Selection procedures for durable resistance in wheat.

Promotor: dr. J.E. Parlevliet, hoogleraar in de plantenveredeling

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# Selection procedures for durable resistance in wheat.

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# Stellingen

I. In veel gevallen wordt niet resistentie tegen pathogenen gemeten, maar een interactie van resistentie met eigenschappen als vroegheid en strolengte.

II. Gezien de duurzaamheid van vele partiële resistenties leent het onderzoek hieraan zich bij uitstek voor programma's in de ontwikkelingslanden waar oogstzekerheid een zeer hoge prioriteit heeft.

III. In Brazilië worden controle rassen in tarwe ten behoeve van de rassenlijst te vaak gewisseld om ook de stabiliteit van de opbrengst (één van de belangrijkste eigenschappen) te kunnen vergelijken.

IV. De subsidie op tarwe heeft in Brazilië de landbouwproductiviteit verlaagd, de kostprijs van tarwe verhoogd en de betalingsbalans sterk negatief beïnvloed.

V. Het gebruik van de term 'crop gains' in plaats van 'crop losses' zou veel overheden stimuleren meer geld beschikbaar te stellen voor resistentie programma's.

VI. Er wordt door veredelingsbedrijven vaak gestreefd naar een nodeloos hoog niveau van resistentie (8 à 9 op de rassenlijst), waar een redelijk niveau (6 à 7) al ruim voldoende zou zijn.

VII. Ten gevolge van de nul-tolerantie voor *Pseudomonas solanearum* in pootaardappelen in Brazilië wordt het oorspronkelijk oerwoud ernstig aangetast.

VIII. Vooral partikuliere veredelingsbedrijven kunnen een belangrijke rol spelen bij de voorziening van hoogwaardig zaaizaad in ontwikkelingslanden.

IX. Op het gebied van plantenveredeling wordt er door de internationale instituten te veel voor en te weinig met de ontwikkelingslanden gedacht.

X. Helaas wordt vaker naar de kwaliteit van de informatieverschaffer gekeken dan naar de kwaliteit van de informatie zelf.

Stellingen behorende bij het proefschrift 'Selection procedures for durable resistance in wheat' door Martinus A. Beek.

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# Contents

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# List of abbreviations and symbols

| | |
|---|---|
| a.i. | active ingredient |
| ANOVA | analysis of variance |
| BSS | Bulk Seed Selection |
| C | degree centigrade |
| CF | Conductivity Factor |
| CIMMYT | Centro Internatioal de Mejoramiento de Maiz y Trigo, Mexico |
| CNPHortaliças | Centro Nacional de Pesquisa de Hortaliças |
| CNPTrigo | Centro Nacional de Pesquisa de Trigo |
| CPACerrado | Centro de Pequisa Agropequária dos Cerrados |
| DC | Decimal Code (Zadoks et al., 1974) |
| ELISA | Enzyme-Linked Immunosorbent Assay Technique |
| EMBRAPA | Empresa Brasileira de Pesquisa Agropecuária |
| EMPASC | Empresa Catarinense de Pesquisa Agropecuária |
| FAO | Food and Agricultural Organization of the United Nations, Rome |
| FECOTRIGO | Federação das Cooperativas Brasileiras de Trigo e soja Ltda |
| FT | Francisco Teresawa Pesquisa e Sementes |
| ft | foot |
| G | Generation |
| $h^2$ | heritability |
| IAPAR | Fundação Instituto Agronômico do Paraná |
| IPAGRO | Instituto de Pesquisas Agronômicas in Porto Alegre |
| IPEAS | Instituto de Pesquisas Agropecuárias do Sul |
| IT | Infection Type |
| LP | Latent Period |
| LS | Line Selection |
| NS | Natural Selection |
| OCEPAR | Organização das Cooperativas do Estado do Paraná |
| ppm | parts per million |
| PR | Paraná |
| r | correlation coefficient (Pearson) |
| RLP | Relative Latent Period |
| RNCOL | Relative Number of Colonies |
| RS | Rio Grande do Sul |
| SL | Southern Latitude |
| SPS | Single Plant Selection |
| US | United States |
| v/v | volume/volume |

Statistics:

| | | |
|---|---|---|
| *** | very highly significant | ($p < 0.001$) |
| ** | highly significant | ($p < 0.01$) |
| * | significant | ($p < 0.05$) |

# 1 The need of breeding for durable resistance in Brazil

## 1.1 Introduction

Brazil has made determined efforts to increase wheat production with a view to self-sufficiency. Wheat imports increased from just over 100 million US dollar around 1970 to over 700 million US dollar in the period 1980-1984 after which the costs of imports decreased. The understandable search for self-sufficiency has been directed more to the expansion of the wheat crop area than to yield increases per ha (Table 1.1). Wheat became cultivated in all areas of Brazil, economically marginal areas not excluded. Many of these areas have climates very conducive to the development of various pathogens. Airborne wheat pathogens can now move easily from wheat crop to wheat crop between 10 and 35 SL forming an enormous epidemiological area. The control of rusts and powdery mildew based on vertical (major gene) resistance appeared to be inadequate due to rapid break downs of resistance. The average duration of such a resistance based on major genes appeared to be a few years only.

In the period 1969-1986 wheat yields in Rio Grande do Sul (RS) varied between 309 and 1,430 kg/ha (Anonymous, 1962-1977; Anonymous, 1978-1986). Extensive experiments of Kugler (1978) showed that wheat yields in Passo Fundo, RS were severely reduced by pests and diseases. Protection with fungicides doubled the yields, protection with insecticides trebled the yields.

Under the auspices of the Food and Agriculture Organization of the United Nations, a number of wheat breeding programmes aimed at durable resistance were initiated in the Third World countries during the 1970's (Robinson & Chiarappa,1977). The main programmes have been executed in Zambia, Morocco and Brazil (Beek 1976,1983; De Milliano,1983; Van der Graaff,1985). The programme in Brazil was carried out from November 1975 to April 1983 at the 'Centro Nacional de Pesquisa de Trigo (CNPTrigo)', The National Wheat Research Centre of EMBRAPA in Passo Fundo, Rio Grande do Sul, Brazil.

Table 1.1. Total wheat imports in tons (x 1000), wheat production in tons (x 1000), wheat area in ha (x 1000) and wheat yields in tons/ha per year in Brazil in three periods (after Anonymous, 1962-1977; Anonymous, 1978-1986).

| Three year period | wheat imports | wheat production | wheat area | yield |
|---|---|---|---|---|
| 1969-1971 | 1,904 | 1,640 | 1,723 | 0.943 |
| 1977-1979 | 3,608 | 2,532 | 3,306 | 0.778 |
| 1984-1986 | 3,481 | 3,912 | 2,773 | 1.382 |

Its purpose was to determine the feasibility of accumulating useful levels of durable resistance to all locally important pathogens of wheat. In addition the programme aimed at producing new wheat cultivars, which combined this durable resistance with other agronomic useful characteristics. To this end a set of selection procedures was compared, which were all based on selection in early generations (see chapter 3, 4 and 7).

# 2 Wheat in Brazil

## 2.1 Wheat production in Brazil

Wheat was introduced in the 16th century in Brazil (Carmo,1911). Its importance increased only slowly, but around 1800 the production of wheat had grown to such an extend that export was possible from Rio Grande do Sul. Due to rust epidemics the wheat yield decreased rapidly after 1813 and so did the exports.

Wheat was introduced again in the second part of the 19th century. Immigration from Europe took place on a large scale. There is no doubt that the immigrants brought wheat seed from their home countries and the wheat cultivars originating from Italy seemed to have had the best adaptation for the conditions of soil and climate of Southern Brazil. In 1920, the wheat acreage was about 100,000 ha which grew to 200,000 ha in 1940. Of this acreage 90% was in Rio Grande do Sul. This state remained the main wheat growing area of Brazil, growing 76% of the cultivated area in 1973, but its relative importance diminished to 45% in 1982. Paraná became a second important state for producing wheat and the latest tendency is a change in the area of cultivation towards states such as Mato Grosso do Sul (Table 2.1).

## 2.2 Main wheat pathogens in Southern Brazil

In Southern Brazil the most important leaf pathogens are: Puccinia recondita Rob. ex Desm. *f.sp.tritici*, *P. graminis* Pers. *f.sp.tritici* Eriks & E. Henn., *Erysiphe graminis* De. *f.sp.tritici* E.Marchal, *Septoria tritici* Rob. ex Desm., *Cochliobolus sativus* (Ito et Kurib.) Drechsl. ex Dastur. On the ears and grains the most common pathogens are: *S. nodorum*, *E. graminis* and *Fusarium graminearum* Schwalbe. Roots are heavily attacked by the Common root rot complex, of

Table 2.1. Wheat acreage in ha (x 1000) per year in various states of Brazil in two periods (Anonymous,1978-1981).

| State | 1981-1982 | 1985-1986 |
|---|---|---|
| Rio Grande do Sul | 1,128 | 1,055 |
| Paraná | 1,082 | 1,596 |
| Mato Grosso do Sul | 132 | 309 |
| São Paulo | 133 | 168 |
| Other states | 37 | 86 |

which *C. sativus* is most important followed by *Fusarium* species and *Colletotrichum graminicola* (Ces) G.W. Wils.. Moreover *Gaeumannomyces graminis* (Sacc.) Arx & Oliver, *var. tritici* Walker can be commonly found on the roots. Two viruses are widely spread: Barley Yellow Dwarf Virus being transmitted by aphids and Soil Borne Mosaic Virus of which *Polymyxa graminis* is the vector.

## 2.3 Wheat breeding in Brazil

Wheat breeding started in 1919 in Southern Brazil. At the initial stages of the wheat breeding programmes, selections were made within existing landraces. These selections were in general well adapted to the climate and acid soil, but very tall and susceptible to the main pathogens such as leaf rust, stem rust and flag smut (Da Silva,1954). The first cultivars selected from land-race cultivars were: Alfredo Chaves 3/21, Alfredo Chaves 6/21, Colonista, Montes Claros, Polyss, Ponta Grossa 142, Salles, Turco, Veadores, III.AC 2, XIII.AP (Lagos,report).

These cultivars formed the basis for the next phase of plant breeding using programmes based on cross breeding. In 1954 a considerable number of new cultivars had already been released to the farmers: Frontana, Trintecinco, Rio Negro, Bagé, Colônias, Patriarca, Trintani, PG 1, Kenia 155, Bandeirante. Petiblanco was a wheat cultivar from La Estanzuela, Uruguay, cultivated in Brazil. All these cultivars were susceptible to leaf rust and stem rust. Breeding programmes were set up for incorporation of leaf rust and stem rust resistance based on the cultivars Kenia 58 and Red Egyptian. The cultivar Sinvalocho from the Argentine was used as another source of resistance to leaf rust (Da Silva,1954). The most successful cultivar of this early breeding work has been Frontana, which was released in 1942 and 24 years later it still represented 67% of the wheat acreage (Tomasini, 1980) and is still grown at present. Two other interesting cultivars from these first cross breeding efforts are BH-1146 and Horto both carrying a good level of partial resistance to leaf rust, which did not break down although they were grown successfully for a long period of more than 30 years.

From 1969 onwards the wheat station of IPEAS at Passo Fundo became the centre for wheat research in Brazil. In 1974 the Ministry of Agriculture transferred all the responsibility of this research to the CNPTrigo of EMBRAPA in Passo Fundo. Other important wheat breeding institutes in Rio Grande do Sul became: FECOTRIGO in Cruz Alta established in 1971 and IPAGRO in Porto Alegre.

In Paraná the breeding programmes have been carried out by IAPAR in Londrina since 1973 and OCEPAR in Curitiba-Cascavel, the cooperative organization in Paraná. Very recent private breeding work started in Ponta Grossa by the company Francisco Teresawa (FT).

In Santa Catarina a breeding programme is conducted on a small scale by EMPASC, the state agriculture research institution.

Every year two meetings are held to compare the cultivar trial data to recommend those cultivars that should be grown in the northern and southern parts of Brazil respectively. These meetings are organized by the group of breeding institutions and companies. Bank credit for farmers has been restricted to those adopting the recommendations of this committee.

# 3 Selection in early generations

## 3.1 Partial resistance

By the definition of Parlevliet (1988), partial resistance is quantitative resistance based on minor genes, which is considered to be much more durable than resistance based on major, race-specific genes. Also Caldwell (1968), Simons (1972) and Parlevliet & Zadoks (1977) agree that polygenically inherited partial resistance provides durable resistance. Selection for partial resistance is often not easy and adequate screening methods are required. Partial resistance is evaluated by measuring the amount of tissue affected by the pathogen and it is this measure that can be influenced by differences in level of disease pressure. The differences of the observed level of tissue affected between host genotypes can be influenced significantly by interplot interference, partial escape effects due to differences in earliness, inoculum density, moment of disease assessment and plant habit, such as tallness (Parlevliet,1988) and so obscure real differences in partial resistance. In the segregating populations the selection for minor genes is even more difficult when a considerable part of the genetic variance is of a non-additive nature and the heritability in the narrow sense of the characteristic under selection is low. The environment and genotype x environment interaction may have a great influence on the efficiency of the screening method. Considering all this together, it is clear that the development of an optimal selection procedure is extremely important and not easy.

The basic procedure of the partial resistance programme has been the concept of Robinson (1973,1976) as described in chapter 4, which is in principle an early (F2) generation selection. However a selection procedure for the accumulation of many minor genes of resistance to several pathogens simultaneously in an early generation is expected to be less efficient than selection in later (F4–F6) generations. Selection for improved levels of partial resistance alone is of little use. This partial resistance has to be embedded in genotypes with a good agronomic value. This means that selection in these early generations should also take care of characteristics like yield, plant length, etc. Especially for a complex characteristic like yield early generation selection may create a problem.

Stable yields through durable resistance is a main objective of the wheat breeding programme in Brazil. First priority has been given in this research programme to those pathogens where the break-down of resistance is most frequent. The traditional brazilian wheat breeding found that race-specific resistance is useless against the biotrofic fungi leaf rust, stem rust and powdery mildew because of its rapid break-down. Therefore partial resistance breeding, avoiding with great care race-specific resistance, was especially directed against these pathogens.

With the necrotrofic pathogens of wheat, *S. nodorum, S. tritici, C. sativus* and *F. graminearum*, the break-down of resistance was not noticed at CNPTrigo. With regard to the pathogens of this group, it was observed that the resistance is predominantly if not solely quantitative in expression. Quantitative resistance is not always non-race-specific as for example demonstrated with *S. tritici* (Eyal et al.,173; Ziv & Eyal,1978). However, considering the durability of resistance already experienced at CNPTrigo, it has not been a concern here and therefore selection was made for quantitative resistance without special measures to avoid possible race-specific resistance as done with the biotrofic fungi.

## 3.2 Selection for yield; A literature review

### *3.2.1 Selection for yield in early generations*

Selection for yield in early generations has been discussed for a long time. It is assumed that yield is controlled by a large number of genes, which means that accumulating most or all desired genes in one selection cycle is not possible. However, through recurrent selection one can accumulate those desirable genes provided their effects are recognizable. Recognizing the superior genotypes in early generations however can be a serious problem as the heritability of the character is generally low especially in early generations. This low heritability is for a small part due to the fact that the additive variance is smallest in the earliest generation (Table 3.1) (Mather,1971). A much larger source of error is the sensitivity of a complex character like yield for environmental variations and fluctuations and the rather large contribution of the genotype x environment interactions hidden in the genotypic variance of experiments, when tested at one location and one year, the normal situation in early generations. The heritability for a complex character like yield increases rapidly after the F2 because the additive variance increases and because of the larger number of plants that are available for evaluating yield.

Table 3.1. Components of genetic variance for independently segregating loci in F2, F3, F4, F$\infty$, in a cross between 2 homozygous lines and the absence of epistasis. D = additive effects, H = dominance effects. (Mather,1971).

| | Variance | | |
|---|---|---|---|
| Generation | Total | Between lines | Within lines |
| F2 | 1/2D + 1/4H | | |
| F3 | 3/4D + 3/16H | 1/2D + 1/16H | 1/4D + 1/8H |
| F4 | 7/8D + 7/64H | 3/4D + 3/64H | 1/8D + 1/16H |
| F$\infty$ | D | D | 0 |

In spite of these problems, most breeders prefer to select early in order not to loose the opportunity of gaining at least some progress from early generation selection.

Immer (1942) found with barley that on a single plant basis the variation of yield of spaced single plants (heterozygous or homozygous) was determined by environmental factors. Similar conclusions were reported by Kalton (1948) with soybeans in F2, F3 and F4 populations of 25 crosses. Variation of grain yield seemed to be almost entirely determined by the environment. Competition effects can be important and were well reviewed by Spitters (1979) and are discussed later in chapter 3.2.2. Bos (1981) concluded from his work on single plant selection in winter rye that both competition and environmental influences can disturb the efficiency of early generation selection considerably. Skorda (1973) concluded that selection of wheat plants in the F2 can only be efficient when the environmental variation is reduced to a minimum, which can be achieved by improving the experimental design. Many others investigated the possibility of direct selection for yield in the early generations too. The results however vary greatly. For wheat Harrington (1940), Busch et al. (1974), Shebeski (1967), De Pauw & Shebeski (1973) and Cregan & Busch (1977) reported positive results from early generation selection. Early generation selection has also often been reported as unsuccessful. Fowler & Heyne (1955) failed to find any positive effects from early generation. Mc. Ginnis & Shebeski (1968) also found that individual F2 plant selection is inefficient for yield. Selected F2 plants from the population were not significantly better than the non-selected population in the following generation. For barley Harlan et al. (1940) and Smith & Lambert (1968) found positive results from early generation testing, while Frey (1954a, 1954b) and Frey & Horner (1955) indicated that in two barley crosses the performance of F2 derived lines was not a reliable selection criterion for either yield or specific weight due to low heritabilities. He and Pederson (1969a, 1969b) suggested that evaluation for yield should not start before F4 or F5 and one should rather select between lines and not within lines. Calculated heritability values for yield in F4 and F5 lines were relatively high. With oats the predictive value of yield tests in the F2 and F3 generation was practically zero following Atkins & Murphy (1949).

From these experiments it can be concluded that single plant selection for yield or testing for yield in very small plots has hardly any effect unless special measures are taken to improve the efficiency of selection in early generations.

The efficiency of selection in early generations apparently tends to be low, due to environmental variance, genotype x environment interactions and genetic effects of a non-additive nature. To meet the problem of great environmental variance many breeders use repeated control plots for instance. Briggs & Shebeski (1968) investigated the use of control plots in wheat breeding nurseries. Yield of control plots at 9 ft distances were significantly correlated, but as the distance between the control plots became larger the correlation decreased rapidly to non-significance (Briggs 1969, Briggs & Shebeski, 1968). Townley-Smith & Hurd (1973) showed that the moving mean of the yields of the adjacent small plots was another suitable approach to reduce the environmental error. How many adjacent plots should be taken together seems to vary from test to test. The use of the moving means has the advantage that it requires less plots to test a set of lines, but one has to harvest them all. Baker & Mc. Kenzie (1967) concluded that one should not expect too much from the repeated control plot approach, and that the use depends on the level of heterogeneity of the soil. It would be better to use an analysis of covariance to avoid over-adjustment. Skorda (1973) used a third approach to reduce the environmental error, the grid selection, by subdividing the selection field and selecting the best plants in each block (grid). Jensen & Robson (1969) used miniature plots so that the number of replicates can be increased. Grading seed can also reduce environmental variation (Christian & Gray 1941).

### *3.2.2 Competition influencing single plant and small plot selection*

Selection in early generations generally means selection in heterogeneous populations, where intergenotypic competition may have important effects. Differences in competitive ability of the genotype were recognized early by Montgomery (1912). Montgomery described competition affecting the survival in mixtures of cereals and found that a good cultivar in a pure stand would not always be the best survivor under competition. ('Montgomery effect'). Jensen & Federer (1965), obtained in general good correlations between the competitive ability of cultivars in the mixture and the performance in a pure stand. However the enhancing effects on yield of taller plants seem to be stronger than the decreasing effects of shorter types (Jensen & Federer,1964). Blijenburg & Sneep (1975) found that the competition ability of eight barley cultivars was correlated with the yield in mono-culture with one exception, which means that there is a chance of underestimating some genotypes with a low competitive ability and a rather high yield potential. These may wrongly be eliminated in bulk populations. This inverse relationship between yield in mixed and pure stands caused by the inability of a genotype to express itself sufficiently in the phenotype was also demonstrated by Wiebe et al. (1963) in barley. Christian & Gray (1941) and Khalifa & Qualset (1974) observed this phenomenon for wheat. Thousand grain weight was in both studies almost unaffected by competition, whereas the number of tillers, number of grains per ear and grain yield were significantly influenced. Also Sakai (1955) reported that the estimation of heritability values without considering interplant competition would lead to an erroneous result. Hamblin & Donald (1974) investigated the relationship between plant form, competitive ability and grain yield in a barley cross. The F5 lines did not show a significant correlation for yield with F3 single plants. However, a significant inverse relationship existed between F5 grain yield on the one hand and plant height in the F3 on the other. The shorter plants could not compete well in the heterogeneous F3, but in the more uniform F5 testing situation their yield potential was realized. In a later study (Hamblin & Rowell,1975), this was confirmed. Because of the confounding effects of competition in heterogeneous populations the breeder should look for testing situations that reduce this problem. For this reason Fasoulas (1973, 1979, 1981) considers very wide spacing preventing all competition, the right condition to select for superior yielding performance in early generations. Heritability would be increased and in order to reduce the environmental error he combined a refined method of grid selection with this wide spacing, the honeycomb design. The value of the honeycomb design method, however, has been questioned by Bos (1983).

Valentine (1979) concluded that the confounding effect of intergenotypic competition for yield is not as great as the confounding effect of plant density. According to him the relative ineffectiveness of single plant selection in the F2 generation can to some extent be explained by the rather wide spacing, where later testing (and commercial growing) is done at much closer spacings. This might be one of the weaker aspects of the honeycomb method of Fasoulas (1973). Where very wide spacings are used, other examples of possible confusing effects because of wide spacing are given by Bartley & Weber (1952), Knott (1972) and Mc. Ginnes & Shebeski (1968). The cereal breeder is interested in genotypes yielding well under high-density conditions. Spitters (1979) therefore recommended selection under light competitive conditions as is normally done with line selection programmes. It can be concluded that in relation to spacing one needs a compromise between the two extremes. If the density is too high intergenotypic competition leads to a situation in which low competitive, high yielding genotypes are discarded (Christians & Gray,1941; Fasoulas, 1973, 1978, 1981), and if we use a very low density, the negative effects of micro-environmental variation and negative competition between plants become much larger than the advantage of loosing intergenotypic competition effects (Chebib et al. 1973; Bos,1983). Competition effects that change the ranking order of genotypes can also come from other factors such as: i) depth of sowing (Darwinkel,1979; Van den Brand & Ten Hag,1979), ii) heterogeneous seed weight (Christian & Gray, 1941; Hamblin & Rowell, 1975; Chebib et al.,1973; Helgason & Chebib,1963), iii) tallness and time of maturity (Christian & Gray,1941) and iv) uniformity of seed bed (Gotoh & Osonai,1959).

*3.2.2.1 Effect of plot size*

Small plots, adjacent to one another are subjected to competition effects. After all single plants represent the smallest possible plots. So the size and lay out of small plots can be of importance as Spitters (1979) showed clearly. He, studying competition and its consequences for selection in barley breeding, recommended the use of 3-row plots and of harvesting all three rows as the most suitable and practical microplot type for selection of yield. Kramer et al. (1982) performed an experiment on plot size, density between rows and the effect of harvesting border rows. Wider spacing for single rows improved the efficiency of selection. Harvest of only the inner rows of a three-row plot gave no improvement above harvesting all three, while the increase from 3- to 6 row plots also did not give much improvement. The three row plots with harvesting of the full plot was again recommended, although discarding the border rows tends to reduce intergenotypic competition, but the harvestable row represents a very much reduced plot causing an increased experimental error. Rich (1973) came to the conclusion that at least 6-row plots are required with 15 to 30 cm between rows for valid yield evaluations. In a one-year experiment 30 cm between rows should be used. The large differences in tallness between the wheat genotypes, and so the strong competition effects were the main reason that large plots were required.

