No.	Race ♀ x Race ♂	Varieties[1] Morden 29	CM9ORR	M69-W59-11-MC62-13-r4	M62-2672-2-r1	M62-2685-14-I
Reciprocal Cross						
Cr65-26-2A-1	3 x 4	A[2]	A, V	A, V	A	V
-2B-1	4 x 3	V	A, V	V, V-	A	V
Cr65-26-3A-2	3 x 4	A	V	A	A	V
-3B-2	4 x 3	V	V	V	A	V
Cr65-26-5A-2	3 x 4	A	V	A	A, V	A
-5B-2	4 x 3	V	V	V	A, A	A
Cr65-26-6A-2	3 x 4	A	V, A	A, A	A, A	A
-6B-2	4 x 3	V	V, A	V, V=	A, V=	A

1/ See note on page A-1

2/ A, avirulent; V, virulent

↕ Reactions of reciprocal F_1 cultures on the same seedlings

APPENDIX TABLE VII. REACTION TYPES OF DIFFERENTIAL VARIETIES TO F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 1 AND 2.

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1	2	3	4	F_1 (♀ x ♂) Cr63-10A (2 x 1)	Cr63-10B (1 x 2)
MI,[1] GH, 5 Mar. 1964	S37-388	3/3[2]	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4
	M29-63-830 (Morden 29)	8/8	0;	3, 4	0;, ON,1	3, 4	3, 4	0;, 1N
	CM9ORR	7/7	0;	0;	3, 4	3, 4	0;	0;
MI, GC 17, 13 Dec. 1965	S37-388	4/4	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3
	M29-63-868 (Morden 29)	2/3	0;, ON	3-	0;, ON	3-	2+, 3_	ON,0;, 1
		1/3	ON	3-, 3	0;	2+, 3-	3	1-N
	CM9ORR	4/4	0;	0;	3-	3-	0;	0;
	M69-64-224 (M69-W59-11-MC62-13-r4)	1/2	1, 2	0;, 1	ON, 1	3-, 3	IN	0;
	M62-64-557 (M62-2672-r1)	1/2	0;	0;	1-N	2+, 3-	1-N	0;
		3/3	3	0;	0;	0;	0;	0;
	M62-64-664 (M62-2685-14-I)	8/8	I	I	I	I	I	I

APPENDIX TABLE VII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races				F_1 (♀ × ♂)			
			1	2	3	4	Cr63-11A (2 x 1)	Cr63-11B (1 x 2)	Cr63-14A (2 x 1)	Cr63-14B (1 x 2)
MI, GH, 5 Mar. 1964	S37-388	3/3	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4
	M29-63-830 (Morden 29)	4/7	ON	3, 4	ON	3, 4	3, 4	ON	3-	ON, IN
		3/7	ON	3, 4	ON	3, 4	3, 4	ON	?	ON, 1N
	CM9ORR	6/6	0;	0;	3, 4	3, 4	0;	0;	0;	0;
MI, GC 17, 13 Dec. 1965	S37-388	3/3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3
	M29-63-868 (Morden 29)	1/4	0;	3	0;	3	3	ON	3	0;
		1/4	0;	3	0;	3-	3	ON, 1	3-, 3	ON, 1N
		1/4	0;	3	0;	3	3	1N, 2	3	four 3- ON, 0;
		1/4	0;	3-, 3	0;	2N, 2+	3	0;, 1, 2	3-	five 3- 0;, 1N
	CM9ORR	1/7	0;	0;	3	3	0;	0;	ON,0;,1	2+, 3-
		5/7	0;	0;	3	3	0;	0;	0;	0;,ON,1,2+
		1/7	0;	0;	3	3	0;	0;	0;	0;

APPENDIX TABLE VII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races				F_1 (♀ × ♂)			
			1	2	3	4	Cr63-11A (2 x 1)	Cr63-11B (1 x 2)	Cr63-14A (2 x 1)	Cr63-14B (1 x 2)
MI, GC 17 13 Dec. 1965	M69-64-224 (M69-W59-11-MC62-13-r4)	1/5	ON	ON, 1	1-N	2+, 3-	2N, 2	0;, 1-N	1-N, two 2+	1-N
		1/5	0;	1, 2, 2+	1-N, 1	3-	2, 2+	0;	1, 2	0;, 1, two 3-
		1/5	0;	2N, 2+	ON	3-, 3	0;, 2	0;	1, 2+	ON, 2+, three 3-
		1/5	0;	0;, 1-N	ON	2+, 3-	1, 2	0;	1, 2	ON, three (2 or 2+)
		1/5	0;	2	1-N, 1	3	1, 2, 2+	0;	1, 2	0;, three 2, one 3-
	M69-64-241 (M69-W59-11-MC62-13-r4)	1/2	0;	ON, 1-N	0;	2+, 3-	2	0;, 1	0;	2N
		1/2	0;	0;, 2	1N	3-	2, 2+	0;, 1	2-	1N
	M62-64-664 (M62-2685-14-I)	6/6	I	I	I	I	I	I	I	I

APPENDIX TABLE VII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-20 -2A-1 (1 x 2)	Cr65-20 -2B-1 (2 x 1)	Cr65-20 -3A-1 (1 x 2)	Cr65-20 -3B-1 (2 x 1)
RI, GC 6, 3 Sept. 1965	S37-388	3/3[2/]	3, 4	3/3 3, 4	3/3 3, 4	3/3 3, 4	3/3 3, 4	3/3 3, 4	3/3 3, 4	3/3 3, 4
	CM9ORR	4/4	0;	3/3 0;	4/4 3, 4	3/3 3, 4	6/6 0;	5/5 0;	6/6 0;	5/5 0;
	M29-64-410 (Morden 29)	5/5	1, 2	5/5 3-,3	5/5 0;	3/3 3,3+	5/5 0;,1,2	4/4 3-,3	4/4 0;,1,2	5/5 3-,3
	M62-64-631 (M62-2685-14-I)		I	I	I	I	I	I	I	I
MI, GC 2, 11 Nov. 1965	S37-388	3/3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3
	M29-63-868 (Morden 29)	1/3	ON	3-, 3	0;, ON	2+N, 3-	ON, 2-	3	1=N	2+, 3-
		1/3	ON	2+, 3-	ON	2+, 3-	ON, 1+	2+N	1-N	3-
		1/3	ON	2+, 3-	ON	2+, 3-	ON	2+, 3-	ON	2+, 3-
	CM9ORR	2/5	0;	0;	3-, 3	3-, 3	0;	0;	0;	0;
		2/5	0;	0;	2+	0;, 1-,1	0;	0;	0;	0;
		1/5	0;	0;	3-, 3	2+	0;	0;	0;	0;
	M62-64-557 (M62-2672-2-r1)	5/5	2+, 3-	0;	0;	0;	0;	0;	0;	0;