*3.2.2.2 Effects of variation in seed density, seed size, and sowing depth*

Whether wheat plots are sown by machine or by hand, a variation of plant density will always occur, resulting in variations in competition between neighboring plants. Smith, H.F. (1937) has shown that stand irregularities arising from this cause are adequately compensated for by differences in tillering, growth and yield of the individual plant. Kariya & Yamamoto (1963) investigated the plant density and selection method for rice. They recommended low density in bulk or F3 derived lines until around F5, minimizing the effect of competition. After this, high planting density should be used for the line selection programme. Bos et al. (1987) found a very significant influence of seed size on yield. In general in a population with variation for plant length there is a tendency of taller plants to produce larger grains than shorter plants. However the shorter plants produce larger grains when the taller plants are topped after flowering. The taller plants also produce larger grains in a population with variation for tallness than in a pure stand. Moreover larger seeds seem to germinate faster and to compete better.

In relation to seed depth very significant differences in yield are found which are due to the effect on the number of tillers per plant. Deeper sowing reduces the number of tillers per plant (Darwinkel 1979; Van den Brand & Ten Hag).

*3.2.2.3 Natural selection*

When generations are advanced by the bulk methods under field conditions, competition between genotypes leads to natural selection. Palmer (1952) reported adverse effects of competition under natural selection. Natural selection for high number of grains per plant increased both the number of grains per plant and number of grains per ear. Since the number of grains per plant was inversely correlated with the grain weight, the grain weight was reduced. As the main factor of natural selection can be considered plant length as discussed in chapter 3.2.2.2.

*3.2.3 Visual selection for yield in early generations*

Yield should be done by measuring the actual grain yield per unit area. In early generations there are often too many entries and too small plots to do this. A visual evaluation if reliable might help. Townley et al. (1973) reported that nine selectors including breeders and technicians were not able to choose the highest yielding wheat lines. The same was observed for oats (Stuthman

& Steidle,1976), wheat (Briggs & Shebeski,1970) and barley (Mc. Kenzie & Lambert,1961). Knott (1972), Lupton & Whitehouse (1955) and Krull et al. (1966) reported slight positive effects of visual selection in wheat and Hanson et al. (1979) in barley. But the number of replicates and assessors had to be increased.

Summarizing the literature it can be concluded that visual selection for yield is rarely an effective selection procedure. The only positive effect of selection seems to be the removal of the lowest yielding genotypes. It functions therefore as a kind of negative mass selection procedure if applied in bulk populations (Frey,1962; Hanson et al.,1962).

### *3.2.4 Indirect selection through yield components*

Yield is polygenically inherited (Kuspira et al.,1957) and because selection for yield in early generations in cereals is in general not effective (Fiuzat & Atkins,1953; Atkins & Murphy, 1949; Jones & Frey,1960; Sidwell et al. 1976; Mc. Neal,1960; Fonseca & Patterson,1968; Alber,1969; Utz et al.,1973; Johnson et al.,1966; Ketata et al.,1976), scientists started to investigate the possibility of indirect selection for yield by selecting for one or more yield components. Thousand grain weight, yield per ear, number of grains per ear and number of ears per plant have been most often considered as traits of indirect selection for yield (Johnson et al.,1966; Ketata et al.,1976; Utz et al.,1973; Fonseca & Patterson,1968; Mc. Neal,1960; Alessandroni & Scalfati,1973).

The best selection responses in early generations have been obtained for the thousand grain weight trait (Palmer,1952; Johnson et al.,1966; Alber,1969; Knott & Talukdar,1971; Utz et al.,1973; Sidwell et al.,1976; Ketata et al.,1976). The heritability of thousand grain weight appears to be high and the trait shows a ready response to mass selection (Bhatt,1972). Only Fonseca & Patterson (1968) obtained low correlations with grain yield and Mc. Neal (1960) found no significant correlation at all between yield and thousand grain weight. Austenson & Walton (1970), however concluded that selection for larger grains would be very effective.

Concerning the other yield components Fonseca & Patterson (1968), Mc. Neal (1960) and Fischer et al. (1977) observed significant positive correlations with yield for the number of grains per ear and the number of tillers per plant. The latter was also observed by Ketata et al. (1976), Lupton et al. (1974), Sidwell et al. (1976) and Hsu & Walton (1971). Yield per ear has been recommended by Alessandroni & Scalfati (1973) on the basis of a low but significant correlation of 0.21 ** between yield per ear in F2 and grain yield in F4.

When selecting for yield components one should be aware of negative associations between them especially at higher densities (Knott et al.,1971). Negative associations have been found between yield per ear and number of ears per plant (Nass,1973), thousand grain weight and tiller number, and between thousand grain weight and number of grains per ear (Sidwell et al.,1976; Fonseca & Patterson,1968). This suggests that simultaneous improvement of yield components might be difficult as such negative correlations among yield components occur frequent in cereals, particularly under various kinds of environmental stress. Adams (1967) showed that interdependency among grain yield components is frequently characterized by such negative associations and suggested a developmental basis for these relationships. Perhaps a certain grain yield per unit area can be realized in various ways, depending on the environmental conditions during the development of the crop, explaining the negative correlations between the components. Because of these negative correlations one should always be cautious when selection is done for one yield component only.

### *3.2.5 Importance of green leaf duration for yield*

The aspects of yield in relation to the importance of green leaf, stem, ear and awn area has been discussed by Watson (1952), Watson et al. (1963), Teare & Peterson (1971), Simpson (1968), Mc. Key (1966), Apel & Lehmann (1970), Loomis & Williams (1969), Bingham (1972), Lupton et al. (1974), Fischer & Kohn (1966), Spiertz et al. (1971), Spiertz (1978), Smocek (1970) and Hsu & Walton (1971). It can be concluded that grain yield depends on the intensity and duration of photosynthesis after anthesis, and most important are the plant's parts above the flag leaf internode The relative contribution of the ear, upperstem internode, flag leaf lamina and flag leaf sheath to the total wheat yield has been estimated as 34%, 12%, 13% and 16% respectively. The second leaf lamina and sheath count for 8% and 10% respectively and the rest of the plant counts for only 7% (Mc. Key,1966). Awns can be important as well (Teare & Peterson,1971).

Fischer & Kohn (1966) obtained for wheat a very high correlation of $r = 0.97^{***}$ between yield and green leaf area duration after flowering. Smocek (1970) predicted that with a selection index method based on the green flag leaf duration area, a maximum genetic advance for yield can be expected. However, the relative importance of this source character varies with the environment (Bingham,1972; Mc. Key,1966). Walton (1971) and Hsu & Walton (1971) found that the flag leaf is important for yield and a long filling period results in larger grains.

It can be concluded that a long green leaf duration after flowering is very important and therefore a very significant yield improvement can be expected from resistance to leaf pathogens.

### *3.2.6 Indirect selection for yield through disease resistance in early generations*

Selection for resistance to leaf pathogens can be considered as the most effective example of indirect selection for yield. Normally negative correlations between the amount of disease and grain yield are found. Selection for resistance means selecting genotypes that a reduced growth and/or development of the pathogen. Generally a yield increase is the consequence.

Selection for resistance is normally far more efficient than selection for tolerance (Parlevliet,1981). Tolerance refers to the ability of the host to endure the pathogen which can only be expressed in a reduced yield loss when it is compared with the yield loss measured on other equally susceptible cultivars. Yield has a low heritability, yield loss, being the difference between two yield estimates, has an even lower heritability. Tolerance is therefore very difficult to measure.

*3.2.6.1 Selection for partial resistance to biotrofic fungi*

The interest in selection for partial resistance to leaf rust, stem rust and powdery mildew increased after the plant breeders became aware that mono-genic, race-specific resistance is in general not durable. Selection for race-specific resistance in early generations is very easy. For partial resistance, being quantitatively expressed and based on minor genes, new breeding procedures have to be established.

The biotrophic fungi leaf rust, stem rust and powdery mildew are wind spread pathogens. Therefore interplot interference is an important aspect that has to be considered when one selects for partial resistance to these pathogens. Interplot interference causes a serious underestimation of the resistance level of the observed plot or plant. It has been one of the main reasons why partial resistance has been ignored so much in the past. Parlevliet & van Ommeren (1975) compared the amount of barley leaf rust in small adjacent plots with those in isolated plots. In the adjacent plots the resistance was underestimated more than a hundredfold. Nørgaard Knudsen et al. (1986) investigated powdery mildew resistance in barley and got reliable results only when interplot interference in the small adjacent plots was reduced by narrow wheat strips between the plots to be evaluated.

The efficiency of early generation selection for partial resistance can be questioned for the same reason as selection for yield in the early generation is ineffective. However, fewer genes are probably involved, while also the environmental variation and environment x genotype interaction may be smaller. Little information is available. The best example comes from barley in relation to barley leaf rust and powdery mildew resistance, being thoroughly investigated by Parlevliet. Screening for partial resistance in barley in a heterogeneous barley population, Parlevliet (1976) found that both single plant selection for affected leaf area in the field and seedling screening for the latent period in the greenhouse were quite effective. The field selection resulted in a slightly better response than the seedling test for the latent period. Later Parlevliet et al. (1980) found that selection for partial resistance to barley leaf rust can successfully be done at any stage of the breeding programme. In the greenhouse a successful demonstration of the accumulation of partial resistance to leaf rust was given with a cross between Vada, representing the highest level of partial resistance among commercial cultivars, and the fairly primitive Cebada Capa. In the F2 the adult plants with the longest latent period were selected. A pedigree selection procedure was followed from F3 to F6 generations, which resulted in genotypes with a relative latent period being much longer than the latent period of Vada (Parlevliet & Kuiper,1985). Also under field conditions the large increase in partial resistance of these selections could be clearly demonstrated (Parlevliet & Van

Ommeren,1985). With a recurrent selection procedure exerting a mild mass selection it has been investigated if partial resistance to leaf rust, powdery mildew, grain yield, thousand grain weight, lodging resistance and earliness can be selected simultaneously (Parlevliet, 1988). With much ease in spite of the mild selection pressure in early generations, lines were obtained after three cycles of recurrent selection with resistance levels higher than vada. This increase in partial resistance was also expressed in a gain in yield. Resistance to powdery mildew higher than any of the European cultivars had also been accumulated. Lodging resistance was not obtained. Observations of Johnson & Wilcoxson (1976) also confirm, that selection for partial resistance to leaf rust in barley can successfully be done in early generations, finding very high heritabilities for 'the area under the disease progress curve' parameter. Selection for latent period was found to be effective while the number of uredosori per square centimetre was not, due to lack of uniformity of the inoculation.

Knott (1982) looked at the polygenic inheritance of stem rust resistance in wheat. This study also gives a very positive perspective on the possibility of accumulating partial resistance. Eight wheat cultivars and lines were crossed producing four double crosses with the objective to accumulate minor genes of resistance. The inheritance study showed that the resistance is recessive involving several genes with small cumulative effects. Early selection was quite effective as the field readings on F2 plants predicted very well the mean rust percentages of their F3 progenies.

Krupinsky & Sharp (1978, 1979) looked at minor gene resistance in wheat to stripe rust. They also concluded that minor gene resistance can easily be accumulated in early generations. Transgressive segregation was demonstrated in the later generations.

*3.2.6.2 Selection for partial resistance to the necrotrophic fungi*

Less is known of the resistance mechanisms to necrotrophics than of those to the biotrophic fungi. In general partial resistance to the wheat pathogens *Septoria nodorum*, *S. tritici*, *Cochliobolus sativus* and *Fusarium graminearum* is expressed quantitatively. Bronnimann (1970,1975) and Scharen & Krupinsky (1978) studied the inheritance of resistance to *S. nodorum* and found quantitative, polygenically inherited effects.

The selection for partial resistance to the necrotrophic fungi has the significant advantage over the biotrophic fungi that interplot interference is in general small. At least it is not large enough to disturb the ranking of resistance in small adjacent plots. On the other hand differences in earliness and tallness have strong disturbing effects on the ranking order of the cultivars for resistance (Little & Doodson, 1974).

*3.2.7 Indirect selection for disease resistance*

When the epidemic development of leaf pathogens occur after flowering the damage to yield can be expected to be mainly directed to the grain size. Therefore it is possible that methods, which separate the light grains from the heavy grains, may form effective mass selection procedures for improving disease resistance and therefore yield.

Derera & Bhatt (1972,1973) investigated the efficiency of mechanical mass selection on genetically heterogeneous wheat populations. After stratifying grains according to grain-size, shifts in means and reductions in variance were observed in the field test for grain weight, grain weight per ear, and grain yield per plot. Homogeneous populations did not give such shifts. Populations selected for large grain size in heterogeneous bulks gave higher grain yields per plot. These results indicate that elimination of pinched grains from stem- or leaf rust affected plants from a segregating population automatically would result in a shift to resistance. Negative effects of stem rust on the grain quality of wheat have been reported by Mangels & Sanderson (1925) and Greaney et al. (1941). Stem rust causes shrivelling of grains and a reduction of specific weight. An increase of stem rust severity from 5% to 90% reduced the yield by 84% and the grain weight by 65%. The specific weight was reduced by 14 lb. (Greaney et al.,1941). High leaf rust levels too reduced the specific weight (Peturson et al.,1948). Even milder infections caused important reductions of these values. Atkins et al. (1966) found significant reductions of specific weight and grain weight by leaf rust as well. Caldwell et al. (1934) reported that leaf rust reduces in the first place the number of grains per ear and secondly grain weight. Specific weight was only slightly effected. Keed & White (1971) reported that in wheat the thousand grain weight and specific weight were affected by leaf- and stem rust. So mass selection for grain weight might eliminate the most susceptible genotypes from a segregating population. In relation to *S. nodorum* Bronnimann (1970) concluded that if one selects for thousand grain weight, one would select both for resistance to *S. nodorum* and for large grains per se.

# 4 The durable resistance breeding programme

The experimental programme was based on Van der Plank's (1963,1968) concept of horizontal resistance. Van der Plank made a sharp distinction between durable and temporary resistance named horizontal- and vertical resistance. Horizontal resistance is defined as race-non-specific and vertical resistance as race-specific resistance. Robinson (1976), elaborating the concept of Van der Plank, suggested that breeding for horizontal resistance should imitate the natural accumulation of durable resistance in maize in tropical Africa after the devastating introduction of *Puccinia polysora* in the 1940's. *P. polysora* was introduced in West Africa in the 1940's reaching East Africa in 1952. The maize populations had never been selected in the presence of this pathogen and appeared to be highly susceptible. The initial damage was extremely high. Efforts to incorporate vertical resistance failed because of its rapid break-down. The maize populations were, as an outbreeding crop, highly heterogeneous. By the exerted natural selection pressure of the pathogen the maize crop accumulated a significant level of horizontal resistance and a decline of the importance of *P. polysora* was soon observed. Therefore, following Robinson's (1973,1976) suggestion, the wheat programme in Brazil initially aimed at changing wheat temporarily from an inbreeding crop to an outbreeding crop in order to facilitate the recombination between the selected plants. The outbreeding situation was created by using a male gametocide. Seeds harvested on the plants treated with male gametocides were to a large extent derived from outcrossing and formed the basis of the population to select from in the next generation. The selection was directed towards those phenotypes that were less affected by the pathogen selected against. By preference plants with less of these pathogens despite a susceptible infection type were taken. The selected plants then were allowed to recombine again. This resistance was identified as partial resistance by Parlevliet (1975) and is the same type of resistance that Van der Plank (1968) and Robinson (1976) meant when they discussed horizontal resistance. The partial resistance is a form of quantitative resistance resulting in a reduced epidemic development of the pathogen despite a susceptible infection type (Parlevliet,1975). If the infection type is not a susceptible one but an intermediate or low one and there is still some epidemic development one could call it incomplete resistance.

# 5 The use of ethrel as a male gametocide

The use of a male gametocide for wheat has been investigated by various researchers (Bennett et al.,1972; Bennett & Smith,1972; Bennett & Hughes,1972; Borghi et al.,1973; Dotlacil & Apltaverova,1978; Fairy & Stoskopf,1975; Hughes,1975; Hughes et al.,1974; Hughes et al.,1976; Jan et al.,1974,1976; Law & Stoskopf,1973; de Milliano,1983; Rowell,1971; Rowell & Miller,1974; Wang & Lund,1975), while the aspects of cross pollination have been reported by de Vries (1971,1973,1974a,1974b). Most of this literature refers to greenhouse experiments in pots. Ethrel (2- chloro ethyl phosphoric acid) was mentioned as a male gametocide of some promise. To find out the most effective treatment in the field a series of experiments was carried out.

## 5.1 Ethrel performance during the off-season in 1976

During the off-season period February-May 1976 the first experiment on ethrel performance in the field was made. Small eight-row plots of 1.5 meter length were sown with seed of a mixture of 6 cultivars: Pel-13738-68, CNT-2, CNT-3, IAS-62, PF-70401 and Coxilha. These cultivars have exactly the same growth cycle. The two inner most rows were used as the female rows and the six remaining rows, three on each side, became the male parent. Spacing was 20 cm between rows, while a 40 cm space was kept between the male- and female strips. The experiment was performed in four replicates with three different ethrel treatments of 1500 ppm, 2000 ppm, 2500 ppm active ingredient (a.i) (1000 l. water per ha) applied at three different growth stages: 41, 45 and 51 DC (Zadoks et al. 1974) Agral 0.05% (v/v) was added as a wetting agent. Gibberellic acid-3 was applied at a rate of 150 ppm (500 l. water per ha) three days after the ethrel application on all the plots to promote ear emergence. At early heading stage

Table 5.1. Number of grains per ear (NG/E) of non-bagged (NB) and bagged (WB) ears and percentage cross pollination (% CP) on a wheat mixture treated with three different concentrations of ethrel (1500 ppm, 2000 ppm and 2500 ppm), at three different growth stages (41, 45 and 51 DC) in February – May 1976.

| | GS 41 | | | GS 45 | | | GS 51 | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1500 | 2000 | 2500 | 1500 | 2000 | 2500 | 1500 | 2000 | 2500 |
| NG/E, NB | 9.3 | 7.8 | 6.4 | 9.8 | 5.9 | 3.5 | 9.9 | 6.7 | 7.3 |
| NG/E, WB | 10.7 | 4.5 | 1.7 | 10.4 | 8.6 | 1.7 | 7.9 | 7.2 | 7.3 |
| % CP | −15.0 | 41.9 | 73.2 | −6.6 | −46.2 | 52.6 | 20.7 | −7.6 | 0.0 |

15 ears were bagged and 35 other ears tagged in the female strips of each plot. As the percentage of cross pollination the difference between the seed set on tagged and bagged ears was taken. The possible effect of bagging on seed set was not taken into consideration. But there appeared to be an effect of bagging as the number of seeds in bagged ears were in some cases higher than in unbagged ears were fertile pollen was assumed to have free access (Table 5.1).

The number of grains per ear on untreated and unbagged plants was counted on a random sample of 200 ears yielding 23.1 grains per ear, indicating that self pollination was normal. Unfortunately poor weather conditions during flowering did not favour cross pollination. This must be considered when evaluating the results. Low temperatures and rain were the main constraints. The general conclusions are: a) Male sterility increases with increasing ethrel concentrations, b) The best effect of ethrel is obtained when it is applied as early as growth stage 41 DC, c) The 2500 ppm concentration is most effective when it is applied at growth stage 41 and 45 DC, however this high concentration gives a strong phytotoxic effect on the leaves and ears. The plants are very much deformed and ear emergence is strongly hindered.

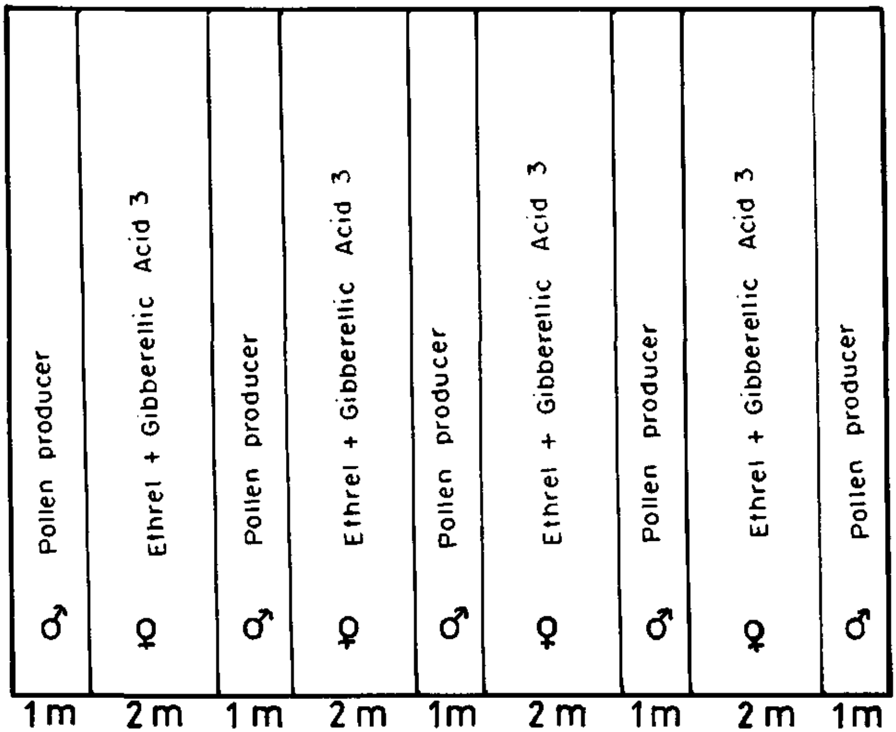



Figure 5.1. Design used to stimulate out crossing in the field.

Table 5.2. Number of grains per ear (NG/E) of non-bagged (NB) and bagged (WB) ears and percentage cross pollination (% CP) on ethrel treated wheat of composite 1 at two different distances from the pollinator parent in June-November 1976.

| | Distance from 0.2 m | male row 1.2 m |
|---|---|---|
| NG/E, NB | 27.6 | 22.8 |
| NG/E, WB | 5.0 | 4.3 |
| % CP | 81.9 | 81.1 |

## 5.2 Ethrel performance on the wheat composite 1 in 1976

The second experiment was performed in 1976 on composite 1 (see chapter 7) during the normal wheat season. The composite 1 was sown in June in a field of 0.5 ha for the first random polycross. For this purpose the field had been divided in male- and female strips of 1 and 2 m wide respectively. Between the strips one row (of 0.20 m) was left unsown (fig. 5.1).

The female strips were sprayed with ethrel, at growth stage 43 DC using a concentration of 2000 ppm a.i. until run-off (1000 l. water per ha). As ethrel hinders ear emergence, four days later 150 ppm gibberellic acid-3 (500 l. water per ha) was applied to promote heading. Pairs of two equal sized ears were tagged of which one was bagged. Fifty pairs were taken at 20 cm distance from the male row and another 50 pairs in the centre of the female row at 120 cm distance from the nearest male row.

A significant amount of cross pollination was observed. The percentage of cross pollination, expressed by the difference between the number of grains of the bagged and tagged only ears, was calculated as 81.9% and 81.1% at the distance of 20 and 120 cm respectively from the male parent (Table 5.2).

## 5.3 Effects of ethrel on pollination

An experiment was performed with cultivar Frontana in order to observe the effects of ethrel on pollen development.