APPENDIX TABLE VII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-20 -2A-1 (1 x 2)	Cr65-20 -2B-1 (2 x 1)	Cr65-20 -3A-1 (1 x 2)	Cr65-20 -3B-1 (2 x 1)
MI, GC 2, 11 Nov. 1965	M69-64-224 (M69-W59-11 -MC62-13-r4)	1/6	0;	ON	ON	2+, 3-	ON	ON	ON	ON
		1/6	0;	ON	ON	2N, 2+, 3-?	ON	ON	ON	ON
		2/6	ON	2N	ON	2+, 3-	ON	ON	ON	ON
		2/6	IN	3-	ON	3-, 3	ON	3-, 3	1-N	3
	M62-64-664 (M62-2685-14-I)	6/6	I	I	I	I	I	I	I	I

1/ MI, multiple inoculation; RI, routine inoculation; GH, greenhouse; GC, growth cabinet.

2/ Numerator indicates the number of seedlings showing particular reaction types, and denominator gives the number of seedlings tested.

APPENDIX TABLE VIII. REACTION TYPES OF DIFFERENTIAL VARIETIES TO F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 1 AND 3

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr63-4A (3 x 1)	Cr63-4B (1 x 3)
MI,[1] GH, 5 Mar. 1964	S37-388	3/3[2]	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4
	CM9ORR	3/7	0;	0;	3, 4	3, 4	3, 4	0;, 1
		4/7	0;	0;	3, 4	3, 4	3, 4	3, 4
	M29-63-830 (Morden 29)	8/8	ON, 1	3, 4	0;,ON,1	3, 4	0;, 1N	0;, 1N
MI, GC 17, 13 Dec. 1965	S37-388	4/4	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3
	M29-63-868 (Morden 29)	2/3	0;,ON,1-	3-	0;, ON	3-	ON, 1-	ON, 1-
		1/3	ON	3-, 3	0;	2+, 3-	1-N	1-N
	CM9ORR	3/4	0;	0;	3-	3-	3-	3-
		1/4	0;	0;	3-	3-	0;, 1, 2, 2+	2N, 2+,3- ?
	M64-64-224 (M69-W59-11-MC62-13-r4)	1/2	1, 2	0;, 1	ON, 1	3-, 3	1, 2	1-
		1/2	0;	0;	1-N	2+, 3-	2N	1N

APPENDIX TABLE VIII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr63-4A (3 x 1)	Cr63-4B (1 x 3)
MI, GC 17, 13 Dec. 1965	M69-64-241 (M69-W59-11-MC62-13-r4)	1/2	1, 2	0;, 1	2	2+, 3-?	2+, 3-?	2+
		1/2	0;	2+	2, 2+	3-	1, 2, 2+	2, 2+
	M62-64-557 (M62-2672-2 r1)	3/3	3	0;	0;	0;	0;	0;
	M62-64-664 (M62-2685-14-I)	8/8	I	I	I	I	I	I

			1	2	3	4	Cr65-33 -1A-3 (1 x 3)	Cr65-33 -1B-1 (3 x 1)	Cr65-33 -5A-1 (1 x 3)	Cr65-33 -5B-1 (3 x 1)
RI, GC 6, 25 Sept. 1965	S37-388	3/3	3,4	3,4	3,4	3,4	3,4	3,4	3,4	3,4
	M29-64-417 (Morden 29)	5/5	1N,1,2	3-,3	0;,1-,1	3,4	1,2	0;,1	1,2	0;, 1
	CM9ORR	4/4	0;	0;	3,4	3,4	3,3+	3,3+	0;	2/5 3 2/5 0;, scattered (2+ or 3-) 1/5 0;
	M62-64-631 (M62-2685-14-I)		I	I	I	I	I	I	I	I

APPENDIX TABLE VIII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-33 -1A-3 (1 x 3)	Cr65-33 -1B-1 (3 x 1)	Cr65-33 -5A-1 (1 x 3)	Cr65-33 -5B-1 (3 x 1)
MI, GC 13,	S37-388	3/3	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-
27 Jan 1966	M29-63-868 (Morden 29)	1/3	ON	2+	ON	2+	1-N	ON	ON	ON
		1/3	ON	2+, 3-	ON	2+, 3-	1-N, few 2 or 2+	ON	ON	ON
		1/3	1-N	2+, 3-	1-N	2+, 3-	1-N, few 1 or 2	ON, 1-N	1-N	ON, few 2
	CM9ORR	2/5	0;	0;	2+, 3-	2+, 3-	2+, 3-	2+, 3-	0;	0;
		2/5	0;	0;, few 2	2+, 3-	2+, 3-	2+, 3-	2+, 3-	0;	0;
		1/5	0;	0;	2+, 3-	2+, 3-	2+	3-, 3	0;	0;
	M62-64-557 (M62-2672-2 -r1)	1/2	1-N	0;	0;	0;	0;	0;	1-N	1-N
		1/2	1-N	0;	0;	0;	ON	ON	1, 2, 2+	1-N, 2
	M69-63-562 (M69-W59-11-MC62-13-r4)	1/3	0;	0;	1-N, 1	2+, 3-	2N, 2+	1N, 2-	0;	0;
		1/3	0;	0;	1N, 2	2+, 3-	1N, 2	1N, 1	0;	0;
		1/3	0;	2+, 3-	1	2+, 3-	2N, 2	1-N	2N, 2, 2+	0;