Ethrel was applied at three different concentrations: 1500 ppm, 2000 ppm and 2500 ppm a.i. at three different growth stages: 41, 45, 51 DC, together with 0 ppm as a control (see chapter 5.1). A sample of 15 ears was collected from the field two days after the treatment from each plot and fixed in 'Newcomer fixer' consisting of 6 parts of isopropilic alcohol, 3 parts acetic acid, 1 part ether, 1 part acetone and 1 part dioxane. Observations were made on the pollen, which were squashed in acetocarmine at the mono- and tri-nucleate stage.

At the mono-nucleate stage not many abnormalities were found, only the number of pores was often higher than normal. Most obvious at the three-nucleate stage was the super contraction of the nuclei and very often two unequal generative nuclei were found. Moreover empty pollen grains and a high reduction of starch granules (20 to 80 %) were observed. The highest percentage of abnormal pollen grains was observed, when ethrel was applied at the rate of 2000 and 2500 ppm at late bootstage (45 DC). A summery of the results is presented in table 5.3.

It was concluded that with cultivar Frontana most of the critical deviations occur at late pollen development, but ethrel should not be applied later than late-bootstage (45 DC).

The number of nuclei can vary considerably ranging between two and eight. Also Bennett & Hughes (1972) reported that ethrel is able to induce an additional mitosis.

## 5.4 Ethrel performance on three wheat cultivars

In this experiment three cultivars, C-33, Frontana and CNT-10 were tested either at one application at growth stage 43 or at two applications at growth

Table 5.3. Percentage pollen with abnormal nuclei (abn), with zero (0), one (1), two equal (2e), two unequal (2u) generative nuclei and one or more than one pores and with super contracted nuclei (SCN), empty pollen grain (EPG) at two pollen development stages and at various growth stages (GS) of the plant and at various ethrel concentrations (Conc). Plants from the field in the season June – November 1978.

| | | | Generative nuclei | | | | | Number of pores | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc | GS | abn | 0 | 1 | 2e | 2u | >2 | 1 | >1 | SCN | EPG |
| Mono-nucleate: | | | | | | | | | | | |
| 0 ppm | | 0.0 | | | | | | 100.0 | 0.0 | | 0.0 |
| 1500 ppm | 45 | 4.0 | | | | | | 96.0 | 4.0 | | 1.0 |
| 2000 ppm | 41 | 14.0 | | | | | | 98.0 | 1.6 | | 11.1 |
| 2000 ppm | 45 | 9.2 | | | | | | 92.0 | 8.0 | | 1.2 |
| 2500 ppm | 41 | 2.7 | | | | | | 98.0 | 2.0 | | 0.7 |
| 2500 ppm | 45 | 42.0 | | | | | | 60.0 | 40.0 | | 2.0 |
| Tri-nucleate: | | | | | | | | | | | |
| 0 ppm | | 10.8 | | 0.6 | 96.0 | 3.4 | | 98.6 | 1.4 | 3.1 | 2.6 |
| 1500 ppm | 45 | 65.5 | | 0.5 | 96.5 | 3.0 | | 99.5 | 0.5 | 64.5 | 0.0 |
| 1500 ppm | 51 | 10.3 | | 0.0 | 99.7 | 0.3 | | 100.0 | 0.0 | 8.0 | 1.3 |
| 2000 ppm | 45 | 88.0 | 2.0 | 4.0 | 90.0 | 4.0 | | 100.0 | 0.0 | 68.0 | 10.0 |
| 2000 ppm | 51 | 20.3 | | 0.3 | 96.4 | 3.3 | | 99.7 | 0.3 | 13.0 | 3.3 |
| 2500 ppm | 41 | 17.3 | | 2.0 | 84.7 | 9.3 | 4.0 | 100.0 | 0.0 | 0.0 | 0.7 |
| 2500 ppm | 45 | 96.4 | 0.4 | 14.0 | 84.0 | 1.6 | | 98.4 | 1.6 | 92.8 | 0.8 |
| 2500 ppm | 51 | 46.7 | | 1.0 | 90.7 | 8.0 | | 96.3 | 3.7 | 35.3 | 0.0 |

stage 39 and 43 DC. The ethrel treatments were: 1) 2000 ppm at growth stage 43, 2) 2500ppm at growth stage 43, 3) 1000 ppm at growth stage 39 + 2000 ppm at growth stage 43, 4) 1500 ppm at growth stage 39 + 2000 ppm at growth stage 43, 5) Control 0 ppm with water.

The plots consisted of 12 rows of 2.50 meter, of which the four innermost rows were treated. Ethrel was applied with a one nozzle, small sprayer using 1000 l water/ha. Gibberellic acid-3 at a 150 ppm concentration, 500 l. water/ha was used to promote ear emergence and a sticker, Ilharequen 2.5 cc/10 l. water was added to the solution.

Per treatment 160 ears were tagged before the application. These ears were selected in relation to the required growth stage. Eighty tagged ears were bagged to measure the efficiency of ethrel as a male gametocide; the other eighty were used for the evaluation of the percentage cross pollination. The number of spikelets per ear and number of grains per ear was counted. Because the number

Table 5.4. Effect of five ethrel treatments on the number of grains per spikelet (NG/S), the percentage cross pollination without the bagging effect (% CP), with the bagging effect (%CP-B), number of spikelets per ear (N S/E) and number of grains per ear (N G/E) on three wheat cultivars in the season June-November 1978.

| Cult var | Treat ment [1] | N G/S | | % CP [2] | % CP-B [3] | N S/E | | N G/E | |
|---|---|---|---|---|---|---|---|---|---|
| | | Bagged | Unbagged | | | Bagged | Unbagged | Bagged | Unbagged |
| C-33 | 1 | 0.10 | 1.16 | 91.4 | 66.4 | 10.8 | 11.9 | 1.08 | 13.80 |
| C-33 | 2 | 0.15 | 0.97 | 84.8 | 59.8 | 11.5 | 11.8 | 1.73 | 11.45 |
| C-33 | 3 | 0.01 | 0.63 | 98.7 | 73.7 | 11.0 | 11.0 | 0.11 | 6.93 |
| C-33 | 4 | 0.02 | 0.46 | 96.7 | 71.7 | 11.4 | 11.6 | 0.23 | 5.34 |
| C-33 | 5 | 1.65 | 2.20 | 25.0 | – | 11.7 | 13.0 | 19.31 | 28.60 |
| Frontana | 1 | 0.25 | 1.37 | 81.7 | 44.8 | 12.9 | 13.5 | 3.23 | 18.50 |
| Frontana | 2 | 0.47 | 1.46 | 67.5 | 30.6 | 13.6 | 13.4 | 6.39 | 19.56 |
| Frontana | 3 | 0.75 | 1.07 | 29.5 | –7.4 | 13.3 | 12.3 | 9.98 | 13.16 |
| Frontana | 4 | 0.14 | 0.66 | 79.0 | 42.1 | 12.2 | 13.1 | 1.71 | 8.65 |
| Frontana | 5 | 1.26 | 1.99 | 36.9 | – | 12.8 | 13.9 | 16.13 | 27.66 |
| CNT-10 | 1 | 0.10 | 0.90 | 88.8 | 65.5 | 10.8 | 11.6 | 1.08 | 10.44 |
| CNT-10 | 2 | 0.01 | 0.78 | 98.5 | 75.2 | 11.1 | 11.8 | 0.11 | 9.20 |
| CNT-10 | 3 | 0.03 | 0.21 | 84.8 | 61.5 | 10.8 | 10.9 | 0.32 | 2.29 |
| CNT-10 | 4 | 0.01 | 0.25 | 96.4 | 73.1 | 10.5 | 10.8 | 0.11 | 2.70 |
| CNT-10 | 5 | 1.70 | 2.21 | 23.3 | – | 11.3 | 11.8 | 19.21 | 26.08 |

[1]) Treatments: 1 = 2000 ppm at growth stage 43 DC
2 = 2500 ppm at growth stage 43 DC
3 = 1000 ppm at growth stage 39 + 2000 ppm at growth stage 43 DC
4 = 1500 ppm at growth stage 39 + 2000 ppm at growth stage 43 DC
5 = 0 ppm (Control)

[2]) Through formula I, see text p. 29.

[3]) Through formula III, see text p. 29.

of grains per ear can vary due to size of the ear, the percentage cross pollination was calculated in this experiment on a seed set per spikelet base.

The number of florets per spikelet for the three observed cultivars was the same.

The following formulae were used:

Expected percentage cross pollination, without loss of grains due to bagging:

$100 - (Tb/Tu \times 100)$ (I)

Expected percentage loss of grains due to bagging:

$100 - (Ub/Uu \times 100)$ (II)

Expected percentage cross pollination taking into consideration loss of grains due to bagging:

$(Ub/Uu \times 100) - (Tb/Tu \times 100)$ (III)

Tb = number of grains per spikelet in ethrel-treated, bagged ears.
Ub = number of grains per spikelet in untreated, bagged ears.
Tu = number of grains per spikelet in ethrel-treated, unbagged ears.
Uu = number of grains per spikelet in untreated, unbagged ears.

This experiment confirmed the results of the previous experiments, showing that ethrel can be efficiently used as a male gametocide when it is applied with 2000 or 2500 ppm at growth stage 43 DC (Table 5.4). The percentage of cross pollination (including the reduction due to bagging) for the two treatments 2000 and 2500 ppm for C-33, Frontana and CNT-10 were: 66.4, 59.8%; 44.8, 30.6%; 65.5, 75.2% respectively. The bagging effect of C-33, Frontana, CNT-10 were 25.0, 36.9 and 23.3% respectively, these differences being significant. The split applications did not give a clear improvement in cross fertilization, while the yield of grains from the unbagged ears was strongly reduced, disqualifying the split application approach. The concentration of 2000 ppm was better than 2500 ppm in relation to the negative side-effects of ethrel (less damage to plant habitus and reduced increases in susceptibility to diseases).

## 5.5 R-111601 performance on composite 1 in 1981

In 1981 an experimental male gametocide, R-111601, became available for testing. The percentage cross pollination obtained by 10,000 ppm a.i. of R-111601 was compared with 2000 ppm a.i. ethrel + 150 ppm gibberellic acid-3. Both gametocides were applied with water at a rate of 1000 l. per ha on composite 1, generation 11 (chapter 7). Directly after the applications tillers were labelled at growth stage 39, 41 and 43 DC in both the treated and untreated strips. Fifty labelled tillers per treatment were bagged, while another fifty remained unbagged, to estimate the percentage of cross pollination.

Both chemicals demonstrated a good capacity to induce male sterility. The highest percentage cross pollination of 46.3% (accounting for the bagging effect)

Table 5.5. Number of grains per ear (N G/E) and percentage cross pollination without the bagging effect (% CP) and with the bagging effect (% CP-B) of wheat composite 1, treated with ethrel, R-111601 and water, at growth stages (GS) 41, 43 and 45 DC in the season June – November 1981.

| Treatment | GS | N G/E | | % CP | % CP-B |
|---|---|---|---|---|---|
| | | Bagged | Unbagged | | |
| Ethrel | 41 | 11.4 | 18.6 | 38.7 | 19.8 |
| | 43 | 5.9 | 21.8 | 72.9 | 46.3 |
| | 45 | 12.9 | 25.4 | 49.2 | 16.4 |
| R-111601 | 41 | 4.7 | 6.4 | 26.6 | 7.7 |
| | 43 | 6.8 | 7.7 | 11.7 | – |
| | 45 | 15.7 | 20.2 | 22.3 | – |
| Water | 41 | 22.7 | 28.0 | 18.9 | |
| | 43 | 20.1 | 27.4 | 26.6 | |
| | 45 | 17.4 | 25.9 | 32.8 | |

was obtained with ethrel applied at growth stage 43 DC confirming the results of earlier experiments, while the best result of R-111601 gave only 7.7% at growth stage 41 DC although good male sterility was obtained with R-111601. Cross pollination was very low, which was partly due to poor weather conditions with much rainfall during flowering. A more important aspect of R-111601 seems to be that it had an effect on female fertility as well, as R-111601 treated plants had a significantly lower seed set than plants which were sprayed with ethrel. R-111601 seems to have some advantages over ethrel. i) The ear emergence was much less affected, ii) The growth stage of the application is much less critical than with ethrel, which is very important when there is a certain variation for earliness. iii) The effect on plant habit is not so strong as with ethrel. The ear looks more yellow and the length of the straw is reduced. It would be interesting to test this product again under more favorable weather conditions or in the greenhouse to investigate if female fertility is really affected.

## 5.6 Discussion

The five experiments indicated that ethrel can be used to enhance cross pollination in wheat. Overall, the most effective application appeared to be an ethrel concentration of 2000 ppm in water at a rate of 1000 l./ha followed by a spray with gibberellic acid-3 at a concentration of 150 ppm when most ears are in the growth stages 41 to 43 DC (early- and mid bootstage). Cultivars may differ in their reaction to this treatment (Table 5.4). Environmental differences too were observed, as in 1976 (Table 5.1) growth stage 41 DC gave the best results, while in 1981 (Table 5.5) growth stage 43 DC was significantly better. Gibberellic acid-3 was very effective in promoting ear emergence.

For the induction of cross pollination and so cross fertilization a male gametocide was essential in the programme described here. During the programme ethrel was the only gametocide commercially available. It does not give full male sterility, but the proportion of outcrossing induced is sufficient for its purpose in this programme. However, there are other unwanted side effects, such as increased susceptibility to diseases and changed plant habits. Moreover, the moment of the application is very critical, which can be a problem in years with a lot of rain or when there is much variation in earliness. Also R-111601 appeared to be far from ideal, which may have been partly due in this experiment to the adverse weather conditions.

Fairey et al. (1975) tested the granular form of ethrel, which may be more suited for practical use than the liquid form. They claim that almost hundred percent male sterility can be obtained without apparent morphological or physiological abnormalities. However, a very high concentration of ethrel is required to obtain such high percentages of male sterility.

A new perspective on the production of a perfect male gametocide has been published by Shell (Anonymous,1986). Fertile pollen contain proline. Shell Research investigated agents interfering with proline biosynthesis finding an analogue of methanoproline (WL-84811), which does not inhibit but interferes very well with pollen viability. New improved male gametocides are of great importance for wheat breeding, with special regard to partial resistance programmes as presented in this paper. Millions of crosses can be made in one day by a small team of 3-4 persons. Hopefully a good commercial hybridizing agent will come on the market within a few years.

# 6 Procedures used to advance wheat generations in the off-season

The normal wheat season in Passo Fundo, Rio Grande do Sul is in the period from June until November. The conditions in the off-season are too poor for a good wheat crop because of too high temperatures, lack of irrigation facilities, and severe insect problems. Therefore only small multiplications, crossing work and single seed descent procedures can be done in this period at CNPTrigo in Passo Fundo.

Advancing generations in the off-season has two main objectives: i) Multiplication, ii) Increasing the level of homozygousity. Several procedures to realize these objectives were investigated.

## 6.1 Multiplication in off-season

The off-season wheat generations advance were performed at: a) CPACerrado / EMBRAPA, Brasília, b) CNPHortaliças / EMBRAPA, Brasília, c) CIMMYT, Mexico.

The off-season crop was planted at the CPACerrado in Brasília, in 1977, 1978, 1980, 1981 and 1982. Brasília is located in the centre of Brazil at an altitude of 1172 meters, at 16°SL. The climate is favorable for wheat production except for dry spells, which do occur even in the rainy season. The research centre has sufficient irrigation facilities. The wheat was always sown in the last week of January and harvested in May. In 1979 the multiplication was done at CNPHortaliças of EMBRAPA in Brasília where the conditions are the same.

The pathogens *Cochliobolus sativus*, powdery mildew, leaf rust, stem rust and insects as stem borers, were the main limiting constraints for high yield. A yield of a thousand kilogram per hectare is normal during the off-season, which can be considered as low.

In 1981 it became possible to multiply at Cimmyt in Mexico, which was interesting because of its much higher multiplication rate. At Cimmyt, more than 5000 kg/ha are easily obtained. Only the seed harvested from the single plant and line selection procedures was sent to Mexico for multiplication. The bulk seed selection procedure was always multiplied in Brasília, because of the high cost of sending seed by air freight from Mexico to Brazil.

## 6.2 Multiplication on water culture

Multiplication in water culture under controlled greenhouse conditions was investigated to find out whether this is an efficient alternative when a high multiplication rate is required.

Table 6.1. Nutrient solution used for the multiplication in water culture.

| | |
|---|---|
| 1 N $KH_2PO_4$ | 1 ml/litre |
| 1 N $KNO_3$ | 5 ml/litre |
| 1 N $Ca(NO_3)_2$ | 5 ml/litre |
| 1 N $MgSO_4 \cdot 7H_2O$ | 2 ml/litre |
| Fe EDTA | 1 ml/litre [1]) |
| Micro | 1 ml/litre [2]) |

[1]) The Fe EDTA is prepared as follows: 33.2 g. EDTA.2Na.$2H_2O$ is dissolved in 89.2 ml (1N) NaOH. 24.9 g. $FeSO_4.7H_2O$ is added after it has been first dissolved in 700 ml water.
[2]) Micro contained $H_3BO_3$ 2.86 g/l; $MnCl_2.4H_2O$ 1.81 g/l; $ZnSO_4.7H_2O$ 0.22 g/l; $CuSO_4.5H_2O$ 0.08 g/l; $H_2MoO_4.H_2O$ 0.02 g/l.

Twenty four F1 plants from hand made crosses of composite 1 Generation 9 (see chapter 7) were multiplied on water culture. The F1 seeds were germinated in the germination chamber with a treatment of Benlate-T. After four days they were transplanted into small containers of 5 l volume with a complete nutrient solution as described in table 6.1.

The multiplication rate was large, ranging from nearly 1200 to some 4000 times (Table 6.2). Compared with the multiplication rate of 10 to 20 times in the off-season field it is a great success. It is interesting to note, that in spite of the poor quality of the F1 seeds not one single grain failed to develop into a vigorous plant.

This very high multiplication rate has three interesting aspects of practical use: a) In a programme selecting for polygenically inherited characters such as partial resistance to several pathogens, one needs large F2 populations; the segregation will be more complete and the chance that plants with many desirable combinations of genes will be present is increased. b) The method can be used for quick and safe multiplication of selected plants during the off-season replacing the Mexico and Brasília generations. c) It becomes possible to produce large quantities of F2, F3, F4 etc. seed, therefore one might replace the ethrel treatments by a series of hand made crossings. By this technique one has 100% F1 seed originating from cross pollination against 60% – 80% obtained with ethrel. After one generation of multiplication in the off-season, large seed quantities are available for the selection procedures to be followed.

Table 6.2. Mean and range of three yield components of 24 F1 wheat plants, grown on water culture.

| Yield component | Mean | Range |
|---|---|---|
| Number of tillers/plant | 55 | 25– 90 |
| Number of grains/plant | 2309 | 1173–4073 |
| Thousand grain weight | 37.6 | 30.1–46.4 |

## 6.3 Advancing through Single Seed Descent

*Single Seed Descent in soil.* Methods for rapidly advancing generations to increase homozygousity during the off-season were tested. Single Seed Descent was tried in wooden boxes with soil carrying 1000 plants per 1 $m^2$. The boxes were put in trays made of a polythene sheet, so that the water level could be controlled. The plants were supported with fishing nets of 3 x 3 cm at 10 cm height and 5 x 5 cm at 90 cm height. The nutrient level was kept very low using a poor soil of almost pure sand.

The generation length of 77 days was achieved in the short day length season. However, severe problems with soil born diseases, poor germination and male sterility, because of unbalanced nutrition occurred. Losses varied between 25% and 70% demonstrating that the system was far from ideal.

*Single Seed Descent in a hydroponic system.* To improve upon the former system a hydroponic approach was tested. In 1981 hydroponic equipment of SCRC-2 systems from Hydrovation Ltd., Leeds, Yorkshire, England was installed. The SCRC-2 has a capacity of 1,250 plants per unit. About 15,000 plants can easily be grown in a greenhouse of 8 m x 12 m space.

The plants grow in channels made of black polythene and the plant nutrition is controlled by three stock solutions: a) Solufeed F; b) Calcium nitrate; c) Nitric acid. A conductivity meter controls the concentration of nutrients in the solution. The solufeed F is a complete fertilizer, including trace elements with a typical composition of: N 6.6% (100% $NO_3$-N); $P_2O_5$ 9.9%; $K_2O$ 37.7%; Mg 2.4%, plus the trace elements Fe, Mn, B, Cu, Zn and Mo. The separate calcium nitrate solution enables local correction for calcium already present in the water supply and also minimizes the chance of calcium phosphate precipitation and loss when solutions are prepared. The diluted nitric acid solution allows for independent pH control and also provides a source of nitrogen.

First the stock solutions are made. 1) Solufeed F by dissolving 25 kg of it in 170 l of water; 2) Calcium Nitrate by dissolving 15 kg of it in 170 l water; 3) Nitric Acid 4%. The pH of solutions varies between 6.03 and 6.39.

Two recipes for making up the complete nutrient solution were used. During the vegetative growth period two parts of calcium nitrate were added to 197 parts of water and mixed well before adding one part of the Solufeed solution (recipe A). If a stock solution is not mixed in very thoroughly before the other is added calcium salts can precipitate making them unavailable to the plants and possibly inhibiting root function. During the generative period one part of calcium nitrate was added to 198 parts of water after which one part of solufeed F solution was added (recipe B).

The soluble salt content was measured with the Kent conductivity meter. The CF (conductivity factor) observed for the complete nutrition level as described (recipe A) came out as CF = 15.1 which corresponds with 1510 microhmos. Under soil conditions a CF between 7 and 13 is recommended for wheat. There is almost a linear relationship between the CF and the amount of nutrient used.

Nutrition levels of 50%, 25% and 12.5% resulted in conductivity values of 8.0, 4.0 and 3.0 respectively.

There are two factors influencing the nutrient uptake of the plants: a) The amount of salts available in the solution, b) The flow of the solution along the roots of the plants. When the concentration is reduced, it can be compensated for with a fast flow. All the aeration at the roots comes from the solution falling from the gravity feed bar into the end closures of the channel and the solution falling back into the sump. Therefore a minimum flow is required to provide the plants with sufficient oxygen. This may explain why the required CF value for normal good plant development was lower than expected.

Plants were grown in 3 cm x 3 cm spaced plant holders at four different nutrition levels to investigate at which level generation advance is most rapid: a) 100%; b) 50%; c) 25%; d) 12.5% of the just described complete solution. Frequent observations were made to keep the nutrient level constant. During the first month one adjustment per week was sufficient. Later, it was necessary to adjust twice a week with $\pm$ 50 ml stock solution solufeed and the corresponding amount of calcium nitrate.

The 100% nutrient level showed phytotoxic effects. The treatment of 50% and 25% nutrient level gave the best results in terms of a normal development of the wheat plant. The 12.5% concentration with a CF of 3.0 showed to be by far the best for rapidly advancing generations. Plants with thin stems without secondary tillers and relatively small ears were obtained. Forty five days after initiating seed germination the first plants began to flower. Ten days later more than 95% of the plants had reached the flowering stage. Three weeks later each plant produced about 20 vigorous seeds. So without additional light in the period October – November it is possible to produce one generation within 70 to 75 days and three generations in the period October to May.

The hydroponic system can become a very important tool in breeding for partial resistance. If the crossings are made during the normal wheat season June – October, F4 lines can be sown in the following season as ear to row plots in the field. It would even be possible to produce homozygous lines in 18 months.

## 6.4 Break down of dormancy

The possibility of growing two or more generations per year is a great advantage for breeding programmes. However special care has to be taken with dormancy being itself a desirable property in wheat as it prevents sprouting in the ear. By heat or gibberellic acid-3 treatments the dormancy can be broken. The temperature treatment, which was applied for large seed lots, consisted of a period of three days heat at 35 °C followed by three days cold at about 10 °C. Bekendam (1965) recommended soaking the seeds overnight in a 0.02 to 0.10% gibberellic acid-3 solution. This has the disadvantage that the seed has to be dried for sowing by machine. An easier method has been described by Don (Report). His gibberellic acid-3 dormancy breaking method is based on a treatment of the seed (1 $cm^3$/100 g seed) with a solution of about 1 g Gibberellic acid-3/litre acetone. The acetone should be allowed to evaporate and the seed can start to germinate that same day.