1/ MI, multiple inoculation; RI, routine inoculation; GH, greenhouse; GC, growth cabinet

2/ Numerator indicates the number of seedlings showing particular reaction types, and denominator gives the number of seedlings tested

APPENDIX TABLE IX. REACTION TYPES OF DIFFERENTIAL VARIETIES TO F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 1 AND 4

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr63-1A (4 x 1)	Cr63-1B (1 x 4)	Cr63-2A (4 x 1)	Cr63-2B (1 x 4)
MI[1]/ GH	S37-388	3/3[2]/	3, 4	3, 4	3,4	3, 4	3, 4	3, 4	3, 4	3, 4
5 Mar. 1964	M29-63-830 (Morden 29)	8/8	0;, ON	3, 4	0;, ON	3, 4	3, 4	0;	3, 4	0;, 1
	CM9ORR	1/7	0;	0;	3, ;4	3, 4	0;	0;	five 3	2, 3
		1/7	0;	0;	3, 4	3, 4	0;	0;	3, 4	2+, 3
		1/7	0;	0;	3, 4	3, 4	0;	0;	0;	3, 4
		4/7	0;	0;	3, 4	3, 4	0;	0;	0;	0;
MI, GC 17,	S37-388	4/4	3, 3+	3, 3+	3, 3+	3, 3+	3, 3+	3, 3+	3, 3+	3, 3+
13 Dec 1965	M20-63-868 (Morden 29)	2/4	0;, ON	3-, 3	0;, ON	3-	3-	0;, 1	3	ON, 2
		2/4	0;, ON,1	3	ON	2+, 3-	3	0;	3	ON, 2
	CM9ORR	2/7	0;	0;	3-	3-	0;, 1, 2	0;, 1, 2	2+, 3-	3-
		1/7	0;	0;	2+, 3-	3, 3+	0;	0;	1+, 2	2+,3- ?
		1/7	0;	0;	3-, 3	3	0;	0;	2, 2+ ?	2+
		1/7	0;	0;	3-	2+, 3-	0;	0;	0;, ON, 2	1, 2
		1/7	0;	0;	2+	2	0;	0;	0;	0;
		1/7	0;	0;	3-, 3	2+	0;	0;	2+	0;, 1, 2

APPENDIX TABLE IX - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr63-1A (4 x 1),	Cr63-1B (1 x 4)	Cr63-2A (4 x 1)	Cr63-2B (1 x 4)
MI, , GC, 17, 13 Dec 1965	Cr64-224 (M69-W59-11-MC62-13-r4)	1/3	ON;, 0;	2N, 2	1, 2	2+, 3-	0;	0;	2+	2, 2+
		1/3	ON	1+N	ON	2+, 3-	2N, 2+	ON, 1	2+, 3-?	1, 2
		1/3	1-N	3-	ON	2+, 3	2+, 3-	2N	2+, 3-?	1, 2, 2
	M62-64-557 (62-2672-2 -r1)	1/4	ON, 3-	0;	0;	0;	ON, 2+	3-	3-	ON, 2+,3-
		1/4	ON, 3	0;	0;	0;	ON, 1, three 3-	2+, 3-	3-, 3	2N, 3
		1/4	ON, 3-	0;	0;	0;	2+, three 3-	three 2+, three 3-	2+, 3-	ON, 3
		1/4	ON, 3	0;	0;	0;	ON, 2+, 3-?	ON, 0;	ON, 2+, 3-	ON, 2+, 3-?
	M62-64-664 (M62-2685-14-I)	8/8	I	I	I	I	I	I	I	I

APPENDIX TABLE IX - continued

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1		2		3		4		F_1 (♀ x ♂) Cr65-25 -8B-1 (1 x 4) (No reciprocal)		Cr65-25 -9A-1 (4 x 1) (No reciprocal)		Cr65-25 -10A-1 (4 x 1)		Cr65-25 -10B-1 (1 x 4)	
RI, GC 6, 15 Sept 1965	S37-388		3/3[2/]	3,4	3/3	3,4	3/3	3,4	3/3	3,4	3/3	3,4	3/3	3,4	3/3	3,4	3/3	3,4
	M29-64-410 (Morden 29)		4/4	1,2	5/5	5,3	5/5	0;	3/3	3,3+	5/5	0;	5/5	2+,3-	4/4	3-,3	3/3	0;,2N, 2, few 2+
	CM9ORR		3/3	0;	2/2	0;	4/4	3,4	3/3	3-,3	3/5 2/5	0; 2+,3-	5/5	3,4	3/4 1/4	0; 0;, 3-	5/5	0;, 2, 2+
	M62-64-631 (M62-2685-14-I)		5/5	I		I		I		I		I		I		I		I
MI, GC 13, 27 Jan 1966	S37-388	2/2	2+, 3-		2+, 3-		2+, 3-		2+, 3-		2+, 3-		2+, 3-		2+, 3-		2+, 3-	
	M29-63-868	4/4	0;, ON		2+, 3-		ON, 0;		2+, 3-		ON		2+, 3-		2+, 3-		ON	
	CM9ORR		Error - plants discarded															
	M69-64-211 (M69-W59-11-MC62-13-r4)	1/2	0;		0;, 2		2		2+		0;, 1		2+		2, 2+		0;	
		1/2	0;		2+		1		2+		1N, 2		2+		2+		2 or 2+	
	M69-63-562 (M69-W59-11-MC62-13-r4)	1/3	0;		1-N		1-N		2+		1-N		2+		0;, few 2		0;	
		1/3	1-N		2+, 3-		ON		3-		1N		3-		3-		1-N	
	M62-64-557 (M62-2672-2 -r_1)	1/3	1N, 2		2+, 3-		ON		3-		1-N		3-		3-		1-N	
		1/3	1-N		ON, 1-N		0;		0;		1-N		3-		3-		3	
		1/3	1-N, 2, 2+		0;		0;		ON		2+,3-?		2+, 3-?		3 or 3+		3 or 3+	
		1/3	1-N		ON, 1-N		0;		0;		1-N		3-		3-		3-	

APPENDIX TABLE IX - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-25 -6B-1 (1 x 4) (No reciprocal)	Cr65-25 -7A-1 (4 x 1)	Cr65-25 -7B-3 (1 x 4)
RI, GC 6, 15 Sept 1965	S37-388		5/5 3, 4	5/5 3, 4	5/5 3, 4	5/5 3, 4	5/5 3, 4	5/5 3, 4	5/5 3, 4
	M29-64-410 (Morden 29)		4/4 1, 2	5/5 3-, 3	5/5 0;	3/3 3, 3+	5/5 0;	5/5 1, 2-	5/5 1N, 2N 2+ few 3-
	CM9ORR		3/3 0;	2/2 0;	4/4 3, 4	3/3 3-,3	5/5 3-	2/5 0; 2/5 0; at very tips few 3- and 3	3/5 0; 1/5 0; at tips few 2+, 3-
	M62-64-631 (M62-2685-14-I)		I	I	I	I	I	1/5 3-, 3 I	1/5 2+, 3- I
MI, GC 13, 27 Jan 1966	S37-388	4/4	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-
	M29-63-868 (Morden 29)	3/3	0;, 1-	2+, 3-	ON, 0;	2+, 3-	ON, 0;	ON, 1	ON, 1N
	CM9ORR	1/3	0;	0;	2+, 3-	2+, 3-	3-	0;,1-, two 2+	1, three 2+
		1/3	0;	0;	3-	3-	2+	2+ or 3-	2+, 3-
		1/3	0;	0;	3-	3-	3	0;, 1, 2	0;, 1

APPENDIX TABLE IX - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-25 -6B-1 (1 x 4) (No reciprocal)	Cr65-25 -7A-1 (4 x 1)	Cr65-25 -7B-3 (1 x 4)
MI, GC 13, 27 Jan 1966	Cr69-64-211 (M69-W59-11-MC62-13-r_4)	1/4	0;	1-N	1-N	2+, 3-	One 2	1-N	2N
		1/4	0;	2+	1-N	2+, 3-	1-N	1N	1-N
		1/4	0;	1N, 2-	1	2+, 3-	1-N	1-N	1-N
		1/4	0;	0;	2N, 2	2+, 3-	2N, 2	1-N, 1	1-N, 1
	M62-64-557 (M62-2672-2 -r_1)	1/3	ON, 1-	0;	0;	0;	0;	3-	3-
		1/3	1-N, 2+	ON	ON	ON	ON	2+, 3-	2+, 3-
		1/3	ON, 1-	0;	0;	ON	ON	2+, 3-	2
	M62-64-664 (M62-2685-14-I)	7/7	I, 0;	I, 0;	I, 0;	I, 0;	I, 0;	I, 0;	I, 0;

1/ MI, multiple inoculation; RI, routine inoculation; GH, greenhouse; GC, growth cabinet

2/ Numerator indicates the number of seedlings showing particular reaction types, and denominator gives the number of seedlings tested

APPENDIX TABLE X. REACTION TYPES OF DIFFERENTIAL VARIETIES TO F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 2 AND 3

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races				F_1 (♀ × ♂)			
			1	2	3	4	Cr63-15A (3 x 2)	Cr63-15B (2 x 3)	Cr63-16A (3 x 2)	Cr63-16B (2 x 3)
MI,[1] GH	S37-388	3/3[2]	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4
	M29-63-830 (Morden 29)	3/7	ON	3, 4	ON	3, 4	ON	3, 4	0;	3
5 Mar 1964		4/7	ON	3, 4	ON	3, 4	ON	3, 4	very poor infection	
	CM9ORR	6/7	0;	0;	3, 4	3, 4	3, 4	3, 4	0;, 1	0;, 1
		1/7	0;	0;	3, 4	3, 4	3	3	3	0;
MI, GC 17,	S37-388	3/3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3	3-, 3	,3-, 3	3-, 3
	M29-63-868 (Morden 29)	1/4	ON	2+, 3-	0;	3	ON	3	0;	3
13 Dec 1965		1/4	ON	3	0;, ON	3	0;, ON	3	ON	3
		1/4	ON	3-	0;	2+, 3-	0;	3-	0;	2+, 3-
		1/4	0;	3	ON	2, 2+	0;	3	ON	3-

APPENDIX TABLE X - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races				F_1 (♀ × ♂)			
			1	2	3	4	Cr63-15A (3 x 2)	Cr63-15B (2 x 3)	Cr63-16A (3 x 2)	Cr63-16E (2 x 3)
MI, GC 17, 13 Dec 1965	CM9ORR	1/5	0;	0;	3-	3-	3-	2+, 3- ?	2	1, 2
		1/5	0;	0;	3-	3-	2+, 3- ?	3-	?	0;, 1-
		1/5	0;	0;	2+, 3- ?	2+, 3-	2+, 3+	ON, 2+	0;	0;
		1/5	0;	0;	3	3-	2+, 3-	2+, 3-	0;, 1	2
		1/5	0;	2, 2+	3	2+, 3-	3-	2+, 3-	2+	1, 2
	M69-64-241 (M69-W59-11-MC62-13-r4)	1/2	0;	0;, 1, 1+	1	2, 2+	1	2+, 3-	ON	2+
		1/2	0;	1-N, 1-	ON, 0;	2, 2+	0;, 2	2+, 3-	ON	1
	M62-64-557 (M62-2672-2-r1)	3/3	ON, 3	0;	0;	0;	0;	0;	0;	0;
	M62-64-664 (M62-2685-14-I)	8/8	I	I	I	I	I	I	I	I