# 7 Breeding material and selection procedures

The experimental breeding programme was initiated in 1976 with a composite based on 18 spring wheat cultivars (composite 1), followed in 1977 by the second and third composite made of 18 and 8 spring wheat cultivars respectively. Composite 3 was an initiative of Mehta from IAPAR (Instituto Agronômico do Paraná), Londrina, PR, and resulted in a joint programme for selection at two different sites with different pathogen populations.

A fourth composite was started in 1980 by a crossing programme under controlled conditions between Composite1Generation9 and 7 spring wheat cultivars investigating how the level of partial resistance of composite 1 is affected by or can be improved after the introduction of new cultivars with a very good plant type and root development but very susceptible to pathogens such as stem rust, leaf rust and powdery mildew. The components of the four composites are shown in table 7.1. These composites formed the basis of the wheat breeding programme for durable resistance. Through the application of the male gametocide ethrel, highly heterogeneous populations were created. The various selection procedures, applied to this material, are described in this chapter.

## 7.1 Selection of cultivars for the composites

The cultivars to compose the wheat composites were selected according to the following criteria:

1. Absence of known fully effective race-specific resistance to stem- and leaf rust. All the cultivars introduced in the programme had to be susceptible with an infection type 3 or 4 on the scale of Stakman et al. (1962). These infection types are based on lesion characteristics; low infection types (0, 1 or 2) have various levels of necrotic or chlorotic tissue surrounding the infection court, while high or susceptible infection types (3 or 4) lack this hypersensitive necrosis / chlorosis response. The difference between a 3 and a 4 refers to the size of the uredium. A large number of cultivars were tested with regard to several races of stem- and leaf rust in order to identify those cultivars that could be introduced in the programme. Also information available at CNPTrigo was used for this purpose.
2. Heterogeneity of genetic material. In a programme aiming at the accumulation of partial resistance to many pathogens simultaneously a high genetic heterogeneity is essential.
3. Indications of the presence of durable resistance as for instance in cultivars BH-1146, Frontana and Horto, which have been grown locally for 30 years or more.

Table 7.1. Cultivars used in the Composite 1, 2, 3 and 4.

| Composite: | | | |
|---|---|---|---|
| 1 | 2 | 3 | 4 |
| BH-1146 | Pergamino Gaboto | IAC-5 | Composite 1, G-9 |
| C-33 | Pat-19 | IAS-20 | Adonis |
| CNT-2 | PF-72248 | Horto | Bastion |
| CNT-3 | CNT-10 | BH-1146 | Kaspar |
| CNT-5 | Londrina | PF-70401 | Melchior |
| Coxilha | Jacuí | Pat-73172 | Selpek |
| Frontana | Maringá | Lerma Rojo 564 | Sicco |
| Horto | Vacaria | CNT-6 | Toro |
| IAS-20 | B-15 | | |
| IAS-58 | Erechim | | |
| IAS-62 | B-7455 | | |
| IAS-63 | Lagoa Vermelha | | |
| Lagoa Vermelha | Horto | | |
| Multiplication-14 | BH-1146 | | |
| Pel. Sl.-1263-69 | Peladinho-1 | | |
| PF-70401 | Peladinho-2 | | |
| S-76 | BR-11354 | | |
| Vila Rica | BR-11356 | | |

4. Equal heading and flowering, so that all cultivars contribute genes to the next generation in the outcrossing programme.
5. Other valuable characteristics, such as adaptation to brazilian conditions of soil and climate. The brazilian soils are acid with pH values between 4.3 and 5.0. The soils are poor in calcium, magnesium and phosphate. Potash is somewhat higher. The organic material is medium to low. The free aluminium level varies between 1 and 6 milli equivalent per 100 grams of soil. The brazilian cultivars have a high tolerance to aluminium toxicity whereas exotic cultivars in general have a low adaptability to this soil condition.
6. Plant type and root development. In general brazilian cultivars can be characterized as tall, susceptible to lodging and having a poor root development. In 1978 an experiment was conducted with the West European cultivars Kaspar, Toro, Adonis, Melchior, Bastion, Sicco and Selpek (FAO,1986). It was demonstrated that these cultivars have a significantly better root development and plant type than the cultivars of composites 1, 2 and 3. The improvement of the level of partial resistance of composite 1 in combination with an improved plant type and root development was sought through controlled crossings between Composite 1, Generation 9 and these seven cultivars.

The selection of cultivars of composite 1 was based solely on information attained from reports and literature. Composite 2 cultivars were chosen on the basis of both reports and specific field observations. Composite 3 was produced by Mehta in Paraná. Composite 4 was a result of experience on plant type and

Table 7.2. Leaf rust (LR) and stem rust (SR) races and their virulence / avirulence factor formulae 1) used in the three composites to eliminate race-specific resistance and to select for partial resistance.

| Race | Virulence /Avirulence factors |
|---|---|
| LR B-2 | 2a,2c,2d,16,17,18,21 /1,3,3allel,10,14a |
| LR B-6 | 2a,3,3allel,16,17,18,21 /1,2c,2d,10,14a |
| SR 11/74 | 8,9e,11,22,24,25,26,27,Tt1,Tt2 /5,6,7a,7b,9b,9d,10,12,13,14,15,16,17 |
| SR 15/65 | 6,7a,13,22,24,25,26,27 /5,7b,8,9a,9b,9d,9e,10,11,12,14,15,16,17,Tt1,Tt2 |

1) The virulence / avirulence formulae describe the resistance genes in the host, 2a,2c,2d,16 etc., for which the pathogen races carry the matching virulence or avirulence factor (Barcellos,1980; Coelho,1980).

root development investigating the possibility of combining these characters with partial resistance.

## 7.2 Leaf and stem rust races used in the selection programme

It is very difficult to select for partial resistance if race-specific resistance is present and operating (Robinson,1976; Parlevliet, 1983; Parlevliet,1988). It follows that race-specific resistance must either be absent or ineffective during the screening process. All race-specific resistance was made ineffective by the proper choice of the inoculum. For stem rust and leaf rust one race of each pathogen was 'designated' for the purpose of the breeding programme and was always used as inoculum at each selection procedure. The races B2 and B6 of leaf rust and 11/74 and 15/65 of stem rust were selected to make the race-specific genes to stem- and leaf rust which might be present ineffective. Composite 1, 3 and 4 were inoculated with the races B6 and 11/74 and composite 2 was inoculated with B2 and 15/65. The virulence / avirulence formulae, from which it can be deducted which resistance genes, if present, will be neutralized, are given in table 7.2.

As there are more than 30 Lr genes and more than 45 Sr genes the formulae do not give the full information that might be present.

## 7.3 Designating the generations

Each generation was numbered irrespective whether selection was practiced or not. The numbering G1, G2, G3, Gn was done according to the number of generations a composite had been advanced. G1 therefore is the equivalent to the first composite in the field obtained after the first crossing (G0) in the field with ethrel. G2 is the subsequent generation. The next generation can be obtained through outcrossing by means of ethrel or by selfing.

## 7.4 The random polycross method

Per composite the original cultivars were thoroughly mixed. Each resulting mixture was sown in a large field and divided into male and female strips (figure 5.1) of 1 and 2 meters width respectively. To induce male sterility the female strips were treated with ethrel. It was applied as an aqueous solution of 2000 ppm ethrel a.i. (1000 l water/ha) at growth stage 41 to 43 DC. Three to four days later 150 ppm gibberellic acid-3 at a rate of 500 l per ha was applied to promote ear emergence. It was estimated that in general about 60 – 80% cross pollination occurred.

## 7.5 Selection procedures

Initially a Single Plant Selection (SPS) procedure in a segregating population (comparable with an F2) was followed. In this procedure based on the concept of Robinson (1976) as discussed in chapter 4 the main ear of the best two plants per $m^2$ (grid selection) was harvested. The best phenotypes were those with the highest level of partial resistance to the complex of pathogens, which were introduced into the field. In the same generation these plants were intercrossed with ethrel. During the off-season the selected plants were multiplied advancing the population to obtain sufficient seed for the next stage (season), when the whole procedure of selecting and outcrossing was repeated. This procedure represents a recurrent selection procedure where single plant selection alternates with recombination. This might mean too much recombination and too little selection pressure. In order to find out if increasing the selection pressure and reducing the frequency of recombination could be a more efficient alternative the programme was divided into four different selection procedures to investigate the most efficient procedure. The four procedures differed from each other in the handling of the population between two cycles of recombination. The procedures (Figure 7.2) are:

1. Single Plant Selection (SPS).
2. Bulk Seed Selection (BSS).
3. Line Selection (LS).
4. Natural Selection (NS).

### *7.5.1 Single Plant Selection*

The procedure consists of a recurrent selection, where each cycle covers two generations, one for selection and outcrossing, one for multiplication.

For selection and outcrossing the populations were sown at a rate of 300 – 350 viable seeds/$m^2$ (first cycle) or 200 – 250 viable seeds/$m^2$ (all other cycles). Slightly denser seed rates than recommended (300 seeds/$m^2$) have the advantage that less tillers per plant are produced, while heading is more uniform favouring an optimal effect of the male gametocide application in the first cycle. The seed rate of 200 - 250 viable seeds gave a plant density that was neither too high so that intergenotypic competition would lead to discard high yielding genotypes nor too low so that a lack of competition would favour the genotypes only growing well under wide spacing conditions.

The first screening was negative and involved the removal of the poorer individuals prior to anthesis in order to eliminate undesirable pollen. About 30% of all plants were removed. This negative screening was conducted in both the male and female strips, as the male sterility of the latter was not absolute. All subsequent screenings were positive and carried out in the female strip only and were based on visual assessments of good health and good appearance. To reduce problems of interplot and interplant interference, all assessments were relative rather than absolute; regardless of the amount of disease, only the least diseased plants were selected. Screening was conducted on a grid system, with the best two or three individuals per $m^2$ being retained; selected plants were labelled with a plastic tag. A final screening was based on individual plants for the quality and health of the grain, both of which were determined in the laboratory. After screening the seed of the selected plants was bulked together and multiplied in the off-season advancing the population one generation (see chapter 6), so that a new cycle of crossing and selection could be started again.

### *7.5.2 Bulk Seed Selection*

Because many diseases have a direct influence on grain development (see chapter 3.2.7), a selection was carried out using weight as a criterion based on the idea that the larger grains came from relatively healthy (resistant) plants. The BSS follows the same procedure as the SPS. However, there was no SPS in the outcrossing generation. All grains from the female strips were harvested in bulk and the selection was applied to those seeds based on the quality of these grains in terms of i) size, ii) specific weight, iii) thousand grain weight and iv) freedom of diseases. Grains from the Ethrel treated strips were graded by means of a) a blower, which separates the light from the heavy grains (figure 7.1), b) a gravity table, c) a dockage tester and d) by visual inspection of the freedom from pathogens. About five percent of the grains were selected. The selected seed was multiplied in the off-season generation, as was done within the SPS procedure, to be used for the next cycle.

### *7.5.3 Line Selection*

The LS procedure was started in the same way as the SPS with one generation of cross pollination with ethrel. After the multiplication as in the preceding two procedures the generations were advanced twice through Single Seed Descent (chapter 6.3). LS started in the G4 generation (fig 7.2), being equivalent to F4 lines. These F4 lines were sown in single short rows, one ear to one row, at a seed rate of ca. 30 seeds per meter row and at a row distance of 16 cm. Five

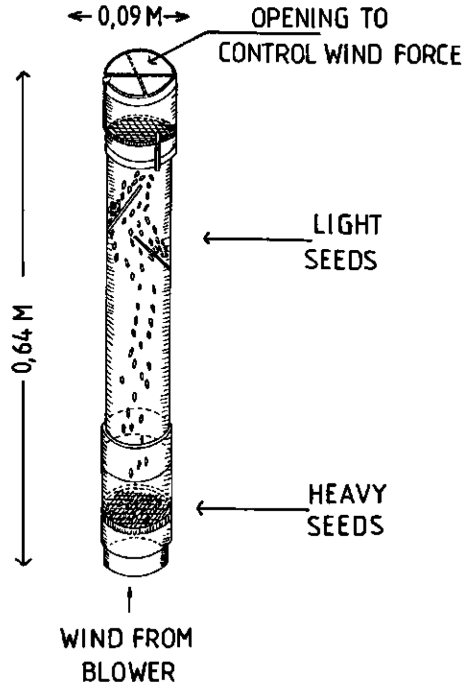


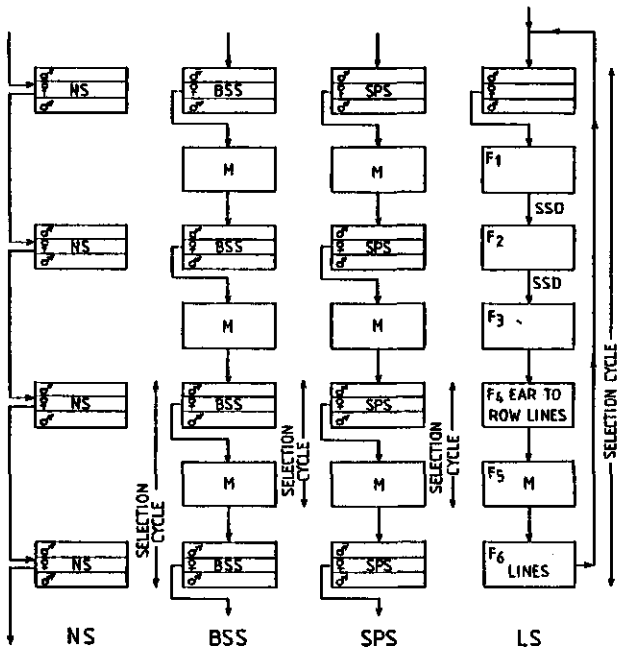
Figure 7.1. Separation of wheat grains on the basis of gravity with a blower.

thousand ear to row plots were grown of each individual composite. The best plants from the best lines were selected. In total, 200 plants were selected from composite 1 and composite 2 each, and 100 from composite 3. After one generation of multiplication in Mexico (chapter 6.1) line selection was repeated in F6. The F6 line selection was done with plots of three rows of 5 m with 16 cm between rows in three replicates. The seed density was the same as recommended in Rio Grande do Sul (60 viable seeds per meter row). The total plot was harvested. Nobre, the commercially most frequently grown cultivar was used as a reference. It occurred after every 20 plots. The best 10% of these lines, considering disease resistance and yield, were selected again and the mixture of these lines can be considered as the result of the line selection procedure to be compared with the populations of the other selection procedures. This material was then ready for a second cycle of outcrossing, followed by line selection.

The LS procedure was initiated in 1979 when the composites 1, 2 and 3 had passed already two, one and one SPS selection cycle respectively (Table 7.3).

### *7.5.4 Natural Selection*

The NS consisted of outcrossing using ethrel after which the harvested hybrid seed was sown again the next season without a multiplication inbetween (Figure




BSS = Bulk Seed Selection
LS = Line Selection
M = Multiplication only
NS = Natural Selection
SPS = Single Plant Selection
SSD = Single Seed Descent

Figure 7.2. Schematic design of selection procedures.

Table 7.3. Number of crossings and selection cycles [1] at the first generation (G1), the intermediate generation when the line selection was started (Gi) and at the end of the programme (Ge), for the four selection procedures of composite 1, 2 and 3.

| | | Number of Crossings | | | Number selection cycles | | |
|---|---|---|---|---|---|---|---|
| | | Comp1 | Comp2 | Comp3 | Comp1 | Comp2 | Comp3 |
| Single Plant Selection | G1 | 1 | 1 | 1 | 0 | 0 | 0 |
| Single Plant Selection | Gi | 3 | 2 | 2 | 2 | 1 | 1 |
| Single Plant Selection | Ge | 6 | 4 | 4 | 5 | 4 | 4 |
| Bulk Seed Selection | Ge | 7 | 4 | 4 | 5(+2)[2] | 4(+1) | 4(+1) |
| Line Selection | Ge | 4 | 2 | 2 | 1(+2) | 1(+1) | 1(+1) |
| Natural Selection | Ge | 5 | – | – | 5 | – | – |

[1]) One crossing (recombination) and one selection cycle form one cycle of recurrent selection.
[2]) In brackets are given the number of crossings and selections already made in the single plant selection procedure.

7.2). The assumption was that the more susceptible a plant is the less seed it will produce in a genetically heterogeneous population.

The number of outcrossings and artificial selections at the beginning (G1), at the intermediate stage G(i) when the LS procedure was started and at the end of the programme (Ge) for the four selection procedures to which the composite 1, 2 and 3 were exposed is shown in table 7.3.

## 7.6 Selection procedure combining partial resistance with an improved plant type

Several experiments were performed on plant type and root development of brazilian and West European wheat cultivars (FAO,1986). It was concluded that the cultivars Adonis, Bastion, Kaspar, Melchior, Selpek, Sicco and Toro are shorter, sturdier, tiller more and have a better developed root system than the Brazilian cultivars, but are extremely susceptible to stem rust and very susceptible to leaf rust and powdery mildew. It was investigated if the partial resistance, already accumulated in Composite 1 Generation 9 could be combined with the above mentioned plant type characters of these seven west european cultivars. A crossing programme of approximately 100 crosses was performed in the greenhouse between these cultivars and Composite 1, Generation 9 and is further referred to as Composite 4. In the F2 generation a SPS was done in the field selecting 0.5 – 1% of the plants. These were strongly multiplied in Mexico in the off-season. The result of this selection procedure, the bulk F4 population, was evaluated.

## 7.7 Exposure to pathogens

Each year selection pressure due to the natural disease pressure of various pathogens existed in the field. The severity of the various diseases varied between the years. With stem rust, leaf rust and powdery mildew the natural population, a mixture of races probably varied over the years as well. The most important pathogens in the period 1976 – 1982 were the following:

**1976:** stem rust and leaf rust; **1977:** leaf rust, *C. sativus*, *F. graminearum*, *S. nodorum*, *S. tritici*, SBMV, BYDV; **1978:** powdery mildew, SBMV, BYDV; **1979:** *C. sativus*, *F. graminearum*, *S. nodorum*, *S. tritici*, SBMV and BYDV; **1980:** *S. nodorum*, *S. tritici*, SBMV an BYDV; **1981:** powdery mildew, leaf rust, stem rust, *F. graminearum* and BYDV; **1982:** leaf rust, stem rust, SBMV, *F. graminearum*, *C. sativus*.

In order to exert the right selection pressure an artificial inoculation was carried out to increase the selection pressure and to make sure that selection was done in the presence of those races of leaf and stem rust and other pathogens that neutralize possible race-specific resistance genes.

Table 7.4. Pathogens, culture medium for inoculum production and concentration of inoculum (Inoc.) for field screening.

| Pathogen | Medium | Inoc. (conidia/ml) |
|---|---|---|
| *S. nodorum* | Potato dextrose agar | 200,000–500,000 |
| *S. tritici* | Pure wheat flower agar | 200,000–500,000 |
| *C. sativus* | Sorghum | 100,000–200,000 |
| *F. graminearum* | Maize or potato dextrose-agar | 100,000–200,000 |
| *P. graminis* | Wheat plants in greenhouse | 100,000–200,000 |
| *P. recondita* | Wheat plants in greenhouse | 100,000–200,000 |
| *E. graminis* | Wheat plants in greenhouse | 100,000–200,000 |

In the field the composites were inoculated with the required races (chapter 7.2) of stem rust and leaf rust and the pathogens *S. nodorum, S. tritici, C. sativus, F. graminearum* and powdery mildew. An aqueous spore suspension was applied by a motorized airblast knapsack sprayer. The inoculum of *S. nodorum, S. tritici, C. sativus* and *F. graminearum* was produced in the phytopathology laboratory, while the isolates of powdery mildew and the leaf- and stem rust races came from multiplication under controlled conditions in the greenhouses of CNPTrigo, Passo Fundo (Table 7.4). At application the inoculum was diluted to one-fourteenth and sprayed on the wheat population at a rate of 100 l of water / ha.

The powdery mildew epidemics, which occurred every year developed probably more from natural inoculum, than from the inoculation. Powdery mildew normally started early, before inoculations were applied and water suspension is not the recommended medium for an efficient inoculation.

The number of inoculations varied from year to year depending on how successful the inoculation had been. Attempts were made to obtain an evenly spread epidemic, being not so severe that 100% of the plants were killed nor so low that selection for escapes was provoked.

Insecticides were never applied. This allowed the possible accumulation of some resistance to aphids and BYDV, which is aphid transmitted. Considering the spotted distribution of aphids in the field, only little progress on aphid and BYDV resistance was expected.

SBMV can be introduced using infected soil. However with such an inoculation one runs the risk that all selection would become for SBMV only, as SBMV attacks are normally extremely severe. It has been observed that SBMV can survive for more than 9 years in the soil. For the selection as described in chapter 7.5 experimental fields were chosen, where SBMV had not been a major problem before and no inoculation was done. SBMV was seen in the selection fields, but never on a large scale.

## 7.8 Selection in Londrina

The SPS procedure of Composite 3 was conducted under two different conditions of pathogens and climate. The climate of Londrina is much warmer and therefore the pathogens *C. sativus* and stem rust are more predominant there, while under the more temperate climatic conditions of Passo Fundo, *S. tritici*, *F. graminearum*, BYDV and SBMV do prevail more. Leaf rust and powdery mildew and *S. nodorum* are equally important at both locations.

# 8 Response to selection for partial resistance to pathogens

## 8.1 Introduction

A large number of experiments was performed to monitor the accumulation of partial resistance to pathogens in successive generations of the breeding programme. Individual experiments were carried out to measure the selection response from the four different selection procedures: Single Plant Selection (SPS), Bulk Seed Selection (BSS), Line Selection (LS) and Natural Selection (NS) in relation to leaf rust, stem rust, powdery mildew, *S. nodorum, C. sativus, F. graminearum,* Common Root Rot, BYDV and SBMV. These evaluations were performed in 1982 at the end of the programme when of all four selection procedures at least one selection cycle was completed.

Evaluating the progress for partial resistance, yield and yield components, comparisons were made between the unselected composite and the latest selection of each of the various selection procedures. Table 7.3 gives the number of crossings (recombinations) per composite and selection cycles per composite, that were made for each of the selection procedures. With regard to the LS and BSS procedures one has to keep in mind that these procedures were started during the programme and had already passed two, one and one cycles, including selection in the SPS procedure of Composite 1, 2 and 3 respectively. For this reason a second control, SPS – Gi (intermediate generation) was incorporated in the programme representing the population from which the BSS and LS procedures were started.

At the final stage of the programme the following populations were compared:

| | | | |
|---|---|---|---|
| Composite 1 | G1 | Unselected population | |
| Composite 1 | G4 | Intermediate population (Gi = G4) | |
| Composite 1 | G12 | SPS | Latest selection |
| Composite 1 | G12 | BSS | Latest selection |
| Composite 1 | G12 | LS | Latest selection |
| Composite 1 | G5 | NS | Latest selection |
| Composite 2 | G2 | Unselected population | |
| Composite 2 | G4 | Intermediate population (Gi = G4) | |
| Composite 2 | G10 | SPS | Latest selection |
| Composite 2 | G10 | BSS | Latest selection |
| Composite 2 | G10 | LS | Latest selection |
| Composite 3 | G2 | Unselected population | |
| Composite 3 | G9 | SPS | Latest selection of Passo Fundo |

| | | | |
|---|---|---|---|
| Composite 3 | G9 | SPS | Latest selection of Londrina |
| Composite 3 | G9 | BSS | Latest selection |
| Composite 3 | G9 | LS | Latest selection |
| | | | |
| Composite 4 | -- | SPS | Latest selection |

Also a few randomly chosen F7 lines derived from the LS procedure of the composites 1, 2, and 3 were tested together in the experiments giving some information on the performance of relatively homozygous and homogeneneous lines in comparison to composites, which are more heterozygous and heterogeneous expressing therefore some dominance and mixture effects. These lines are: RH-18, RH-82040 and RH-83150 originating from composite 1, RH-82371 from composite 2 and RH-82428 from composite 3.

The experiments on response to selection for partial resistance are discussed per pathogen in the chapters 8.2. – 8.8. Crop loss assessments have been used to estimate the yield improvement as a result of increased resistance levels. Over the years response has been measured from fungicide, from insecticide and from their combined applications. In total five successive crop loss experiments have been performed. These experiments were carried out in 1976, 1978, 1979, 1981 and 1982 and are discussed in chapter 8.9.

## 8.2 Leaf rust

### *8.2.1 Evaluation of partial resistance through field experiments*

In 1981 and 1982 two field experiments were performed to measure the selection response of the various selection procedures. In 1981 only the SPS procedure was compared with the BSS procedure as the LS was not yet ready for evaluation. In 1982 all four selection procedures were compared together. In reality these experiments form part of the crop loss experiments as discussed in chapter 8.9.

Experiment I and II.

In 1981 a crop loss experiment was performed with four different treatments: i) No plant protection, ii) Fungicides, iii) Insecticides and iv) Fungicides + Insecticides. The aim was a full control of pests and/or diseases. The design was a latin square (Schuster et al., 1979) with four replicates. The populations that entered the experiment were: Composite 1 G2 Unselected population; Composite 1 G10 SPS Latest selection; Composite 1 G10 BSS Latest selection; Composite 2 G2 Unselected population; Composite 2 G8 SPS Latest selection; Composite 2 G8 BSS Latest selection. The plots measured 7 x 3.60 m and were sown at a seed rate of 300 viable seeds per $m^2$. In order to reduce interplot interference borders of rye of ca one m wide were sown between the plots. One leaf rust

Table 8.1. Leaf rust assessment in 1981 on six wheat populations.

| Population | | leaf rust assessment [1]) |
|---|---|---|
| Composite 1 G2 | Unselected population | 22.3 b[2]) |
| Composite 1 G10 | SPS latest selection | 6.7 a |
| Composite 1 G10 | BSS latest selection | 3.4 a |
| | | |
| Composite 2 G2 | Unselected population | 12.3 b |
| Composite 2 G8 | SPS latest selection | 5.0 a |
| Composite 2 G8 | BSS latest selection | 3.9 a |

[1]) Obtained as follows (% leaf area affected of flag leaf + 2 x % leaf area affected of 2nd leaf)/3

[2]) The leaf rust assessment carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).

assessment on the flag leaf and second leaf was made at growth stage 75 DC. Thirty plants per plot were assessed using the scale of James (1971, figure 8.1).

In 1982 an identical experiment was carried out in which all four selection procedures were compared. The leaf rust assessment was made at growth stage 49 DC on the upper two leaves. Twenty plants per plot were assessed. The composites tested in this experiment are those described in chapter 8.1.

Due to the circumstances 1982 has been a very difficult year for leaf rust evaluations. The break-down of race-specific resistance of the main grown wheat cultivar CNT-10 caused a leaf rust epidemic as never had been experienced before in Rio Grande do Sul. Interference coming from the surrounding fields, which were planted with CNT-10, was extremely strong, making borders with oats ineffective to reduce this phenomenon.

In 1981 a moderate level of leaf rust occurred in the field, being sufficient to observe the percentage leaf area affected at growth stage 75 DC. Both the SPS and BSS procedures showed a clear and significant improvement of partial resistance whereby the BSS procedure tended to show a slightly higher level of resistance than the SPS procedure. There was no difference between the plots that had been treated with insecticides and the plots that had no plant protection at all. Therefore both treatments have been taken together to give the data in table 8.1. The infection level on the 2nd leaf was almost twice as severe as on the flag leaf and presented clearer the differences between the unselected populations and both selection procedures. For this reason and because the 2nd leaves are larger, the leaf rust infection has been expressed as:
(% leaf area affected of flag leaf + 2 x % leaf area affected of 2nd leaf) / 3. (Table 8.1).

The 1982 experiment showed that all three selection procedures SPS, BSS and LS accumulated partial resistance to leaf rust (Table 8.2). For the statistical analysis computation was done with the transformed data into: ln (% leaf area affected of flag leaf + 2 x % leaf area affected of 2nd leaf) / 3. The test value for normality of error was seen by the Skewness, Kurtosis and D-value in the

Table 8.2. Leaf rust assessment in 1982 on sixteen wheat populations.

| Population | | leaf rust assessment [1] |
|---|---|---|
| Composite 1 G1 | Unselected population | 23.0 a [2] |
| Composite 1 G4 | Intermediate population | 13.4 bc |
| Composite 1 G12 | SPS latest selection | 12.1 bc |
| Composite 1 G12 | BSS latest selection | 14.9 bc |
| Composite 1 G12 | LS latest selection | 10.5 c |
| Composite 1 G5 | NS latest selection | 16.9 ab |
| Composite 2 G2 | Unselected population | 21.8 a |
| Composite 2 G4 | Intermediate population | 15.9 ab |
| Composite 2 G10 | SPS latest selection | 12.8 ab |
| Composite 2 G10 | BSS latest selection | 11.7 ab |
| Composite 2 G10 | LS latest selection | 11.4 b |
| Composite 3 G2 | Unselected population | 18.6 ab |
| Composite 3 G9 | SPS latest selection PF [3] | 15.0 abc |
| Composite 3 G9 | SPS latest selection Lo [4] | 19.2 ab |
| Composite 3 G9 | BSS latest selection | 11.9 bc |
| Composite 3 G9 | LS latest selection | 10.5 c |
| Control cultivar CNT-10 | | 35.8 d |

[1]) Obtained as follows: (% leaf area affected of flag leaf + 2 x % leaf area affected of 2nd leaf)/3.

[2]) The leaf rust assessments carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).

[3]) PF = Passo Fundo.

[4]) Lo = Londrina.

SAS Univariate programme showing an improvement when the logarithmic transformation is applied. Also the Duncan's multiple range test, was carried out using the ln transformed values.

The unselected populations of composite 1, 2, and 3 had 23.0, 21.8 and 18.6% leaf rust respectively, while the best selection procedure, the LS procedure gave 10.5, 11.4 and 10.5% leaf rust respectively (Table. 8.2). However interplot interference made it difficult to distinguish well between different levels of partial resistance and the three selection procedures where not significantly different in the Duncan's multiple range test (P = 0.05). The tendency of the BSS procedure being very efficient was also seen in this experiment. For composite 2 and 3 the response has been almost as good as the LS, while BSS resistance level in composite 1 was hardly less than that of the SPS. The natural selection was statistically not discernable from the unselected composite although there was somewhat less leaf rust. The Londrina SPS gave almost the same result as the Passo Fundo SPS procedure.

The composite 4 SPS latest selection was also compared in this experiment and and showed a very good selection response of 9.4% affected leaf area. It is expected that the West European cultivars with a complete different origin introduced in composite 1 some different genes of partial resistance to leaf rust.

Experiment III.

In 1982 forty five F-6 lines from the LS procedure were evaluated for leaf rust resistance. The lines were planted in small adjacent plots of four rows of 3 m length with three replicates. Cultivar CNT-9, a cultivar with the same level of susceptibility as CNT-10, was planted as a control because this was the standard cultivar in the official EMBRAPA/ CNPTrigo trials and also the latest generations of the SPS selections of the three composites (chapter 8.1) were used as controls to make a comparison with material from the partial resistance programme possible. The leaf rust assessment was done at growth stage 73-75 DC using the scale of James (1971).

A good level of partial resistance was observed in the majority of the selected lines being far superior to that of the control CNT-9 (Table 8.3) with 46.3% leaf rust and ca 30 lines were also better than the three SPS latest selections of composite 1, 2 and 3 which had a leaf rust infection of 10.1%, 9.8% and 12.0% respectively. The multiple range test of Duncan ($P=0.05$) indicated that 27 lines were significantly better than the composite 1, SPS procedure. The interplot interference was less of a problem in this experiment, because it was planted on the border of the experimental area with the main wind direction coming from outside.

From the experiment it can be concluded that even in a year with an extreme high level of leaf rust it is possible to have an effective level of partial resistance in the field. Even resistance levels lower than 1% were observed. The F7 line RH-82040, line RH-82371 and line RH-82403 from composite 1,2 and 3 gave 1.2%, 9.9% and 7.4% leaf rust respectively. These lines were further investigated by de Milliano et al. (1986) where it has been demonstrated that the resistance found in Brazil is also highly effective against five isolates from the Netherlands representing at least four races of leaf rust.

Table 8.3. Number of wheat lines at five levels of leaf rust assessed at growth stage 73-75 DC. The level of leaf rust was evaluated in the same way as given in the tables 8.1 and 8.2.

| Level of leaf rust: | < 1% | 1–5% | 5–10% | 10–15% | > 15% |
|---|---|---|---|---|---|
| Number of lines: | 3 | 15 | 19 | 6 | 2 |

Percentage leaf rust of controls: CNT-9 = 46.3%; Composite 1 G12, SPS = 10.1%; Composite 2 G10, SPS = 9.8%; Composite 3 G9 SPS = 12.0%.

Table 8.4. Number of F2 progenies with a mean percentage of leaf area affected by leaf rust in the flag and in the second leaf in five classes of affected leaf area.

| Affected leaf area | Flag leaf | Second leaf |
|---|---|---|
| < 2.1 | 10 | 7 |
| 2.1– 4.0 | 7 | 2 |
| 4.1– 6.0 | 9 | 3 |
| 6.1–10.0 | 1 | 13 |
| 10.1–15.0 | 0 | 2 |

Experiment IV

Considering the composites as a gene pool in which the gene frequencies change during the selection process, it is important to know how much genetic variation is still present in the population after several cycles of recurrent selection. It has been attempted to obtain a certain information on this with composite 2 after four cycles of SPS procedure. Twenty seven randomly taken F1 plants from the Composite 2, SPS, G8 were multiplied on a per plant basis in Mexico where high multiplication rates can be obtained (see chapter 6). The F2 progenies were sown in Passo Fundo in plant to row plots of 2 m (120 plants per plot). Ten cultivars, randomly taken from those that made up Composite 2 were planted for comparison with the F2 progenies and to obtain information on the environmental variation. The leaf rust severity was evaluated at growth stage 75 DC on the upper two leaves. Per plot twenty three randomly taken plants were assessed.

The F2 lines showed a continuous variation for the percentage leaf rust observed in the field. The variation between the F2 progenies was large. The mean over all F2's was also much lower than the mean of the ten control cultivars, which was about 10% for the flag leaf and about 17% for the second leaf. The average resistance level of the F2 progenies ranked from below 2.1% to 15 % (Table 8.4). As the variation between the F2 progenies appeared to be still very large, even larger than the variation within F2 progenies, one can conclude that there is still a considerable amount of variation for partial resistance present in the composite population, although a substantial response to selection already did occur.

### *8.2.2 Evaluation of partial resistance through latent period*

The latent period (LP) was investigated for its value to measure partial resistance. Together with Paniagua et al. (1984) differences in LP for several Brazilian and Paraguayan wheat cultivars with correlated field resistance were found. LP evaluations were carried out as described by Parlevliet (1975). It can be as-

Table 8.5. Relative latent period (RLP) of 10 wheat cultivars for leaf rust races 1 and 4 at the seedling stage compared with the amount of leaf rust in the field at growth stage 30 and 59 DC.

| Cultivar | RLP race 1 | RLP race 4 | Growth stage 30: number of uredo-sori per tiller | Growth stage 59: average % leaf rust on upper three leaves |
|---|---|---|---|---|
| Coxilha | 100 | 100 | 9.2 | 14.8 |
| CNT-5 | 100 | 104 | 6.0 | 7.8 |
| PF-70401 | 100 | – [1]) | 7.2 | 8.5 |
| Pel SL 1263-69 | 104 | 111 | 6.0 | 6.5 |
| C-33 | 104 | 112 | 2.0 | 8.5 |
| IAS-20 | 107 | 115 | 3.0 | 5.8 |
| CNT-3 | 109 | 110 | 5.5 | 6.5 |
| S-76 | 113 | 132 | 8.5 | 17.2 |
| BH-1146 | 127 | 126 | 0.5 | 4.2 |
| Frontana | 128 | 121 | 0.0 | 3.2 |

[1]) = *Infection type* X

sessed at the seedling stage and on adult plants and is evaluated by measuring the period from inoculation until 50% of the total number of uredosori have appeared in a marked area on the middle part of the leaf. In total three experiments were performed.

Experiment I

In 1976 ten of the cultivars that made up Composite 1 were inoculated at the seedling stage with two different leaf rust races, races 1 and 4 (Races were not yet identified by B numbers). That year race 1 was the predominant race in the field and also used to inoculate the composite in the field.

The inoculum was sprayed with water and wetting agent Tween (one drop per l) over the one week old seedlings (from emergence) at ca 16.00 hr and there after the plants were kept wet until the following day (08.00 hr) in a humid dark growth chamber. During incubation the plants remained in the growth chamber at 17 C, with a day length of 12 hr and 90% humidity. Four seedlings per cultivar were evaluated.

The cultivar PF-70401 race 4 combination gave an X-infection type (IT). All other combinations showed a susceptible IT (3 or 4).

A field experiment was done with the same cultivars in order to assess the partial resistance at growth stages 30 and 59 DC. The lay out of the experiment was a factorial design with randomized blocks and four replicates. The plot size was 5.0 x 3.2 m with a border of one row wheat. All cultivars had the same earliness. The percentage affected leaf area was estimated with the assessment scale of James (1971) (see figure 8.1). Ten tillers per plot were evaluated. The epidemic was based on a natural infection with predominantly race 1.

The LP with race 1 ranged from 7.5 days for Coxilha to 9.6 days for Frontana. With race 4 the LP ranked from 7.2 days for Coxilha to 9.5 days for S-76. The latent periods were transformed into relative values (Table 8.5). The Duncan's multiple range test (P = 0.05) showed that the cultivars S-76, BH-1146 and Frontana have a significantly longer LP than the remaining cultivars for both races. The interaction between cultivar and race was significant due to the longer LP of S-76 with race 4. The origin of this interaction has not been investigated. However this interaction resembles the interaction type which Parlevliet & Van Ommeren (1985) found with leaf rust in barley.

The leaf rust assessment at growth stage 30 DC in the field of these 10 cultivars gave a significant correlation of −0.70 * with the LP, race 1. The second observation at growth stage 59 DC was almost significant with a correlation coefficient of -0.42 only. This correlation was probably low due to interplot interference in the field and the unexpected high disease incidence of cultivar S-76.

As a general conclusion, it was found that LP is a fair parameter of measuring partial resistance. It has, moreover the advantage that it is a monocyclic test in the sense that the pathogen develops only one cycle until the observation is done. In such a test interplot interference and the effects of race mixtures that might occur in the field are avoided.

Experiment II.

Eight cultivars (Table 8.6) all with a susceptible IT (IT 3 or 4) were tested for their LP at the seedling stage and on the young flag leaf (growth stage 41 DC). Also the number of uredosori per unit leaf area was counted. The inoculations were made with race B-11 in the same way as in the previous experiment. After inoculation the adult plants were directly transferred to the greenhouse where they stayed under normal daylight conditions. The seedlings remained in the growth chamber at 20 C, 12 hr of light during the day time and 90% humidity.

For the seedling test, the middle part of the leaf was marked before the first uredosori became visible. In the same way fifteen flag leaves per cultivar of the adult plants were labelled and an area of ca 5 cm marked in the middle part of the leaf for the LP assessments. Daily countings were made on the number of uredosori that appeared in the marked area and the LP was measured as the time from inoculation until the moment that 50% of the uredosori had appeared.

For a comparison of the latent period with the partial resistance level to leaf rust in the field, the cultivars Jacuí and Cotiporã were tested in a field experiment during four years from 1978 until 1981. These cultivars were used in a crop loss trial with plots of 20 $m^2$ in 1978 and 1979. The plot size was increased to 50 $m^2$ in 1980 and 1981. At stem elongation race B-11 was inoculated with a motorized air blast knapsack sprayer. Each assessment was made on the four individual top leaves of ten random selected plants with the scale described by James (1971).

The best partial resistance was found in the cultivars Horto, Jacuí and Mascarenhas. The LP appeared to be better expressed at the adult stage than at the

Table 8.6. Relative latent period (RLP) and relative number of uredosori (RNU) per unit leaf area of leaf rust race B-11 on eight different wheat cultivars at the seedling stage and at the flag leaf at growth stage 41 DC.

| | RLP | | RNU per unit leaf area | |
|---|---|---|---|---|
| Cultivar | Seedling | Adult plant | Seedling | Adult plant |
| Cotiporã | 100 | 100 | 100 | 51 |
| Maringá | 103 | 101 | 62 | 51 |
| CNT-5 | 106 | 103 | 34 | 51 |
| CNT-1 | 104 | 106 | 83 | 100 |
| IAS-61 | 104 | 114 | 43 | 34 |
| Mascarenhas | 120 | 123 | 48 | 18 |
| Jacuí | 126 | 119 | 42 | 8 |
| Horto | 126 | 137 | 3 | 6 |

seedling stage (table 8.6) as the variance for LP in the adult plants was some 40% greater than the variance for LP in the seedlings.

A correlation of 0.88 ** was observed between the relative LP at the seedling stage and at growth stage 41 DC. Also the relative number of uredosori per unit leaf area at the seedling stage and in the flag leaf were significantly correlated with the LP. These correlation coefficients between the relative number of uredosori per unit leaf area and relative LP were -0.69 * and -0.73 * for seedlings and adult plants respectively.

From this experiment it can be concluded that Jacuí and Horto are very valuable for partial resistance breeding programmes.

In 1981 another test on the latent period of some cultivars tested within the scope of the national wheat cultivar trial also confirmed the partial resistance of Jacuí. A seedling test with race B-12 gave for Cotiporã, Maringá, CNT-1, Mascarenhas and Jacuí relative LP values of 107, 102, 100, 116 and 120 respectively.

Both parameters LP and number of uredosori per unit leaf area can be used to evaluate partial resistance to leaf rust. The adult plants tend to give better results than the seedlings, but for testing large numbers of adult plants in the greenhouse much space is required.

The difference in LP was confirmed with field observations. During five years Jacuí showed a highly significant level of partial resistance in comparison to the very susceptible cultivar Cotiporã (Table 8.7).

Experiment III.

Six cultivars of composite 1 were tested for two components of partial resistance to race B-6 at the seedling and adult plant stage following the same methodology as described in the previous experiments. Also the number of uredosori

Table 8.7. Percentage leaf area affected by leaf rust of two cultivars in four years at two or three growth stages (GS).

| Cultivar | 1978 | | | 1979 | | 19801981 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | GS 59 | GS 77 | GS 91 | GS 45 | GS 91 | GS 37 | GS 45 | GS 69 | GS 71 | GS 77 |
| Jacuí | 0.0 | 1.1 | 2.5 | 0.1 | 1.0 | 0.0 | 0.0 | 0.6 | 0.0 | 0.8 |
| Cotiporã | 1.1 | 4.5 | 8.5 | 1.0 | 10.0 | 0.3 | 1.7 | 12.5 | 0.9 | 7.8 |

per unit leaf area was evaluated. All data are based on ten plants per cultivar.

The relative LP of BH-1146 and Horto were at both the seedling stage and the adult plant stage 39 DC significantly longer than those of the remaining 4 cultivars (Table 8.8). The results confirm the partial resistance that had been found for race B-11 with Horto and race 1 and 4 with BH-1146. The ranking order of the cultivars for the relative number of pustules per unit leaf area is the inverse of the relative LP. The correlation coefficients between both parameters were highly significant being at seedling stage −0.90 ** and at the adult plant stage −0.97 **.

In general it can be concluded that partial resistance is well expressed by both LP and infection frequency with special regards to adult plants. This agrees with the series of studies with barley of Parlevliet reviewed by this author (Parlevliet, 1988).

### *8.2.3 The response to selection evaluated by latent period*

LP can be seen as an efficient parameter to measure partial resistance to leaf rust (chapter 8.2.2). Therefore the improvement for leaf rust resistance obtained

Table 8.8. Relative latent period (RLP) and relative number of uredosori (RNU) per unit leaf area of leaf rust race B-6 on six wheat cultivars at the seedling stage and at the flag leaf at growth stage 41 DC.

| Cultivar | RLP | | RNU per unit leaf area | |
|---|---|---|---|---|
| | Seedling | Adult plant | Seedling | flag leaf |
| Coxilha | 100 | 100 | 100 | 100 |
| IAS-20 | 107 | 103 | 57 | 80 |
| Maringá | 106 | 107 | 69 | 76 |
| CNT-5 | 108 | 114 | 38 | 45 |
| BH-1146 | 116 | 141 | 11 | 7 |
| Horto | 129 | 144 | 3 | 8 |

Table 8.9. Relative latent period (RLP) of wheat seedlings of three composites at three stages (Gu, Gi, Ge [1])) *of four selection procedures: Single Plant Selection (SPS), Bulk Seed Selection (BSS),* Line Selection (LS) and Natural Selection (NS) after inoculation with either leaf rust race B-12 or B-18.

| Selection procedure | | Composite 1 /race B-12 | Composite 2 /race B-18 | Composite 3 /race B-12 |
|---|---|---|---|---|
| Gu[1]) | Unselected population | 100 bc[2]) | 100 c | 100 d |
| Gi | Intermediate population | 104 b | 104 c | – |
| Ge | SPS latest selection PF[3]) | 103 b | 105 c | 108 bc |
| Ge | SPS latest selection Lo[4]) | – | – | 105 c |
| Ge | BSS latest selection | 113 a | 126 a | 116 a |
| Ge | LS latest selection | 110 a | 111 b | 115 a |
| Ge | NS latest selection | 96 c | – | – |

[1]) Gu = G unselected: G1 for composite 1; G2 for composite 2 and 3.
Gi = G intermediate selection: G4 for composite 1 and 2.
Ge = G end selection: G12 for SPS, BSS, LS in composite 1, G5 for NS in composite 1; G10 in composite 2; G9 in composite 3.
[2]) Latent periods carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).
[3]) PF = selection in Passo Fundo
[4]) Lo = selection in Londrina

through the four selection procedures SPS, BSS, LS and NS described in chapter 8.2.1 was also evaluated by measuring the LP at the seedling stage. Comparison between the unselected composites and the final result of each selection procedure permits another evaluation of how efficient each procedure was.

The seedlings, grown in the greenhouse under disease-free conditions, were transferred to the growth chamber seven days after emergence, where they were inoculated with race B-12 for composite 1 and 3, and race B-18 for composite 2 populations. The inoculation was performed as described in 8.2.2 experiment I. The growth chamber conditions during incubation were: 17 °C, 85% humidity and 12 hr daylight. Before the uredosori started to appear, 40 first leaves per population were marked for daily countings to be made as described in chapter 8.2.2. experiment I. LP was assessed as the time from inoculation until 50% of the uredosori had appeared in the marked area.

The response to selection appeared to differ significantly between the various selection procedures for the three composites according to the analysis of variance and the Kruskal Wallis (1952) chi square approximation. Considerable progress was made by the BSS and LS procedure, while the SPS procedure showed only a small improvement, which was not significant (Table 8.9). The relative LP of the BSS procedure showed for composites 1, 2 and 3 a response from 100 to 113, 126 and 116 respectively. For the LS procedure this relative LP increased for composites 1,2 and 3 to 110, 111 and 115 respectively. Up to the intermediate control (Gi) stage of the programme the increase of the relative LP was small and not significant. One selection cycle with the LS procedure gave a significant improvement while three selection cycles in the SPS procedure did not. The same result was seen for the BSS procedure in comparison to the SPS procedure. The results are almost identical for the three different composites 1, 2 and 3. This confirms the field data (8.2.1) where LS was superior to SPS in all three composites while BSS was better than SPS in two of the three composites (Table 8.2). The NS procedure showed even a tendency towards a shorter LP.