APPENDIX TABLE X - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races				F_1 (♀ × ♂)			
			1	2	3	4	Cr63-17A (3 x 2)	Cr63-17B (2 x 3)	Cr63-18A (3 x 2)	Cr63-18B (2 x 3)
MI, GH	S37-388	3/3	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4
5 Mar 1964	M29-63-830 (Morden 29)	5/8	ON	3	0;, ON	3, 4	0;, ON	3	ON	3
		3/8	ON	3	ON	3, 4	- - - - - very poor infection - - - - -			
	CM9ORR	1/6	0;	0;	3, 4	3, 4	3, 4	3	3	3
		1/6	0;	0;	3, 4	3, 4	3, 4	3, 4	0;, 1	3-
		1/6	0;	0;	3, 4	3, 4	3, 4	2+, 3-	1	2+, 3-
		1/6	0;	0;	3, 4	3, 4	3	3	0;	0;, 1
		2/6	0;	0;	3, 4	3, 4	2, 2+	0;, 1	0;, 1	0;, 1, 2

APPENDIX TABLE X - continued

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-24-4A-1 (3 x 2)	Cr65-24-4B-1 (2 x 3)	Cr65-24-5A-1 (3 x 2)	Cr65-24-5B-1 (2 x 3)
RI, GC 6, 15 Sept 1965	S37-388		5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4
	M29-64-410 (Morden 29)		4/4 1,2	5/5 3-,3	5/5 0;	3/3 3,3+	5/5 0;	5/5 3-,3	5/5 0;	5/5 2+,3-
	CM9ORR		3/3 0;	2/2 0;	4/3 3,4	3/3 3-,3	5/5 3	5/5 3-,3	5/5 3	5/5 3-,3
MI, GC 13, 27 Jan 1966	S37-388	3/3	2+, 3-	2+, 3	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-
	M29-63-868 (Morden 29)	3/3	0;, 1-	2+, 3-	ON, 1-	2+, 3-	ON, 1-	2+, 3-	ON, 0;	2+, 3-
	CM9ORR	1/6	0;	0;	3	3, 3+	3+	3	3	3
		1/6	0;	0;, 2	2+, 3-	2+, 3-	2+, 3-	3-, 3	3	3
		1/6	0;	0;, 1	3-, 3	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-
		2/6	0;	0;	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-
		1/6	0;	0;	2+	2+	2+	2+	2+	2+
	Cr69-64-211 (M69-W59-11-MC62-13-r4)	1/4	0;	0;	1-N,1	2+	1N, 2	2+, 3-	1-N, 1	2+, 3-
		1/4	0;	2	ON, 2	2+, 3-	1-N,2	2+, 3-	1-N	2+, 3-
		1/4	1-N	2+, 3-	ON	2+, 3-	1-N	2+, 3-	ON	3-, 3
		1/4	2N,2	3-	ON	2+, 3-	1-N	2+, 3-	ON	3-, 3

APPENDIX TABLE X - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races				F_1 (♀ × ♂)			
			1	2	3	4	Cr65-24 -4A-1 (3 x 2)	Cr65-24 -4B-1 (2 x 3)	Cr65-24 -5A-1 (3 x 2)	Cr65-24 -5B-1 (2 x 3)
MI, GC 15, 27 Jan 1966	M62-64-557 (M62-267-2 -r1)	1/2	2N, 2+	0;	0;	0;	0;	0;	1-N	1-N
		1/2	1N, 2-	0;	0;	0;	0;	0;	0;	0;
	M62-64-664 (M62-2685-14-I)	8/8	I	I	I	I	I	I	I	I

			1	2	3	4	Cr65-4 -1A-2 (2 x 3)	Cr65-4 -1B-1 (3 x 2)
MI, GC 17, 28 Feb 1966	S37-388	4/4	3, 3+	3, 3+	3, 3+	3, 3+	3, 3+	3, 3+
	CM9ORR	1/6	0;	0;	3-	0;, few 2	3-	3-
		1/6	0;	0;	3	0;, 2	2+, 3-	3
		1/6	0;	0;	3, 3+	0;, few (2, 2+)	0;, 1, 2, 2+	2+, 3-, 3
		1/6	0;	0;	3	3-	0;, 1, 2, 2+	3-
		1/6	0;	0;	3, 3+	1, 2, few (3- or 3)	0;, 1, 2+	2+, 3-
		1/6	0;	0;	0;, ON	0;	ON, 0;	1

APPENDIX TABLE X - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-4 -1A-2 (2 x 3)	Cr65-4 -1B-1 (3 x 2)
MI, GC 17,	M29-63-868 (Morden 29)	3/3	ON	3, 3+	ON	3-	2+, 3-	2+, 3-
28 Feb 1966	M69-63-562 (MC62-13-r_4)	1/2	0;	0;	0;	ON, 0;	1, 2, few 2+	0;, 1
	M69-64-211 (M69-W59-11-MC62-13-r_4)	1/2	0;	0;	0;, ON	0;	ON, 0;	1
		1/3	ON	ON	ON	2	2, 2+	2+
		1/3	0;	ON	1-N	ON, 1+	2, 2+	2+
	M62-64-555 (M62-2672-2 -r_1)	1/3	0;	0;	0;, few 2	0;, 1	1+, 2	2
		1/1	3	0;	0;	0;	0;, five 3-	0;
	M62-64-557 (M62-2672-2 -r_1)	1/3	3	0;	0;	0;	0;	0;
		1/3	3-	0;	0;	0;	0;, three 3-	0;, ON
	M62-64-664 (M62-2685-14-I)	1/3	2+, 3-	0;	0;	0;	ON, few 2+	ON
		7/7	I	I	I	I	I	I

1/ MI, multiple inoculation; RI, routine inoculation; GH, greenhouse; GC, growth cabinet

2/ Numerator indicates the number of seedlings showing particular reaction types, and denominator gives the number of seedlings tested.