The SPS in Londrina gave almost the same response as the selection in Passo Fundo, with relative LP's of 105 and 108 respectively. This was expected, as the climatic conditions for leaf rust development are very similar at the two locations.

The results of the two selection procedures, SPS and LS can be explained by the effects of early and late generation selection described in chapter 3. The single plant selection procedure is in reality an early generation selection. In the F2 generation plant differences are still masked by heterozygosity and environmental effects, making efficient selection difficult. Moreover the interplot interference (or better interplant interference) renders the selection of single plants in a heterogeneous population very difficult. With LS in the F4 and F6 generation the environmental variation is highly reduced because one can base the assessment on a large number of plants and the additive genetic variance in the F4 and F6 is much higher in the F2 (Table 3.1).

These results suggest that the heritability of partial resistance to leaf rust is moderate. This is not fully in agreement with the results in barley to leaf rust (Parlevliet et al.,1980) in wheat to stripe rust (Krupinsky & Sharp, 1978, 1979) and in wheat to stem rust (Knott,1982), who found relatively high selection responses from early generation selection.

The good response from the BSS procedure indicates that there was a good correlation between partial resistance to leaf rust and thousand grain weight and/or specific weight. In the same way as Derera & Bhatt (1972,1974) made in heterogeneous populations, seed selection in the highly heterogeneous populations, which gave a significant selection response of leaf rust resistance.

The homozygous F7 lines RH-18, RH-82040 and RH-82428 also showed an increased level of partial resistance with relative LP's of 116, 113 and 110 respectively, while the lines RH-83150 and RH-82371 did not. The predominantly grown wheat cultivar CNT-10 was used as a general control in the experiment resulting in a relative LP of only 85, 89 and 88 when it is compared with the unselected control of composite 1, 2 and 3. This low latent period explains also the extremely high susceptibility of cultivar CNT-10 after its breakdown of race-specific resistance in 1982. It also shows that the composites did carry a significant amount of partial resistance at the start.

Table 8.10. Percentage stem (leaf sheath) area affected by stem rust of eighteen wheat cultivars and one composite.

| Cultivar | % stem rust | Cultivar | % stem rust |
|---|---|---|---|
| Vila Rica | 4.5 a[1]) | Horto | 16.8 def |
| Frontana | 7.0 ab | Coxilha | 17.0 def |
| CNT-5 | 8.2 abc | IAS-62 | 18.5 ef |
| IAS-63 | 10.0 abc | Pel SL 1263-69 | 18.5 ef |
| Lagoa Vermelha | 10.5 bc | CNT-2 | 21.5 fg |
| C-33 | 11.2 bcd | S-76 | 22.8 fg |
| Multiplication 14 | 12.8 bcde | IAS-58 | 26.8 gh |
| Composite 1 | 13.8 cde | PF-70401 | 29.0 h |
| CNT-3 | 14.2 cde | BH-1146 | 29.0 h |
| IAS-20 | 14.2 cde | | |

[1]) The percentage stem rust carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).

## 8.3 Stem rust

### *8.3.1 Evaluation of the partial resistance*

Latent period (LP) was tested as a parameter to evaluate the level of partial resistance. No variation for LP between cultivars such as observed for leaf rust were found. Therefore it was investigated if different levels of partial resistance could be distinguished under field conditions.

In 1976 eighteen cultivars of composite 1 and this composite were sown in plots of 16 m² in a randomized block design with four replicates. The plots were exposed to the natural stem rust inoculation; which consisted for 66% of race 11/74 and 33% of race 15/65 and only traces of other races (Coelho,1980). To the predominant race all cultivars had a susceptibility infection type 3 or 4 in the seedling stage. At growth stage 85 DC ten tillers per plot (for the composite 30 tillers) were assessed for stem rust infection with the key described by James (1971), (Fig 8.1).

There appeared to be significant differences in the amount of stem rust present on the various cultivars (Table 8.10). Cultivars with a relatively high level of partial resistance were: Vila Rica, Frontana, CNT-5, IAS-63 and Lagoa Vermelha with 4.5, 7.0, 8.2, 10.0, 10.5% stem rust respectively, while BH-1146 and PF-70401 were the most susceptible cultivars with 29% stem rust. Because cultivar IAS-63 carries a low-infection type resistance against the second race 15/65 it could be expected that the resistance observed in this cultivar is partly due to the mixture effect of the two stem rust races occurring in the field. However it is very unlikely that this mixture effect has influenced the experiment because the cultivar Vila Rica, Frontana, CNT-5 Lagoa Vermelha have also a susceptible infection type 4 to stem rust race 15/65, while PF-70401 is resistant against this race. Differences between blocks were not significant indicating that the stem

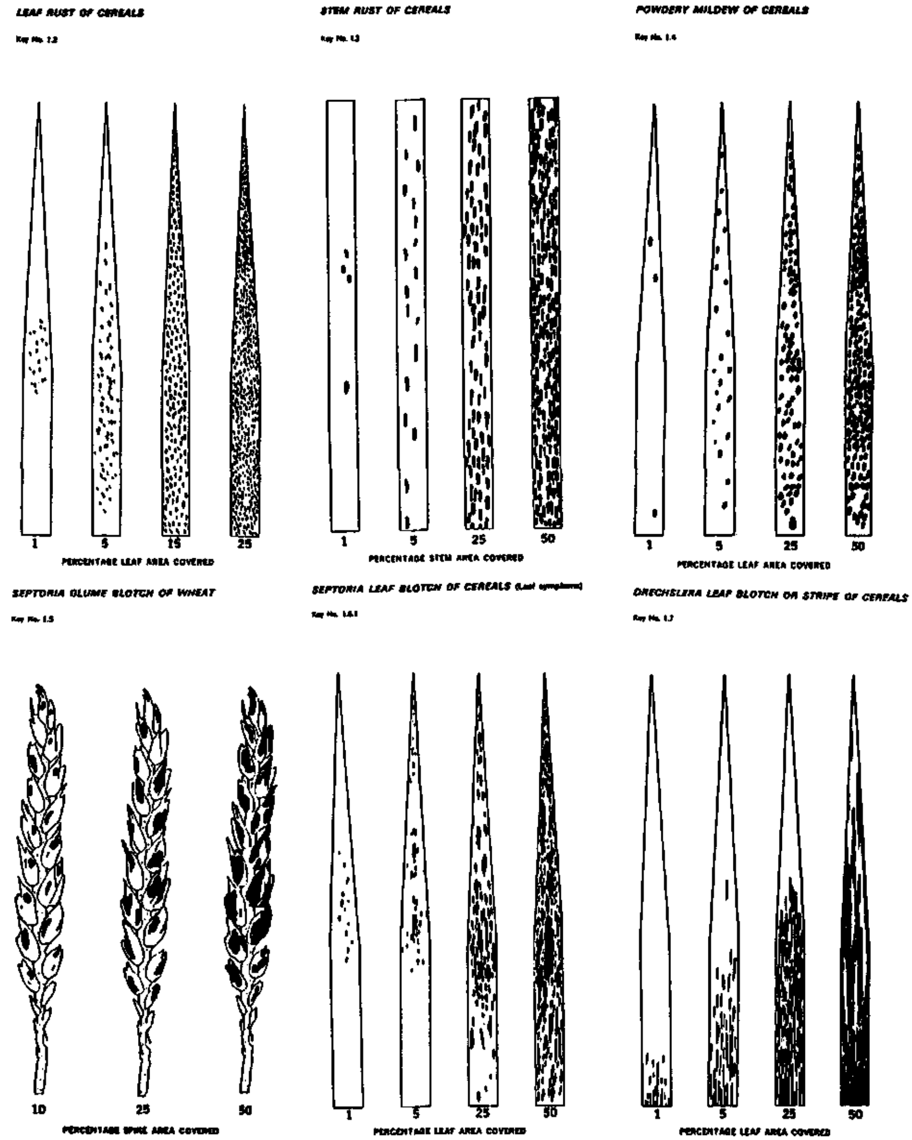



Figure 8.1. Assesment keys for leaf rust, stem rust, powdery mildew, *S. nodorum* on the ear, *S. nodorum* and *C. sativus* after James (1971).

rust was well spread over the experimental area. The composite itself had 13.8% stem rust, slightly but not significantly less then the average of the eighteen cultivars which was 16.2%.

Possibly the differences between cultivars could have been even larger, if the plot size had been greater and the inoculum pressure from the surrounding fields less, reducing interplot interference (Parlevliet & van Ommeren,1975).

Vila Rica and Frontana are the two best cultivars as far as stem rust resistance is concerned. The ability of Frontana to retard the development of stem rust has also been reported by Wilcoxson et al. (1975) in an evaluation of wheat cultivars done in the U.S.A. This is again, as mentioned in chapter 8.2.2. for leaf rust, an example of partial resistance that is expressed at quite different locations in the world.

It is interesting to note that BH-1146, the most susceptible cultivar in this test appeared to be relatively resistant in another experiment in 1978. In that experiment BH-1146 was compared with the West-European spring wheat: Adonis, Bastion, Kaspar, Melchior, Selpek, Sicco and Toro. These cultivars had never been selected for stem rust resistance because this pathogen is not a problem at the locations where they were selected. This suggests that the susceptible cultivars PF-70401 and BH-1146 still carry some partial resistance genes to stem rust.

### *8.3.2 The response to selection*

To evaluate the response to selection with the four selection procedures, SPS, BSS, LS, NS, the composites described in chapter 8.1, were compared in an experiment with a factorial design. The plot size was 2.0 x 2.2 m with borders of flax at all sides to reduce interplot interference. Four replicates were used. The plots were regularly sprayed with Indar and Milgo in order to control leaf rust and powdery mildew. Stem rust race 11/81 was introduced in the plots sown with populations of composite 1 and 3, while race 15/65 was introduced in the plots sown with composite 2 populations. For composites 1 and 3 race 11/74 had always been used for the screening of partial resistance. However this race was not sufficiently available in 1982. Race 11/81 is almost identical to race 11/74. All cultivars from which the original composites 1 and 3 were derived have a susceptible infection type to race 11/81. Introduction through spraying with an inoculum suspension was done at growth stage 51 DC. Fifteen randomly taken main tillers were assessed per plot at growth stage 75 DC.

A considerable and significant progress in partial resistance was observed as table 8.11. shows. The statistical analysis was carried out on the logarithmically transformed data as this transformation did improve the normality of the error considerably (Stephens,1974). Both the SPS and LS procedure were very efficient to accumulate partial resistance to stem rust. The final result of the SPS was for composite 1 equal to the result of the LS procedure. For composite 2 the SPS resulted even in a slightly higher partial resistance level, while for composite 3 the LS procedure was slightly better. It should be considered that the LS procedure of composite 1 increased in one screening cycle only, the resistance level from 9% (the partial resistance level of G4 when both selection procedures started) to 2.8%, while through the SPS this result was obtained after three selection cycles. The same consideration can be made for the compo-

Table 8.11. Stem rust assessment in 1982 on seventeen wheat populations.

| Population | | % stem rust |
|---|---|---|
| Composite 1 G1 | Unselected population | 16.0 a[1]) |
| Composite 1 G4 | Intermediate population | 9.0 b |
| Composite 1 G12 | SPS latest selection | 3.1 d |
| Composite 1 G12 | BSS latest selection | 13.1 c |
| Composite 1 G12 | LS latest selection | 2.8 de |
| Composite 1 G5 | NS latest selection | 14.9 a |
| Composite 2 G2 | Unselected population | 13.9 a |
| Composite 2 G4 | Intermediate population | 10.3 b |
| Composite 2 G10 | SPS latest selection | 2.5 e |
| Composite 2 G10 | BSS latest selection | 7.3 c |
| Composite 2 G10 | LS latest selection | 4.8 d |
| Composite 3 G2 | Unselected population | 18.4 a |
| Composite 3 G9 | SPS latest selection PF[2]) | 3.2 d |
| Composite 3 G9 | SPS latest selection Lo[3]) | 14.0 b |
| Composite 3 G9 | BSS latest selection | 8.8 c |
| Composite 3 G9 | LS latest selection | 2.4 e |
| Composite 4 -- | SPS latest selection | 1.0 |
| Cultivar CNT-10 | | 6.8 |

[1]) The leaf rust assessments carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).
[2]) PF = Passo Fundo.
[3]) Lo = Londrina.

sites 2 and 3 (see Table 7.3). The considerable selection response obtained with the SPS for composite 1, 2 and 3, is indicative for a high heritability. This agrees with the stem rust observations of Knott (1982) who also observed a high selection response in early generations.

The BSS procedure did not give much of a selection response, much less than was obtained against leaf rust. Possibly stem rust had in the screening populations not such a strong influence on grain formation as leaf rust. This is probably the case due to the fact that the stem rust epidemics start too late to damage the grain formation seriously, while leaf rust epidemics always start much earlier (just after tillering) in the season.

Also the SPS procedure in Londrina did not show much of a selection response. During the selection procedure the population was exposed to natural stem rust epidemics in Londrina, while in Passo Fundo stem rust was artificially introduced, probably resulting in a much higher stem rust pressure in Passo Fundo.

The NS did not accumulate any significant level of partial resistance at all.

The partial resistance level of Composite 4 was higher than was expected considering that the seven newly introduced cultivars Adonis, Bastion, Kaspar, Melchior, Selpek, Sicco and Toro in this composite are very susceptible to stem rust. However the crossings made with these cultivars resulted in even higher levels of partial resistance what must be due to the effect of an accumulation of minor genes originating from composite 1 and the cultivars coming from a complete other origin carrying still a few different minor genes of partial resistance to stem rust. The selection pressure in composite 4 has been very severe what might explain the large response of this selection as well.

In this experiment homozygous F7 lines too were evaluated for their partial resistance level. The lines RH-82040 and RH-83150 selected through the LS procedure from composite 1 had 3.3 and 4.3% stem rust respectively. RH-18, with 2.0% infection, was even significantly better than the latest selection of the SPS procedure. The homozygous lines RH-82371 and RH-82428 obtained from the LS procedure of composite 2 and 3 had 3.4 and 1.7% stem rust infection respectively. This latter line had a partial resistance significantly better than that of the SPS and LS procedures. The main grown cultivar CNT-10 was included in the experiment. CNT-10 had 6.8% stem rust infection.

## 8.4 Powdery mildew

Two different experiments have been performed to measure the response to selection for resistance to powdery mildew. The first is an evaluation of the percentage powdery mildew on adult plants grown in pots in the greenhouse, while the other refers to a test with detached seedling leaves.

### *8.4.1 The response to selection through greenhouse experiment*

The response of the four different selection procedures SPS, BSS, LS and NS for partial resistance to powdery mildew has been tested in an experiment on adult plants in the greenhouse.

Of each of the composite populations 40 plants were raised, four per pot, in a greenhouse. The powdery mildew was allowed to develop freely on them. A small number of homozygous F7 lines (also 40 plants per line) derived from the LS procedure and the cultivar CNT-10 were included in this test as well. The composite populations were those described in chapter 8.1. The inoculum came from pots with adult plants standing in the same greenhouse. These adult plants belonged to the tested populations (2 pots of each population). This procedure gave the guarantee that the powdery mildew inoculum matches with the tested populations. The greenhouse conditions were perfect for the powdery mildew development with a mild temperature at night, sufficient humidity and protected from rain. A very uniform infection was obtained from the seedling stage onwards over the whole experiment. The experiment can be considered as a polycyclic test as the powdery mildew could develop freely for several generations during the development of the plants to be tested. At growth stage 61

DC, 40 plants per treatment were assessed for the percentage leaf area affected on the flag leaf and second leaf using the scale of James (1971) (Figure 8.1).

There was an excellent correlation between the assessments of the flag leaf and those of the second leaf, r being 0.98 ** (P=0.01). The error of the mean percentage leaf area affected appeared to be not normally distributed. Logarithmic transformation showed near-normally distributed errors for all composites. The statistical analysis were therefore performed on the transformed data.

The response to selection differed significantly between the various selection procedures for the three composites according to the analysis of variance and the Kruskal Wallis (1952) chi square approximation. Both SPS and LS were very efficient procedures to accumulate partial resistance to powdery mildew (Table 8.12). The LS procedure was even significantly better than the SPS procedure for composite 1 and 3. Comparisons should be made between the intermediate generation G4 for composite 1 and 2, when the LS procedure was started, the G2 for composite 3 and the latest selections of the SPS and LS procedures. This means that one line selection cycle gave as good or even a better selection response than three SPS cycles (see Table 7.3).

Also the BSS procedure gave a positive selection response. It has not been as efficient as the SPS and LS procedures, but a significant accumulation of partial resistance was observed. The explanation of this selection response must be the fact that powdery mildew epidemics always start early when the wheat is at a growth stage of about 25 DC. Reducing the green leaf area in this and later stages, powdery mildew must have an impact on the grain filling. Moreover powdery mildew helps other pathogens to penetrate what makes the effect of powdery mildew on yield and yield components stronger. For the same reason the small positive selection response of the NS procedure can be explained. The response to selection through SPS was smaller at Londrina than at Passo Fundo (Table 8.12, composite 3).

The composite 4, with 2.0% powdery mildew, had the highest level of partial resistance. This accumulation might be due to the accumulation of minor genes from wheats of completely different origins (brazilian and west european wheat cultivars).

The homozygous F7 lines RH-18, RH-82040 and RH-83150 were with 1.6%, 1.2% and 3.0% powdery mildew respectively, significantly more resistant than the composite 1 latest selection of the LS procedure from which composite they were taken. The lines RH-82371 and RH-82428 presented 7.0% and 8.0% powdery mildew infection respectively and were more susceptible than the LS latest selection of composites 2 and 3 to which these lines belong.

Cultivar CNT-10 was extremely susceptible with 49.4% infection what agrees with the field observations.

### *8.4.2 Response to selection assessed through detached leaf tests*

Wolfe (1982) developed a detached leaf test for the measurement of partial resistance to powdery mildew in wheat. This method was used to evaluate the

Table 8.12. Powdery mildew assessment on sixteen wheat populations measured on adult plants in the greenhouse.

| Population | Powdery mildew assessment[1]) | |
|---|---|---|
| Composite 1 G1 | Unselected population | 48.0 a[2]) |
| Composite 1 G4 | Intermediate population | 43.4 ab |
| Composite 1 G12 | SPS latest selection | 8.1 d |
| Composite 1 G12 | BSS latest selection | 16.0 c |
| Composite 1 G12 | LS latest selection | 4.6 e |
| Composite 1 G5 | NS latest selection | 34.0 b |
| Composite 2 G2 | Unselected population | 25.7 a |
| Composite 2 G4 | Intermediate population | 8.9 b |
| Composite 2 G10 | SPS latest selection | 4.2 c |
| Composite 2 G10 | BSS latest selection | 8.4 b |
| Composite 2 G10 | LS latest selection | 4.5 c |
| Composite 3 G2 | Unselected population | 25.1 a |
| Composite 3 G9 | SPS latest selection PF[3]) | 5.2 d |
| Composite 3 G9 | SPS latest selection Lo[4]) | 16.0 b |
| Composite 3 G9 | BSS latest selection | 6.3 cd |
| Composite 3 G9 | LS latest selection | 3.2 e |
| Composite 4 | SPS latest selection | 2.0 |
| Control cultivar CNT-10 | | 49.4 |

[1]) Obtained as follows: (% leaf area affected of flag leaf + % leaf area affected of 2nd leaf)/2.

[2]) The powdery mildew assessments carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).

[3]) PF = Passo Fundo.

[4]) Lo = Londrina.

response to selection in the various composite populations. The method has the advantage that it is reliable and fast. Leaf segments are inoculated and the amount of powdery mildew is measured after the first generation powdery mildew colonies have appeared. An advantage is that interplant interference effects as must have occurred in the first experiment (chapter 8.4.1) are eliminated in this monocyclic test.

Seedlings of the populations to be tested were raised in a pathogen free greenhouse. After eight days, the leaves were cut in three equal parts of ca 2 cm. These seedlings were placed, with the adaxial surface upward, on plain agar (6 g of agar per l water), containing 150 ppm benzimidazole. The three segments of each seedling were placed in the same order in three petri dishes, so that each seedling could be exposed to three different sources of inoculum. Per composite population 25 seedlings were inoculated. The three inoculum sources were:

1. A bulk isolate from plants of the highly susceptible cultivar IAS-54. This powdery mildew population was maintained and multiplied in the greenhouse to be used for inoculations in the breeding programme.
2. A bulk isolate from plants of the original cultivars of each composite 1, 2 and 3.
3. A bulk isolate from plants of the latest selection of the LS procedure of each composite 1, 2 and 3.

Powdery mildew populations 2 and 3 came from the greenhouse experiment, described in chapter 8.4.1.. As a susceptible reference check, six leaves of the highly susceptible cultivar IAS-54 were placed in each petri dish to correct for different amounts of inoculum per inoculation. This procedure made comparisons between all the inoculations possible. The inoculation itself was done in a settling tower, with a capacity of 16 petri dishes (400 genotypes) per inoculation. The inoculum was blown in from 1.00 m above the dishes and two minutes were given for the conidia to settle on the leaves. By this methodology all the tested genotypes should receive an equal amount of inoculum. The petri dishes were kept at ca 18 °C with a day length of 12 hr. After eight days the leaf segments were evaluated by means of a binocular for the number of colonies per unit leaf area. Figure 8.2 gives a visual review of the testing procedure.

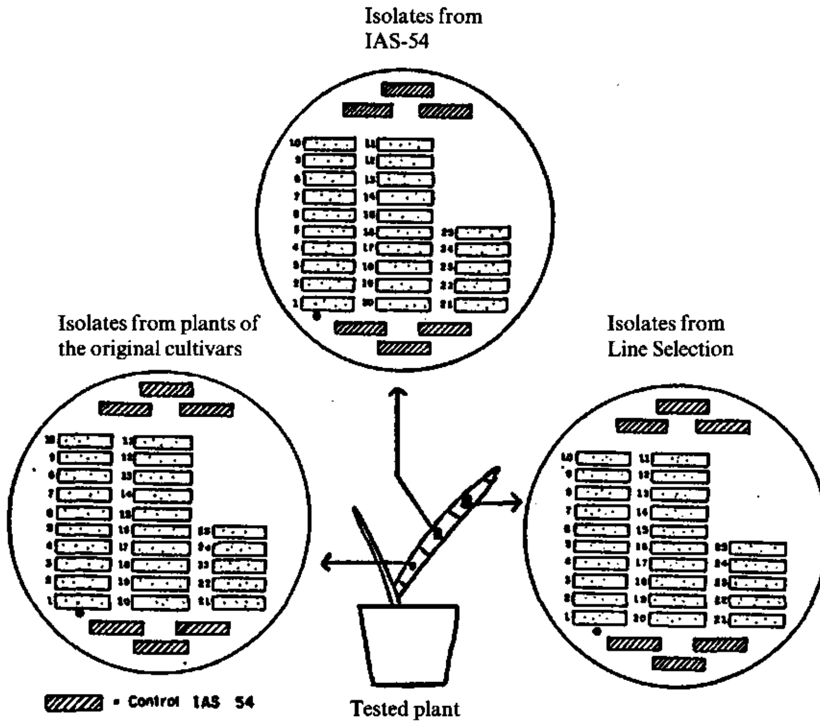



Figure 8.2. Design detached leaf test of powdery mildew resistance. Picture represents three petri dishes for one selection procedure.

Table 8.13. Powdery mildew on seventeen wheat populations measured at the seedling stage by the detached leaf test (experiment 1) measured as the average relative number of colonies per unit leaf area (RNCOL) for three different powdery mildew bulk isolates.

| Population | | Powdery I | mildew bulk II | isolates[1]) III |
|---|---|---|---|---|
| Composite 1 G1 | Unselected population | 79 ab[2]) | 111 ab | 72 ab |
| Composite 1 G4 | Intermediate population | 72 ab | 122 a | 62 abc |
| Composite 1 G12 | SPS latest selection | 65 bc | 68 d | 54 bc |
| Composite 1 G12 | BSS latest selection | 85 ab | 49 e | 50 c |
| Composite 1 G12 | LS latest selection | 44 d | 46 e | 51 c |
| Composite 1 G5 | NS latest selection | 91 a | 73 cd | 77 a |
| Composite 2 G2 | Unselected population | 89 a | 135 a | 97 a |
| Composite 2 G4 | Intermediate population | 62 b | 70 bc | 44 b |
| Composite 2 G10 | SPS latest selection | 52 bc | 55 c | 51 b |
| Composite 2 G10 | BSS latest selection | 50 bc | 77 b | 60 b |
| Composite 2 G10 | LS latest selection | 41 c | 57 c | 46 b |
| Composite 3 G2 | Unselected population | 75 a | 75 a | 106 a |
| Composite 3 G9 | SPS latest selection PF[3]) | 52 b | 50 b | 38 bc |
| Composite 3 G9 | SPS latest selection Lo[4]) | 52 b | 25 c | 19 d |
| Composite 3 G9 | BSS latest selection | 43 b | 54 b | 53 b |
| Composite 3 G9 | LS latest selection | 42 b | 43 b | 33 c |
| Composite 4 | SPS latest selection | 51 | 69 | 58 |
| Control IAS-54 | | 100 | 100 | 100 |

[1]) I = Bulk isolate from IAS-54; II = Bulk isolate from original cultivars of each composite; III = Bulk isolate from the latest selection of the LS procedure of each composite (see text 8.4.2).
[2]) Transformed mean numbers of colonies are significantly different according to Duncan's multiple range test (P = 0.05) if they carry no similar letters.
[3]) PF = Passo Fundo.
[4]) Lo = Londrina.

All results have been transformed into the relative number of colonies per unit leaf area (RNCOL), whereby the number of colonies per unit leaf area of the susceptible cultivar CNT-54 was set at 100. The average value of the RNCOL was calculated for each of the tested composites (Table 8.13). Transformation of the data to the square root of the RNCOL + 0.5 was applied to improve the normal distribution of the data for the analysis of variance and Duncan's multiple range test.

The analysis of variance indicated that the effects of the selection procedure were highly significant for composite 1, 2 and 3. The bulk isolate effect was significant for composite 1 and 2, while the selection procedure x bulk isolate interaction was only significant for composite 1 (Table 8.14, first experiment).

The results confirm very well what was observed in the previous experiments. The Duncan's multiple range test showed that with the 'Bulk isolate from IAS-

Table 8.14. F-values of two experiments on selection response of four different selection procedures (Sel. Pr.) for partial resistance to three bulk isolates (BI) of powdery mildew on composite 1, 2 and 3.

| | F value of ANOVA first experiment | F value of ANOVA second experiment |
|---|---|---|
| Composite 1: | | |
| Sel. Pr. | 12.85 ** | 147.99 ** |
| BI | 20.68 ** | 1.85 ns |
| Sel. Pr. x BI | 4.74 ** | 2.68 ns |
| Composite 2: | | |
| Sel. Pr. | 17.44 ** | 24.03 ** |
| BI | 18.20 ** | 7.00 ** |
| Sel. Pr. x BI | 0.87 ns | 0.43 ns |
| Composite 3: | | |
| Sel. Pr. | 37.66 ** | 34.05 ** |
| BI | 1.04 ns | 9.59 ** |
| Sel. Pr. x BI | 2.41 ns | 0.07 ns |

Table 8.15. Powdery mildew on six wheat populations measured at seedling stage by the detached leaf test (experiment 2) measured as the average relative number of colonies per unit leaf area (RNCOL) for two different powdery mildew bulk isolates.

| Population | | Powdery mildew bulk isolates [1]) II | III |
|---|---|---|---|
| Composite 1 G1 | Unselected population | 75 a[2]) | 99 a |
| Composite 1 G12 | LS latest selection | 17 b | 15 b |
| Composite 2 G2 | Unselected population | 93 a | 80 a |
| Composite 2 G10 | LS latest selection | 60 b | 46 b |
| Composite 3 G2 | Unselected population | 114 a | 87 a |
| Composite 3 G9 | LS latest selection | 64 b | 51 b |
| Control IAS-54 | | 100 | 100 |

[1]) II = Bulk isolate from original cultivars of each composite; III = Bulk isolate from the latest selection of the LS procedure of each composite (see text 8.4.2).

[2]) Transformed mean numbers of colonies are significantly different according to Duncan's multiple range test (P = 0.05) if they carry no similar letters.

54' the LS procedure gave the best selection response being for composite 1 significantly better than for the SPS procedure and for composite 2 and 3 equally good. Considering the fact that the number of selection cycles from the moment that the LS procedure was started was one only for the LS and three for the SPS procedure there is no doubt that the LS is superior. A significant accumulation of partial resistance by the BSS procedure was observed again. In the previous experiment the response of the BSS was the strongest for composite 3 followed by composite 2 and 1. The same effect was observed in the detached leaf test, however for composite 1 the BSS procedure was not significantly different from the unselected composite 1. The NS showed an increased level of susceptibility at the seedling stage.

A significant 'population selection procedure' x 'bulk isolate' interaction was found in composite 1. The isolate from the unselected population gave slightly more colonies on plants of this unselected population and the isolate from the LS population gave slightly more colonies on the plants of the LS population than was expected. This indicates that a race-specific effect could have been present. To investigate this aspect of the interaction effect in some more detail a second experiment was performed using the same methodology. In this test 40 seedlings of the unselected composites 1,2 and 3 and the latest selection populations of the LS procedure were inoculated with isolates taken from the same composites as in the previous experiment (bulk isolates II and III). When the powdery mildew inoculum was collected the plants were at growth stage 65 DC. Great care was given to collect uniformly over all the plants of the various populations.

The response to selection due to LS again was very significant (Table 8.15). The selection procedure and inoculum effects were always significant. The interaction effects, however were not significant (Table 8.14, second experiment).

### *8.4.3 Conclusion*

The progress in partial resistance assessed in 8.3.1 on adult plants in a polycyclic test was reasonably well confirmed by the assessment made on detached seedling leaves in a monocyclic test, whereby the adult plant test appeared to discriminate better, which is not surprising.

The results indicate that partial resistance can rather easily be accumulated indicating a fairly high heritability. With the exception of NS, significant progress was made by the applied selection procedures. The LS appeared to be the most efficient, followed by the SPS. Seven crossings and five selection cycles of BSS increased the resistance level to some extent. The introduction of other minor genes in composite 4 resulted in high levels of partial resistance.

## 8.5 *Septoria nodorum* and *Cochliobolus sativus*

### *8.5.1 The response to selection*

The selection response of the SPS, BSS, LS and NS procedures for partial resistance to *S. nodorum* and *C. sativus* has been evaluated on the populations described in chapter 8.1 in a greenhouse experiment with adult plants. A small number of homozygous F7 lines derived from the LS procedure and the cultivar

CNT-10 were included in the test as well. Per entry 40 plants were raised in pots with four plants per pot. After heading, at growth stage 59 DC, the four main tillers in each pot were tagged with wool before they were inoculated so that later these labelled tillers with the right growth stage at inoculation would be assessed. The inoculation was done in an inoculation room. The inoculum was prepared at a concentration of 500,000 conidia/ml for *S. nodorum* and 100,000 conidia/ml for *C. sativus*. Iharaguen at 3 drops/l was added as a sticker; 8 l of inoculum was applied on 240 pots (= 960 plants). The inoculation was made with a pressure point sprayer. For the *S. nodorum* evaluation the plants were kept in the inoculation room for 48 hr at 22°C, while for the *C. sativus* evaluation the plants stayed only 24 hr at 24°C. A mist blower kept the humidity at 100%. After inoculation, the plants were brought back to the greenhouse and evaluated for *S. nodorum* resistance after 14 days and for *C. sativus* after seven days. The ear, flag leaf, second leaf of the tagged tillers were assessed with the scale of James (1971) (Figure 8.1).

Septoria nodorum resistance.

The percentage leaf area affected on the first and second leaf are significantly correlated with that of the ear, r=0.62**. However the differences in partial resistance level were easier to discern through the leaf assessments. A high correlation between the infection on the flag and second leaf of r = 0.87 ** was observed, and therefore the average of both leaves was taken. A logarithmic transformation was used, because the standard error was highly correlated with the mean (r=0.88**). After transformation in ln[(% leaf area affected of flag leaf + % leaf area affected of 2nd leaf)/2 + 1] and ln(%affected ear + 1) the error appeared to be normally distributed.

Both the Duncan's multiple range test (Table 8.16) and a non-parametric test of Kruskal Wallis (1952) were used to compute the significance of differences between selection procedures within each group of composites. Highly significant differences were found.

All three selection procedures SPS, BSS and LS resulted in a very positive selection response (Table 8.16). The SPS was significantly better for the accumulation of partial resistance than the LS in the composites 2 and 3, while in composite 1 the LS procedure was more efficient. It was observed that composite 1 had already accumulated a significant amount of partial resistance in its first selection cycle. The intermediate generation G4 of the SPS of composite 1 had 8.6% *S. nodorum* while the unselected composite had 12.2%. This increase in resistance level was obtained in one SPS cycle in 1977 what was an extremely good year for the development of *S. nodorum*. The first SPS of composite 2 was made in 1978 what has been a very unfavorable year for this pathogen. It was seen that in that year one SPS did not accumulate any partial resistance. This suggests that the early generation selection can be very efficient provided the climatic conditions favour the development of *S. nodorum* in the field. The artificial inoculation, in spite of having been applied under favorable conditions

Table 8.16. *S. nodorum* on seventeen wheat populations assessed on the leaf and ear of adult plants in the greenhouse.

| Population | | *S. nodorum* assessment | |
|---|---|---|---|
| | | leaf [1]) | % ear area affected |
| Composite 1 G1 | Unselected population | 12.2 a[2]) | 5.3 a |
| Composite 1 G4 | Intermediate population | 8.6 b | 2.5 cd |
| Composite 1 G12 | SPS latest selection | 4.4 c | 2.0 bc |
| Composite 1 G12 | BSS latest selection | 3.2 d | 1.4 cde |
| Composite 1 G12 | LS latest selection | 3.2 d | 0.8 de |
| Composite 1 G5 | NS latest selection | 11.0 a | 3.0 b |
| Composite 2 G2 | Unselected population | 9.4 a | 3.8 a |
| Composite 2 G4 | Intermediate population | 9.4 a | 2.7 a |
| Composite 2 G10 | SPS latest selection | 3.9 c | 1.3 c |
| Composite 2 G10 | BSS latest selection | 4.4 bc | 1.8 c |
| Composite 2 G10 | LS latest selection | 5.4 b | 1.9 c |
| Composite 3 G2 | Unselected population | 8.5 a | 3.1 a |
| Composite 3 G9 | SPS latest selection PF[3]) | 3.8 d | 1.0 b |
| Composite 3 G9 | SPS latest selection Lo[4]) | 6.5 ab | 3.5 a |
| Composite 3 G9 | BSS latest selection | 4.6 dc | 1.9 ab |
| Composite 3 G9 | LS latest selection | 6.1 bc | 2.3 ab |
| Composite 4 | SPS latest selection | 5.6 | 6.4 |
| Cultivar CNT-10 | | 7.8 | 5.4 |

[1]) Obtained as follows: (% leaf area affected of flag leaf + % leaf area affected of 2nd leaf)/2.
[2]) The *S. nodorum* assessments carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).
[3]) PF = Passo Fundo.
[4]) Lo = Londrina.

every year, was not sufficient to obtain the required selection pressure.

The BSS procedure appeared to be quite efficient to accumulate partial resistance to *S. nodorum*. For composite 1, with 3.2% leaf area affected, it was even the best selection procedure, while for composite 2 and 3 this procedure was as efficient as LS procedure. The results agree much with the experience of Brnnimann (1970,1974), who found a high correlation between thousand grain weight and *S. nodorum* resistance. Also Pierobom (pers. comm.) obtained very good results with a selection system based on the weight of grains. The NS procedure gave no selection response on the leaves and only a small effect on the ear.

The resistance level of composite 4 was slightly but not significantly lower than the latest SPS selection of composite 1, from which it had been derived. A drop of partial resistance level was expected. Because of the favorable climatic conditions for *S. nodorum* in the south of Brazil plant breeders have wittingly or unwittingly been selecting for resistance against this pathogen. The best available resistance exists in Brazilian germplasm and with that, also the unselected composite 1 has a certain resistance level, which is much higher than that of the West European cultivars introduced into composite 1 to make composite 4. The homozygous F7 lines RH-18, RH-82040, RH-83150, from composite 1, RH-82371 from composite 2 and RH-82428 from composite 3 gave 1.9, 3.2, 5.8, 5.5 and 7.0% leaf area affected respectively. Only RH-18 was outstanding with a significant better resistance level than the LS population from which it had been derived.

The latest selections of the SPS and LS procedures tended to be taller than the unselected populations. This agrees with observations made by Brnnimann (1970,1974).

Cochliobolus sativus resistance.

For the same reason as discussed with *S. nodorum*, a transformation in ln[(% leaf area affected of flag leaf + % leaf area affected of 2nd leaf)/2 + 1] and ln(% ear area affected + 1), was needed to improve the normality of the error, measured by the D-value of Stephens (1974), sufficiently for an analysis of variance. The F-values from ANOVA and chi square approximation values of the non-parametric test of Kruskal Wallis (1952) indicated significance of the selection response for partial resistance to *C. sativus* on the leaf and ear except for the ear response of composite 2.

Considering the results (Table 8.17) of the three composites 1, 2 and 3 together it is not possible to indicate which selection procedure was best. In composites 1 a significant accumulation of partial resistance was obtained after one SPS in the field in 1977. The intermediate generation presented 7.4% leaf area affected against 17.1% of the unselected population. The year 1977 has been, besides for the *S. nodorum* epidemic also very favorable for *C. sativus* resulting in this positive selection response. That the climatic conditions are important can be seen in the result of the first SPS in composite 2. In 1978 the weather did not favour the development of this pathogen and no resistance was accumulated in the G4 intermediate population. After the first SPS not more resistance was accumulated in composite 1 except by the BSS procedure. The populations of the BSS procedure have always been multiplied in Brasília where *C. sativus* is a major problem (see chapter 6). This automatically means some screening for *C. sativus* resistance. The LS procedure as a whole did not give a remarkable response. However the homozygous F7 line RH-18, derived from the LS procedure, had an extremely high level of resistance with only 2.8% leaf area and 0.6% ear area affected. Other lines of composite 1, RH-82040 and RH-83150 were much less resistant with 6.6 and 9.2% leaf area and 1.6 and 2.6% ear area affected respectively, a level not significantly different from the LS population from which they were taken.

The NS procedure had a positive effect. It has been reported that *C. sativus* can induce sterility what may explain this selection response.

Table 8.17. *Cochliobolus sativus* on seventeen wheat populations assessed on the leaf and ear of adult plants in the greenhouse.

| Population | | C. sativus assessment | |
|---|---|---|---|
| | | leaf[1]) | % ear area affected |
| Composite 1 G1 | Unselected population | 17.1 a[2]) | 2.6 abc |
| Composite 1 G4 | Intermediate population | 7.4 d | 1.6 cde |
| Composite 1 G12 | SPS latest selection | 9.2 cd | 1.2 e |
| Composite 1 G12 | BSS latest selection | 5.0 e | 1.5 e |
| Composite 1 G12 | LS latest selection | 8.5 bc | 1.8 abcd |
| Composite 1 G5 | NS latest selection | 8.3 bcd | 1.4 b |
| Composite 2 G2 | Unselected population | 8.2 a | 1.4 a |
| Composite 2 G4 | Intermediate population | 7.8 ab | 1.7 a |
| Composite 2 G10 | SPS latest selection | 5.8 b | 1.7 a |
| Composite 2 G10 | BSS latest selection | 8.2 a | 1.7 a |
| Composite 2 G10 | LS latest selection | 7.4 ab | 1.7 a |
| Composite 3 G2 | Unselected population | 12.4 a | 2.9 a |
| Composite 3 G9 | SPS latest selection PF[3]) | 9.0 ab | 1.8 bc |
| Composite 3 G9 | SPS latest selection Lo[4]) | 10.2 a | 1.6 bc |
| Composite 3 G9 | BSS latest selection | 8.0 b | 1.8 b |
| Composite 3 G9 | LS latest selection | 4.4 c | 1.1 c |
| Composite 4 | SPS latest selection | 10.4 | 3.0 |
| Cultivar CNT-10 | | 9.0 | 2.4 |

[1]) Obtained as follows: (% leaf area affected of flag leaf + % leaf area affected of 2nd leaf)/2.
[2]) The C. sativus assessments carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).
[3]) PF = Passo Fundo.
[4]) Lo = Londrina.

Composite 4 resulted in an increased susceptibility level what is probably due to the susceptibility of the introduced west european cultivars and lack of efficient selection.

In composite 2 no resistance was accumulated except a small effect for the SPS procedure. It is possible that this composite does not have much genetic variation or either the selection procedures failed to exploit it. Also the line RH-82371 did not show any increased resistance level.

Composite 3, which has for its genetic background much in common with composite 1, did show a significant selection response for the BSS and LS procedure. Again it is expected that the multiplication in Brasília has been beneficial for the accumulation of partial resistance in the BSS procedure. Further the LS has been more efficient than the SPS procedures in years with a low selection pressure. The SPS procedure did not give any selection response in Passo Fundo nor in Londrina, where a good selection response was expected because of the climatic conditions of Londrina, which favor the development of *C. sativus* more resulting in a higher selection pressure. The line RH-82428 with 10.7% leaf area affected did not represent an improved resistance level.

### *8.5.2 Response to selection for resistance to* Cochliobolus sativus *on grains*

It was investigated whether the field selection for partial resistance also had improved the resistance to infection of the grains. To this end a field experiment was performed in 1982. Seed of the composite populations (described in 8.1) was sown in plots of 1.8 x 7.0 m in eight replicates in a randomized block design. Each plot was separated from the neighboring plots by strips of oats to reduce interplot interference. When the wheat was just ripe, a random sample of 25 ears per plot (200 ears per composite population) was taken and carefully analyzed for the percentage infected grains. No artificial inoculation was carried out. Identification analysis in the laboratory of CNPTrigo of the pathogens revealed that *C. sativus* and *F. graminearum* were the main pathogens on the grains. *S. nodorum* was almost not found in this experiment.

The data have not been transformed as a test of the normality of error displayed an almost normal distribution. The analysis of variance indicated highly significant differences (P= 0.01) for the selection procedures of composites 1, 2 and 3. In general the results agree well with those of the leaf assessments of the previous chapter (Table 8.18). The composite 1 and composite 3 populations, which presented the best selection response for the leaf assessment, had a correlation of 0.62 * with the percentage infected grains of this field experiment. For the composite 2 populations this correlation was not significant, but the tendency of the results is still the same.

Again the progress of the first SPS in 1977 of composite 1 was seen. The percentage infected grains was reduced from 20.0 to 13.3%. After this stage of the programme more resistance was accumulated only by the LS and BSS procedure resulting in 7.9 and 9.8% infected grains respectively. The loss of resistance observed on the leaves of composite 4 was not perceived on the grains. The NS was with 17.7% infected grains again slightly more resistant than the unselected population although the difference in this experiment was not significant.

As in the previous experiment the first SPS in 1978 did not improve the resistance level in composite 2 after which stage the SPS procedure gave the best result though not significantly better than the LS and BSS procedures. In composite 3 all three selection procedures gave a positive selection response with a tendency of the SPS of Passo Fundo and Londrina to be the least successful.

The F7 lines RH-82040, RH-82371 and RH-82428 gave 6.4, 9.0 and 6.6% infected grains respectively, the same resistance level as the LS composite populations to which they belong.

Table 8.18. Percentage of grains infected (GI) with *C. sativus* of seventeen wheat composite populations.

| Population | | % GI |
|---|---|---|
| Composite 1 G1 | Unselected population | 20.0 a[1] |
| Composite 1 G4 | Intermediate population | 13.3 bc |
| Composite 1 G12 | SPS latest selection | 12.2 c |
| Composite 1 G12 | BSS latest selection | 9.8 cd |
| Composite 1 G12 | LS latest selection | 6.7 d |
| Composite 1 G5 | NS latest selection | 17.7 ab |
| Composite 2 G2 | Unselected population | 16.1 a |
| Composite 2 G4 | Intermediate population | 14.7 a |
| Composite 2 G10 | SPS latest selection | 7.2 c |
| Composite 2 G10 | BSS latest selection | 7.3 c |
| Composite 2 G10 | LS latest selection | 10.8 bc |
| Composite 3 G2 | Unselected population | 17.3 a |
| Composite 3 G9 | SPS latest selection PF[2] | 10.4 bc |
| Composite 3 G9 | SPS latest selection Lo[3] | 12.2 b |
| Composite 3 G9 | BSS latest selection | 9.7 bc |
| Composite 3 G9 | LS latest selection | 7.7 c |
| Composite 4 | SPS latest selection | 8.6 |
| Cultivar CNT-10 | | 12.7 |

[1]) The *C. sativus* assessments carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).
[2]) PF = Passo Fundo.
[3]) Lo = Londrina.

## 8.6 *Fusarium graminearum*, response to selection

The field experiment evaluating the resistance of the grains to C. sativus (8.5.2) was used to evaluate the resistance of the grains to F. graminearum at the same time.

The percentage infected (pink coloured) grains were counted for each of the populations (chapter 8.1).

The first SPS selection cycle gave a significant improvement in resistance in the composites 1 and 2 (Table 8.19). However, part of this selection response was lost in the following generations of selection. At the end only the BSS for the composite 1 with 3.0% and LS procedure for composite 2 with 3.8% infected grains gave a significant higher resistance level than the unselected composites with 6.0 and 5.6% infected grains respectively. In composite 3 only the LS gave a significant selection response. The lines RH-82040, RH-82371 and RH-82428 had 5.1, 3.4 and 5.1% infected grains respectively of which only RH-82428 was more resistant than the unselected composite from which they came.

The selection response appeared to be moderate which may be caused by i)

Table 8.19. Percentage of grains infected (GI) with *F. graminearum* of seventeen wheat composite populations.

| Population | | % GI |
|---|---|---|
| Composite 1 G1 | Unselected population | 6.0 a[1]) |
| Composite 1 G4 | Intermediate population | 3.3 bc |
| Composite 1 G12 | SPS latest selection | 4.6 abc |
| Composite 1 G12 | BSS latest selection | 3.0 bc |
| Composite 1 G12 | LS latest selection | 3.7 abc |
| Composite 1 G5 | NS latest selection | 5.5 ab |
| Composite 2 G2 | Unselected population | 5.6 a |
| Composite 2 G4 | Intermediate population | 3.6 b |
| Composite 2 G10 | SPS latest selection | 4.8 ab |
| Composite 2 G10 | BSS latest selection | 4.8 ab |
| Composite 2 G10 | LS latest selection | 3.8 b |
| Composite 3 G2 | Unselected population | 7.3 a |
| Composite 3 G9 | SPS latest selection PF[2]) | 6.1 ab |
| Composite 3 G9 | SPS latest selection Lo[3]) | 4.3 ab |
| Composite 3 G9 | BSS latest selection | 5.1 ab |
| Composite 3 G9 | LS latest selection | 3.0 b |
| Composite 4 | SPS latest selection | 4.3 |
| Cultivar CNT-10 | | 6.5 |

[1]) The *F. graminearum* assessments carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).
[2]) PF = Passo Fundo.
[3]) Lo = Londrina.

a lack of genetic variation for resistance to *F. graminearum* in the original cultivars of the composites, ii) a low selection pressure in most years so that often escapes rather than resistant genotypes were selected, iii) a moderate heritability for this characteristic, or iv) a combination of these factors.