APPENDIX TABLE XI REACTION TYPES OF DIFFERENTIAL VARIETIES TO F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 2 AND 4

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-22 -1A-1 (2 x 4)	Cr65-22 -1B-1 (4 x 2)	Cr65-22 -2A-1 (2 x 4)	Cr65-22 -2B-1 (4 x 2)
RI,[1] GC 6,	S37-388	5/5[2]	3, 4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4
	M29-64-410 (Morden 29)	4/4	1, 2	5/5 3-,3	5/5 0;	3/3 3,3+	5/5 3,4	5/5 2+,3-,3	5/5 3,3+	4/4 2+,3-
15 Sept 1965	CM9ORR	3/3	0;	2/2 0;	4/4 3,4	3/3 3-,3	3/5 3-,3 1/5 0;, 2+,3- 1/5 0;	2/5 3,4 2/5 0;, at tips (2+,3-) 1/5 0;,2+	3/6 0;,2 2/6 0;, at tips 3 1/6 2+	2/5 0; 3/5 0;, at tips 3
MI, GC 2,	S37-388	3/3[2]	3+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-
	M29-63-868 (Morden 29)	1/3	1-N	2+, 3-	0;	2+, 3-	2+, 3-	2+, 3-	3-	2N, 2+
11 Nov 1965		1/3	1-N	2+, 3-	0;	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-
		1/3	0;, ON	2+, 3-	0;	2+N, 2+	ON, 2+	2+, 3-	2+N, 2+	2+N, 2+
	CM9ORR	1/4	1-N	1-N	3	2+, 3-	2+, 3-	2+, 3-	2N, 2	2N, 2
		3/4	0;	0;	3-	2+, 3-	0;, 1N few 2	0;, 1, few 2	0;, 1, few 2	0;, 1, few 2

APPENDIX TABLE XI - continued

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1	2	3	4	F_1 (♀ x ♂) Cr65-22 -1A-1 (2 x 4)	Cr65-22 -1B-1 (4 x 2)	Cr65-22 -2A-1 (2 x 4)	Cr65-22 -2B-1 (4 x 2)
MI, GC 2, 11 Nov 1965	M69-64-224 (M69-W59-11-MC62-13-r_4)	1/2	ON	ON, few 2	ON	2N, 2+ few 3-?	2	2	1N	1N
		1/2	1-N	3-, 3	1-N	2+, 3-	2+, 3-?	3-, 3	3-, 3	2N, 2+
	M62-64-557 (M62-2672-2 -r_1)	6/6	2N, 2+,3-	0;	0;	0;	0;	0;	0;	0;
	M62-64-664 (M62-2685-14-I)	6/6	I	I	I	I	I	I	I	I

			1	2	3	4	Cr65-22 -3A-1 (2 x 4)	Cr65-22 -3B-1 (4 x 2)
RI, GC 6, 15 Sept 1965	S37-388	5/5	3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4
	M29-64-410 (Morden 29)	4/4	1,2	5/5 3-,3	5/5 0;	3/3 3,3+	6/6 3-,3	5/5 3,4
	CM9ORR	3/3	0;	2/2 0;	4/4 3,4	3/3 3-,3	2/4 0; 1/4 0;,1,2 1/4 0;, at tip 3	2/4 3- 1/4 0;, at base 2+,3-
	M62-64-631 (M62-2685-14-I)		I	I	I	I	I	I

APPENDIX TABLE XI - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-22 -3A-1 (2 x 4)	Cr65-22 -3B-1 (4 x 2)
MI, GC 2,	S37-388	3/3	3+	3+	3+	3+	3+	3+
	M29-63-868 (Morden 29)	1/3	ON	2+, 3-	ON	2+, 3-	2+, 3-	?
11 Nov 1965		1/3	ON, 2	2+, 3-	ON	2+, 3- ?	2+, 3-	2+, 3-
		1/3	ON	?	ON	1, 2+	2+, 3-	2+, 3-
	CM9ORR	4/6	0;	0;	3-	3-	0;, 1, 2, 2+	0;, 1, 2, 2+
		1/6	0;	0;	2+, 3-	2+, 3-	0;, 1, 2+	0;, 1, 2
		1/6	0;	0;	2+	2	1-N, 1	1-N, 1
	M69-64-224 (M69-W59-11-MC62-13-r_4)	1/3	0;	ON	ON	2+, 3-	ON	ON
		2/3	0;	ON	ON	2+	ON	ON, 1
	M62-64-557 (M62-2672-2-r_1)	5/5	2+, 3-	0;	0;	0;	0;	0;
	M62-64-664 (M62-2685-14-I)	8/8	I	I	I	I	I	I
MI, GC 17,	M29-63-868 (Morden 29)	1/3	Inoculation failed	3, 4	ON	3-	3-	3-
7 Mar 1966		2/3	"	2+, 3-	ON	2+, 3-	2+, 3-	2+, 3-

APPENDIX TABLE XI - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-22 -3A-1 (2 x 4)	Cr65-22 -3B-1 (4 x 2)
MI, GC 17,	CM9ORR	1/6	Inoculation failed	0;, few 2	3, 4	4	3	2+, 3-
7 Mar 1966		1/6	"	0;	3	3-, 3	2+, 3-?	3-
		1/6	"	0;	3-	3-	3-	2+, 3-
		1/6	"	0;, 1, 1+	3-	3-	2+	2+
		1/6	"	0;	3, 4	0;, 2+, 3-?	0;, 1, 2	0;
		1/6	"	0;	3	2 or 2+	0;, 1, 2	0;

			1		2		3		4		Cr65-25 -2A-1 (4 x 2)		Cr65-25 -2B-1 (2 x 4)	
RI, GC 6,	S37-388	5/5	3, 4	5/5	3, 4	5/5	3, 4	5/5	3, 4	5/5	3, 4	5/5	3, 4
	M29-64-410 (Morden 29)	4/4	1, 2	5/5	3-, 3	5/5	0;	3/3	3, 3+	5/5	2+, 3-	5/5	3-, 3
15 Sept 1965	CM9ORR	3/3	0;	2/2	0;	4/4	3,4	3/3	3-, 3	5/5	3-, 3	3/3	3+
	M62-64-631 (M62-2685-14-I)		I		I		I		I		I		I

APPENDIX TABLE XI - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-25 -2A-1 (4 x 2)	Cr65-25 -2B-1 (2 x 4)
MI, GC 2, 11 Nov 1965	S37-388	4/4	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-
	M29-63-868 (Morden 29)	3/3	ON	3-, 3	ON, 0;	2+, 3-	2+, 3-	3-, 3
	CM9CRR	1/3	0;	0;	2+, 3-	2+, 3-?	2+, 3-?	2+, 3-
		1/3	0;	0;	2+, 3-	2+	2+	2+, 3-
		1/3	0;	0;	2+, 3-	?	2+, 3-	2+, 3-
	M69-64-241 (M69-W59-11-MC62-13-r_4)	1/6	ON	1-N	1N	3-	2+, 3-	3
		1/6	ON	ON	3	3	3	3
		4/6	ON	ON, 1-	2+, 3- ?	2+, 3- ?	2+, 3- ?	2+, 3-
	M62-64-557 (M62-2672-2-r_1)	3/3	3-, 3	0;	0;	0;	0;	0;
	M62-64-664 (M62-2685-14-I)	7/7	I	I	I	I	I	I

APPENDIX TABLE XI - continued

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-25 -2A-1 (4 x 2)	Cr65-25 -2B-1 (2 x 4)
MI, GC 17,	Cr29-63-868	4/4	Inoculation failed	3-	ON	2+, 3-	2+, 3-	2+, 3-
7 Mar 1966	CM9CRR	3/6	"	0;	3, 4	3, 4	3, 4	3, 4
		1/6	"	0;	3, 4	0;, 1, 2	3	3
		1/6	"	0;	3-	2+, 3-	3-	2+, 3- ?
		1/6	"	0;	2+, 3-	0;, 1, 2	2+, 3-	2+, 3-

1/ MI, multiple inoculation; RI, routine inoculation; GH, greenhouse; GC, growth cabinet.

2/ Numerator indicates the number of seedlings showing particular reaction types, and denominator gives the number of seedlings tested.

APPENDIX TABLE XII REACTION TYPES OF DIFFERENTIAL VARIETIES TO F_1 UREDIAL CULTURES DERIVED FROM CROSSES BETWEEN RACES 3 AND 4

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-26 -2A-1 (3 x 4)	Cr65-26 -2B-1 (4 x 3)
RI,[1] GC 6,	S37-388	5/5[2]	3, 4	5/5 3, 4	5/5 3, 4	5/5 3, 4	5/5 3, 4	5/5 3, 4
25 Sept 1965	M29-64-417 (Morden 29)	5/5	1N, 1, 2, few 2+	6/6 3-, 3	6/6 0;, 1-, 1	6/6 3- 3	6/6 0;, 1, 1+	6/6 3+
	CM9ORR	4/4	0;	5/5 0;	5/5 3, 4	6/6 3, 4	2/5 3- or 3 3/5 0;, 2+, scattered 3- or 3	4/4 3, 4
	M62-64-631 (M62-2685-14-I)	5/5	I	6/6 0;	6/6 I	8/8 I	7/7 3+	6/6 3
MI, GC 2,	S37-388	4/4[2]	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-
11 Nov 1965	M29-63-868 (Morden 29)	3/3	ON	3-, 3	ON, 0;	2+, 3-	0;, ON	2+, 3-, 3
	CM9ORR	1/3	0;	0;	2+, 3-	2+, 3- ?	2, 2+	0;, 1
		1/3	0;	0;	2+, 3-	2+	1, 2	0;, 1
		1/3	0;	0;	2+, 3-	?	2	1N, 1
	M69-64-224 (M69-W59-11-MC62-13-r_4)	1/2	1N	3	0;	3	ON	3
		1/2	ON	ON	1-	2+, 3-?	ON	2N, 2+

APPENDIX TABLE XII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-26 -2A-1 (3 x 4)	Cr65-26 -2B-1 (4 x 3)
MI, GC 2,	M69-64-241 (M69-W59-11-MC62-13-r_4)	1/6	ON	1-N	1-N	3-	ON	2N, 2+
		1/6	ON	ON	3	3	3	3
11 Nov 1965		4/6	ON	ON, 1-	2+, 3-	2+, 3-	ON	2+, 3- ?
	M62-64-557 (M62-2672-2-r_1)	3/3	3-, 3	0;	0;	0;	0;	0;
	M62-64-664 (M62-2685-14-I)	7/7	I	I	I	I	3-, 3, 3+	3-, 3, 3+
MI, GC 17,	M29-63-868 (Morden 29)	4/4	Inoculation failed	3-	ON	2+, 3-	ON	2+, 3-
7 Mar 1966	CM9CRR	3/6	"	0;	3, 4	3, 4	3, 4	3, 4
		1/6	"	0;	3, 4	0;, 1, 2	1, 2, 2+	0;, 1, 2
		1/6	"	0;	3-	2+, 3-	0;, 1	2+, 3-?
		1/6	"	0;	2+, 3-	0;, 1, 2	0;	0;, 1

APPENDIX TABLE XII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-26 -3A-2 (3 x 4)	Cr65-26 -3B-2 (4 x 3)	Cr65-26 -5A-2 (3 x 4)	Cr65-26 -5B-2 (4 x 3)
RI, GC 6,	S37-388		5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	3,4
25 Sept 1965	M29-64-417		5/5 1N,2, few 2+	6/6 3-,3	6/6 0;, 1-,1	6/6 3-,3	5/5 0;	5/5 3,3+	6/6 0;, 1	5/5 3-, 3
	CM9ORR		4/4 0;	5/5 0;	5/5 3, 4	6/6 3,4	5/5 3,3+	6/6 3,4	5/5 3	5/5 3
	M62-64-631 (M62-2685-14-I)		5/5 I	6/6 0;	6/6 I	8/8 I	6/6 3+	6/6 3+	5/5 I	6/6 I
MI, GC 2,	S37-388	3/3	3-	3-	3-	3-	3-	3-	3-	3-
	M29-63-868	1/4	1-N, 1	3	ON	2+, 3-	0;, ON	2+, 3-	ON	2+, 3-
11 Nov 1965		1/4	?	3-	ON	2+, 3-	0;, 1, 2	2+, 3-?	?	2+, 3-
		1/4	ON	3	ON	2+N	ON	2+, 3-?	ON, 0;	2+, 3-?
		1/4	ON	2+, 3-?	ON	2+, 3-?	0;	?	ON, 0;	2+, 3-?
	CM9ORR	1/5	0;	0;	3	2+, 3-	3	3	3-	3
		3/5	0;	0;	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-	2+, 3-
	M69-64-224 (M69-W59-11-MC62-13-r4)	1/5	0;	0;	2+, 3-	2	2+, 3-	2, 2+	2	2, 2+
		1/3	1-N	0;	ON	2+, 3-	?	3	ON	3
		1/3	0;	0;	ON	2+	ON	2+, 3-	ON	2+
		1/3	ON	2+, 3-	ON	3-	2, 2+	2+, 3-	1N	3-, 3

APPENDIX TABLE XII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-26 -3A-2 (3 x 4)	Cr65-26 -3B-2 (4 x 3)	Cr65-26 -5A-2 (3 x 4)	Cr65-26 -5B-2 (4 x 3)
MI, GC 2, 11 Nov 1965	M69-64-241 (M69-W59-11-MC62-13-r_4)	2/2	ON	1, 2	1, 2	2+, 3-?	1, 2	2+, 3-	1N	2+, 3-
	M62-64-557 (M62-2672-2-r_1)	2/5	3	0;	0;	0;	0;	0;	0;	0;
		1/5	3+	ON, 0;	ON, 0;	ON, 0;	ON, 0;	0;	2+, 3-?	0;
		1/5	3	0;	0;	2N	2N, 2+	ON, 2	2+, 3-	0;
		1/5	1-N	0;	0;	0;	0;	0;	2+, 3-	0;
	M62-64-664 (M62-2685-14-I)	1/8	I	I	I	I	3	3	I	I
		3/8	I	I	I	I	2+, 3-	2+, 3-	I	I
		4/8	I	I	I	I	2, 2+	2, 2+	I	I
MI, GC 17, 7 Mar 1966	M29-63-868 (Morden 29)	3/3	Inoculation failed	3-, 3	ON	2+, 3-, 3	ON	3	ON	3-, 3

APPENDIX TABLE XII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seedlings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-26 -3A-2 (3 x 4)	Cr65-26 -3B-2 (4 x 3)	Cr65-26 -5A-2 (3 x 4)	Cr65-26 -5B-2 (4 x 3)
MI, GC 17,	CM9CRR	4/8	Inoculation failed	0;	3, 4	3, 4	3, 4	3, 4	3, 4	3, 4
7 Mar 1966		1/8	"	0;	3-	2+	3, 3+	3	3-	3
		1/8	"	0;	3-	3-	1N, 2 three 3-	3-	3-	3
		1/8	"	0;	3-	2, 2+	2+, 3-	2+, 3-?	2+, 3-?	3-
		1/8	"	0;	3	2	2, 2+, 3-	2+, 3-	2+	2+

			1	2	3	4	Cr65-26 -6A-2 (3 x 4)	Cr65-26 -6B-2 (4 x 3)
RI, GC 6,	S37-388	5/5	3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4	5/5 3,4
	M29-64-417 (Morden 29)	5/5	1N,2, few 2+	6/6 3-,3	6/6 0;, 1-, 1	6/6 3-,3	5/5 0;	5/5 3
25 Sept 1965	CM9CRR	4/4	0;	5/5 0;	5/5 3,4	6/6 3,4	2/5 3+ 2/5 0;,1, 2,2+ at tips 3- 1/5 0;,1	2/5 3 3/5 0;, at tips 2+, 3-
	M62-64-631 (M62-2685-14-I)		I	I	I	I	I	I

APPENDIX TABLE XII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-26 -6A-2 (3 x 4)	Cr65-26 -6B-2 (4 x 3)
MI, GC 2,	S37-388	3/3	3-	3-	3-	3-	3-	3-
	M29-63-868 (Morden 29)	2/4	ON, 1-N	3	ON	2, 2+	ON	3
11 Nov 1965		2/4	ON	2+, 3-	ON	2, 2+	ON	2+, 3-
	CM9ORR	1/3	0;	0;	3-	3-	1-	0;, 2
		2/3	0;	0;	3-	2, 2+	0;,1,2	1, 2
	M69-64-224 (M69-W59-11-MC62-13-r4)	4/6	ON	ON	ON	2+	ON	2+
		1/6	1-N	3	ON	3	ON	3
		1/6	ON	3	ON	2+, 3-?	ON	3-
	M69-64-241 (M62-W59-11-MC62-13-r4)	1/2	ON	0;	1-N	2+	ON	2+
		1/2	0;	0;	2N	2+	ON	2
	M62-64-557 (M62-2672-2-r1)	1/2	3	0;, 1, 2	0;	ON, 2	m ON, 2	ON, 1
		1/2	2+, 3-	0;	0;	0;	ON, 1	2+
	M62-64-664 (M62-2685-14-I)	6/6	I	I	I	I	I	I

APPENDIX TABLE XII - continued

Method of inoculation, environment, and date tested	Varieties	No. of seed-lings	Parent races 1	2	3	4	F_1 (♀ × ♂) Cr65-26 -6A-2 (3 x 4)	Cr65-26 -6B-2 (4 x 3)
MI, GC 17,	M29-63-868	3/3	No infection	3, 3+	ON	3-, 3	3, 4	3, 4
7 Mar 1966	CM9ORR	3/6	"	0;, few 1	3, 3+,4	3, 3+, 4	3, 4	3, 4
		3/6	"		3, 3+	0;, 1, 2, 2+	0;, 1	0;, 1

1/ MI, multiple inoculation; RI, routine inoculation; GH, greenhouse; GC, growth cabinet.

2/ Numerator indicates the number of seedlings showing particular reaction types, and denominator gives the number of seedlings tested.