## 8.7 Common Root Rot, response to selection for resistance

Common root rot is caused by a complex of pathogens in which *C. sativus* plays a major role followed by several *Fusarium* species and *Colletotrichum graminicola* (Diehl, 1979).

During the selection process in the field, a final test was always done on the SPS and LS populations. The plants selected (for other traits) were pulled upwards. If they came easily, they had a poorly developed root system and were abandoned. If they held firmly in the soil, they were kept. Resistance was as-

Table 8.20. Assessment classes of wheat root systems affected by common root rot.

| Scale value | level | Description |
|---|---|---|
| 0 | clean | no lesions |
| 2 | slight | 1–25% of root area lesioned |
| 5 | moderate | 25–50% of root area lesioned |
| 10 | severe | > 50% of root area lesioned |

sumed to be closely associated with a healthy root development in the soil and so with resistance to pulling.

In a field experiment the response to selection was evaluated. The composites described in chapter 8.1. and a small number of lines were grown in plots of 2.0 x 7.0 m in four replicates on a field that had carried wheat for three years in succession followed by one year of rape-seed. This one year rape seed is insufficient to eliminate common root rot pathogens (A minimum of 2-3 years of other crops is recommended to reduce the amount of inoculum in the soil sufficiently).

At growth stage 75 DC, 100 plants/plot were pulled out without damaging the roots too much. The plants were taken to the greenhouse, where the root system of the 8,800 plants were carefully washed.

The assessment was performed in the phytopathology laboratory by putting the root system one by one in a white plastic tray and grouping them in four different categories (Table 8.20) on the basis of the area of the root system that is affected.

Per plot a percentage disease severity (PDS) was calculated by using the equation of Tinline (1975):

$$PDS = \frac{\Sigma \text{ (Scale value x N° plants in scale)}}{\text{Total N° plants x Maximum scale value}} \times 100$$

The error of the PDS-values was nearly normally distributed (W test of Shapiro & Wilk, 1965), indicating that this assessment procedure gave data that were statistically well accessible. The results of the experiment are presented in table 8.21. The progress of resistance to Common Root Rot at the initial stage of the selection procedure is interesting to note. This is probably because the most susceptible genotypes, which evidently do not produce a good grain, due to a disturbed root system were eliminated. Also it should be noted that the first selections were made under non-rotation. Until 1978, CNPTrigo did not apply a rotation system for their wheat breeding programme. Wheat after wheat was planted on the same location every year.

The LS procedure seems to have resulted in a slightly higher resistance level than the SPS procedure; the BSS populations always showed a higher disease severity. The latest selection of the composite 4 SPS procedure demonstrated a good progress, as expected. This composite was especially made for the pur-

Table 8.21. Percentage disease severity (PDS) of Common Root Rot on the root system of seventeen wheat composite populations.

| Population | | PDS |
|---|---|---|
| Composite 1 G1 | Unselected population | 43.9 b[1] |
| Composite 1 G4 | Intermediate population | 23.7 a |
| Composite 1 G12 | SPS latest selection | 21.7 a |
| Composite 1 G12 | BSS latest selection | 26.3 a |
| Composite 1 G12 | LS latest selection | 22.6 a |
| Composite 1 G5 | NS latest selection | 37.0 b |
| Composite 2 G2 | Unselected population | 39.6 c |
| Composite 2 G4 | Intermediate population | 21.2 a |
| Composite 2 G10 | SPS latest selection | 19.0 a |
| Composite 2 G10 | BSS latest selection | 28.6 b |
| Composite 2 G10 | LS latest selection | 16.3 a |
| Composite 3 G2 | Unselected population | 39.0 b |
| Composite 3 G9 | SPS latest selection PF[2] | 27.5 a |
| Composite 3 G9 | SPS latest selection Lo[3] | 24.2 a |
| Composite 3 G9 | BSS latest selection | 29.2 a |
| Composite 3 G9 | LS latest selection | 22.5 a |
| Composite 4 | SPS latest selection | 17.2 |
| Cultivar CNT-10 | | 16.2 |

[1]) The Common Root Rot assessments carrying different letters are significantly different according to Duncan's multiple range test ($P = 0.05$).
[2]) PF = Passo Fundo.
[3]) Lo = Londrina.

pose of a root improvement besides a better plant type. The NS procedure did not differ much from the unselected composite.

With a PDS of 25.8 and 21.5% the lines RH-82040 and RH-82428 were as resistant as the LS population, while RH-82371 with 33.6% remained as susceptible as the unselected composite.

In view of these results two approaches are possible to reduce the damage from common root rot; i) Planting the breeding material in the field under a rotation system, exerting a minimum selection pressure for soil borne diseases. This is an approach of management practices to solve the soil borne disease problems, which has the advantage of being able to exert a higher selection pressure for other characteristics. ii) Planting the breeding material without rotation, exerting also a selection pressure for soil borne problems, which may affect the intensity of the selection pressure for the remaining characteristics, but ensures that future cultivars have enough resistance to survive soil borne disease problems. A balance should be maintained between what can be solved by agronomy and what can be done by plant breeding. Priorities should be established in the breeding programme. There is often a tendency that almost everything can be solved by plant breeding, but one forgets that it may take a long time to solve all those wishes this way.

## 8.8 Viruses

### *8.8.1 Soil Borne Mosaic Virus*

The EMBRAPA/CNPTrigo research station has one experimental area which is especially set up for the evaluation of germplasm for Soil Born Mosaic Virus (SBMV) resistance. Infected soil had been evenly distributed and incorporated in this area and wheat is grown continuously on it. Together with the virologist of CNPTrigo, Caetano, the composite populations discussed in chapter 8.1. were tested here for resistance to SBMV.

The composite populations and lines were planted in small plots of 3 m length in two replicates. The evaluation was made at growth stage 73 DC using the scale from 0 to 9 developed by Caetano (Table 8.22).

The classes 0, 1 and 2 are considered as resistant, 3 and 4 as moderately resistant, 5 and 6 as moderately susceptible, 7, 8 and 9 as susceptible.

In general little progress has been made by the various selection procedures. This was expected as only the first two years 1976 and 1977 wheat was grown in areas without rotation where the chance of SBMV epidemics is greater. In 1979 CNPTrigo started a wheat rotation for the experimental fields.

A certain level of resistance was already present at the initial stage of the

Table 8.22. Assessment key for wheat affected by Soil Born Mosaic Virus.

| Scale | Description |
|---|---|
| 0 | No leaf symptoms. |
| 1. | Weak mosaic symptoms on leaves after plants have been cut. |
| 2. | Weak mosaic symptoms on leaves of some tillers; unevenly and sparsely distributed over the plants. |
| 3. | Weak mosaic symptoms on leaves on almost all the tillers; occurring uniformly throughout the plants. |
| 4. | Weak mosaic symptoms on leaves on all the tillers. |
| 5. | Weak mosaic symptoms on leaves on all the tillers and in some plants coalescing into chlorotic areas. |
| 6. | Strong mosaic symptoms on leaves. |
| 7. | Strong mosaic symptoms on leaves, frequently coalescing and showing chlorotic stripes, however not over the entire plant. |
| 8. | Strong mosaic symptoms on leaves, frequently coalescing, plant showing chlorotic stripes over the entire plant. |
| 9 | Strong mosaic symptoms on leaves, frequently coalescing and showing chlorotic stripes and rosette formation. |

selection procedures coming from the cultivars Horto, IAS-62, IAS-58 and PF-70401 (Caetano,1977) but the selection procedures failed in general to accumulate higher levels of resistance against SBMV (Table 8.23). The selection pressure has always been low or absent and moreover SBMV resistance did not have a high priority. It was remarkable that the best results were obtained by the NS procedure of composite 1 and the SPS procedure of Londrina. These composite populations presented weak mosaic symptoms on the leaves, while the unselected composite showed strong mosaic symptoms on the leaves.

### *8.8.2 Barley Yellow Dwarf Virus*

Barley Yellow Dwarf Virus (BYDV) is a persistent virus transmitted by several aphids: *Metopolophium dirhodum* (Walk.), *Rhopalosiphum maides* (Fitch), *R. padi* (L), *R. rufiabdominalis* (Sasaki), *Schizaphis graminum* (Rond), *Sipha flava* (Forbes) and *Sitobion avenae* (F) (Caetano, 1972,1973). Systemic insecticides have only a partial effect in protecting the wheat against this virus in the field because it only prevents the transmission of virus from one plant to the other within the protected area but it does not protect against aphids carrying BYDV that enter from outside. They transmit the virus first and die afterwards as a result of the systemic insecticide. Resistance therefore is the most important plant protection measure against BYDV.

Symptoms of BYDV vary greatly from no symptoms at all to yellow chlorotic leaves, which sometimes turn into a reddish colour. Moreover the vigour of the plant is strongly reduced, the ears may be partly sterile and the root system is often less developed. Caetano (pers. comm.) found no significant correlation between the percentage yellow leaf area and yield reduction in a large experiment consisting of some 50 wheat cultivars. Therefore assessing the amount of leaf yellowing does not assess resistance, but assesses the combined effects of resistance and tolerance to symptom expression. Yield comparisons are made between inoculated and not inoculated wheat plants.

The test evaluating the response of the four selection procedures SPS, BSS, LS and NS was performed in a screenhouse, which was insect free. The test had primarily a demonstrative value and was performed without replicates. Twenty five plants of each composite population were inoculated with BYDV at the second leaf growth stage and compared with twenty five non-inoculated plants. As a vector the aphid *R. padi* was used. The aphids came from the insectarium where they were reared on BYDV infected plants. Ten aphids were put in small cages on each plant to be inoculated where they remained for three days. After inoculation insecticides were applied on the experiment with weekly intervals. Only the yield loss, (difference between inoculated and non-inoculated plants) was assessed.

From the results (table 8.23) it can be seen that LS was the most efficient procedure in reducing the yield reduction from BYDV. No ELISA tests were done, therefore it is unknown if the plants, which remained without symptoms, were infected with BYDV or not. Considering the fact that for the LS procedure of composite 1 and 2 no yield reduction at all was observed, it is likely that with a selection pressure of ten aphids per seedling the plants of these LS latest selection populations did not become infected, what can be different when more aphids per seedling are used for inoculation. The LS was most effective in composites 1 and 2 and mildly so in composite 3. The SPS selection was only effective in 1977 when there was a severe epidemic of BYDV (composite 1, SPS-G4). The BSS had a positive effect in all three composites anc was as a whole more effective than SPS.

The selected homozygous F7 lines derived from the LS procedure also showed a high level of resistance to BYDV. The lines RH-18, RH-82040, RH-83150 from composite 1 had a yield reduction of 15.5, 4.4 and 12.6% respectively, while the unselected composite 1 had 29.4% yield reduction. The unselected composites 2 and 3 gave 29.2 and 21.9% yield loss, while the lines RH-82371 and RH-82428 selected from these composites appeared to be very resistant, yielding 1.4 and 1.0 % more than the not inoculated plants respectively.

Table 8.23. Soil Borne Mosaic Virus (SBMV) symptom levels expressed in scale values and yield reduction in percentage due to Barley Yellow Dwarf Virus (BYDV) of 17 wheat composite populations and two cultivars.

| Population | | SBMV[1]) | BYDV |
|---|---|---|---|
| Composite 1 G1 | Unselected population | 6 | − 29.4 |
| Composite 1 G4 | Intermediate population | 5 | − 5.7 |
| Composite 1 G12 | SPS latest selection | 5.5 | − 18.7 |
| Composite 1 G12 | BSS latest selection | 5 | − 18.2 |
| Composite 1 G12 | LS latest selection | 7 | + 4.2 |
| Composite 1 G5 | NS latest selection | 4 | − 21.9 |
| Composite 2 G2 | Unselected population | 6 | − 29.2 |
| Composite 2 G4 | Intermediate population | 5 | − 12.8 |
| Composite 2 G10 | SPS latest selection | 6 | − 19.6 |
| Composite 2 G10 | BSS latest selection | 7 | − 9.4 |
| Composite 2 G10 | LS latest selection | 6 | + 1.8 |
| Composite 3 G2 | Unselected population | 6 | − 21.9 |
| Composite 3 G9 | SPS latest selection PF[2]) | 6.5 | − 20.7 |
| Composite 3 G9 | SPS latest selection Lo[3]) | 4.5 | − 27.3 |
| Composite 3 G9 | BSS latest selection | 7 | − 1.5 |
| Composite 3 G9 | LS latest selection | 6.5 | − 13.8 |
| Composite 4 | SPS latest selection | 5.5 | − 7.6 |
| Cultivar CNT-10 | | 5 | − 42.0 |
| Cultivar BR-6 | | 8 | − |

[1]) Scale values on a scale of 0 (no symptoms) to 9 (very strong symptoms).
[2]) PF = Passo Fundo.
[3]) Lo = Londrina.

## 8.9 Yield response due to increased levels of resistance

### *8.9.1 Crop loss assessment in the unselected composite 1 in 1976*

In 1976, when this programme started, the first of a series of crop loss assessment experiments was conducted. The objective of these experiments was to evaluate the yield losses due to pests and diseases in the unselected composite 1 in comparison with the two main cultivars. It is an indication of how much yield improvement could be gained from indirect selection for yield through resistance breeding.

The unselected composite 1 was compared against the two current major wheat cultivars IAS-54 and Nobre in a factorial experiment with randomized blocks and four replicates. The plot size was 6.0 x 3.2 m, the seed rate was 60 germinating seeds per m row. Four different treatments were applied: i) no plant protection, ii) protection with fungicides, iii) protection with insecticides, iv) protection with fungicides + insecticides. A full pest control was the aim of the pesticide treatments. The pesticide treatments were: 1. Seed treatment with Bayleton, 300g/100 kg seed. 2. Soil treatment after sowing with Aldrin 5%, 20 kg/ha. 3. At tillering (25 DC): a) Mancozeb, 2.5 kg/ha; b) Pyrimor 50%, 300 g/ha. 4. At bootstage (45 DC): a) Mancozeb, 2.5 kg/ha; b) Pyrimor 50%, 300 g/ha. 5. At flowering (65 DC): a) Mancozeb, 2.5 kg/ha + Benomyl, 0.5 kg/ha; b) Pyrimor 50%, 300 g/ha. All the applications were done with 250 l water per ha. Differences in yield between no plant protection and the three treatments should give the estimate of the losses due to diseases, pests and diseases and pests combined, provided that the fungicides and insecticides have a complete neutral effect on the plant in the absence of diseases and pests.

One disease assessment was made at growth stage 59 DC. In each plot, the upper three leaves of 10 tillers were assessed for the percentage leaf area covered with powdery mildew and total necrotic leaf area. The assessment key according to James (1971) (Figure 8.1) was used. For the assessment of aphids a standardized way of shaking the aphids from the plants into a water trap was applied. This method is quick and counting can afterwards be performed in the laboratory. Aphids have been counted as a total number without differentiation to species and winged or non-winged forms.

The yields increased dramatically with increasing protection; both fungicides and insecticides had a significant effect (Table 8.24). The data indicated a yield loss of 44% due to fungi, 64% due to insects and viruses, and 72% caused by fungi, insects and viruses together. Considering that the average yield in Rio Grande do Sul in 1976 was 938 kg/ha this experiment is a good reflection of the reality. Only a limited number of the farmers applied fungicides and insecticides as recommended by CNPTrigo/EMBRAPA, keeping the average yields so low.

The averages of Composite 1, IAS-54 and Nobre disease assessments (table 8.25) show the efficiency of the chemical treatment which was for the fungicide applications far from complete underestimating the yield loss caused by fungi.

Table 8.24. Yields in kg/ha of the Composite 1, G0, Unselected population (C-1, G0, UP) and two cultivars with and without fungicides and insecticides in 1976.

| | No plant protection | Fungicides | Insecticides | Fungicides + Insecticides | Average |
|---|---|---|---|---|---|
| C-1, G0, UP | 730 d[1]) | 1,061 cd | 1,871 ab | 2,157 a | 1,455 |
| IAS-54 | 544 d | 1,331 bcd | 1,650 abc | 2,441 a | 1,491 |
| Nobre | 659 d | 1,053 cd | 1,852 ab | 2,233 a | 1,449 |
| Average | 644 | 1,149 | 1,791 | 2,277 | |

[1]) The treatments within and between columns carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).

Table 8.25. Average number of aphids per tiller (NAT), percentage powdery mildew (PM) and percentage total necrotic leaf area (TNLA) on the flag leaf, second leaf and third leaf at growth stage 59 DC of three wheat entries Composite 1, G0, Unselected population, IAS-54 and Nobre without plant protection (No P P), with fungicides (F), with Insecticides (I) and Fungicides + Insecticides (F + I) in 1976.

| | | Flag leaf | | Second leaf | | Third leaf | |
|---|---|---|---|---|---|---|---|
| | NAT | PM | TNLA | PM | TNLA | PM | TNLA |
| No P P | 13.7 a[1]) | 2.4 a | 9.6 a | 6.9 a | 35.6 a | 23.2 a | 76.7 a |
| F | 14.7 a | 1.9 a | 4.8 b | 2.9 b | 17.6 b | 6.9 b | 57.2 b |
| I | 1.4 b | 2.5 a | 3.5 b | 7.4 a | 13.4 b | 18.5 a | 38.7 b |
| F+I | 1.6 b | 2.0 a | 2.8 b | 3.5 b | 6.5 b | 7.0 b | 27.7 c |

[1]) The NAT, PM and TNLA assessments carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).

It explains in part the yield loss that was experienced. At the moment of the disease assessment the flag leaf did not yet give a sufficient differentiation for the fungicide and insecticide treatments, but a good association between yield and disease assessment was found for the second leaf assessment, which leaf together with the flag leaf is most important for grain filling.

### *8.9.2 Response to selection after one generation of Single Plant Selection in Composite 1*

In 1978 the yield response from one generation Single Plant Selection (G4, chapter 8.1) was tested against the Composite 1, G1, unselected population. The cultivars Nobre and Jacuí were included in this test as well. The design of the experiment was a factorial one in randomized blocks with four replicates similar to the one in 1976 (chapter 8.9.1).

The plot size was 4.0 x 9.0 m, the seed rate was 60 germinating seeds per m row. Borders of oats with a width of 2.0 m were sown to reduce interplot interference. Four different treatments were applied: i) no plant protection, ii) protection with fungicides, iii) protection with insecticides, iv) protection with fungicides + insecticides. A full pest control was the aim of the pesticide treatments. The pesticide treatments were: 1. At stem elongation (30 DC): Bayleton, 1 kg/ha. 2. At flag leaf just visible (37 DC): a) Bayleton, 1 kg/ha; b) Pyrimor 50%, 150 g/ha. 3. At flag leaf collar visible (39 DC): a) Pyrimor 50%, 150 g/ha. 4. At late bootstage (49 DC): a) Sulphur, 2 kg/ha + Dithane, 2.5 kg/ha + Bayleton, 0.5 kg/ha; b) Pyrimor 50%, 150 g/ha. 5. Beginning anthesis (61 DC): a) Dithane, 2.5 kg/ha + Sulphur, 2 kg/ha + Benlate, 0.5 kg/ha; b) Pyrimor 50%, 150 g/ha. 6. At medium milk stage (75 DC): a) Dithane, 2.5 kg/ha + Sulphur, 2 kg/ha + Bayleton, 0.5 kg/ha; b) Pyrimor 50%, 150 g/ha. All the applications were done with 250 l water per ha.

At early boot stage (40 DC) the number of aphids per tiller (30 main tillers per plot) and at flowering (65 DC) leaf rust (15 main tillers per plot) were assessed.

A significant yield improvement as a response of one generation SPS was obtained (Table 8.26). In 1978 Rio Grande do Sul experienced its highest mean yield since 15 years of 1237 kg/ha. This experiment too reflects a very good wheat year in which the total yield loss to fungi, pests and virus together accounted for only 16%. The fungicide treatments did not give a significant yield improvement, while insecticides and fungicides + insecticides increased the yield with 8 and 16% respectively. Only a small effect of the pathogens and pests was seen. For leaf rust at flowering stage the Composite 1, G1, unselected population had 0.8, 1.9 and 0.4% affected leaf area on the first, second and third leaf respectively while the G4, SPS population had 0.1, 0.8 and 0.1 %. It is expected that the yield response to selection is partly due to the resistance to BYDV (see chapter 8.6) which has been a major constraint in 1978.

The different chemical treatments together reflect the response to selection quite well which has been calculated as 10%.

Table 8.26. Yields in kg/ha of the Composite 1, G1, unselected population (C-1, G1, UP), Composite 1, G4, SPS population (C-1, G4, SPS) and two cultivars with and without fungicides and insecticides in 1978.

| | No plant protection | Fungicides | Insecticides | Fungicides + Insecticides | Average |
|---|---|---|---|---|---|
| C-1, G1, UP | 2,545 | 2,603 | 2,730 | 3,113 | 2,748 (100) b |
| C-1, G4, SPS | 2,939 | 2,756 | 3,106 | 3,310 | 3,028 (110) a |
| IAS-54 | 2,070 | 2,130 | 2,382 | 2,569 | 2,288 ( 83) d |
| Nobre | 2,511 | 2,310 | 2,629 | 2,725 | 2,544 ( 93) c |
| Average | 2,516 c[1]) (100) | 2,450 c (97) | 2,712 c (108) | 2,929 a (116) | |

[1]) The treatments carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).

Table 8.27. Yield in kg/ha, thousand grain weight (TGW) in g, and specific weight (SW) in g of the Composite 1, G1, unselected population (C-1, G1, UP), Composite 1, G6 after two Single Plant Selections (C-1, G6, SPS) and one cultivar without chemical control in 1979.

| | Yield | TGW | SW |
|---|---|---|---|
| C-1, G1, UP | 753 b[1]) | 25.4 a | 68.8 a |
| C-1, G6, SPS | 862 a | 26.6 a | 71.3 a |
| Nobre | 760 b | 26.7 a | 68.5 a |

[1]) Data are significantly different (P = 0.05) according to Duncan's multiple range test if they carry no similar letters.

### *8.9.3 Response to selection by two generations of Single Plant Selection in Composite* 1

In 1979 the response to selection was evaluated in an experiment, similar to those described in chapter 8.9.1. and 8.9.2. However, in this experiment only the treatment 'without plant protection' was investigated. The Composite 1, G6, SPS (with two cycles of the SPS procedure) was compared with the Composite 1 unselected population.

The plot size was 4.0 x 10.0 m, the seed rate was 60 germinating seeds per m row. Borders of oats with a width of 2.0 m were sown to reduce interplot interference. Nobre, the main wheat cultivar of Rio Grande do Sul was included as a reference.

Disease observations were made at late boot stage (45 DC) for the number of aphids per tiller, leaf rust and powdery mildew + *S. nodorum* + *S. tritici* + *C. sativus* combined on the first three leaves, and at medium milk growth stage (75 DC) for the total necrotic leaf area on the first three leaves, stem rust on the stem and *S. nodorum* + *C. sativus* on the ear. Fifteen tillers per plot were assessed.

The results reflect largely the difficulties experienced by the farmers in 1979. The average yield in Rio Grande do Sul was one of the lowest in its history with 448 kg/ha. The main reason has been the adverse weather conditions in combination with a severe disease pressure. The weather conditions have been extremely adverse because of i) a frost on the 19th of September, when the wheat had just headed, b) an exceptional rainfall in the month of October of 417 mm which was more than twice the mean rainfall of 183 mm. For this reason the yield data are only of limited value to evaluate the selection response. The yields of the Composite 1 after two SPS cycles were some 14% higher, the grains slightly bigger combined with a higher specific weight than the unselected population (Table 8.27). This increase is paralleled by a decrease in the amount of pathogen in the selected population and confirms, what has been discussed in the chapters 8.2 to 8.8 where SPS gave often a good response to selection. The necrotic leaf and ear area has been preferred as a parameter in these experiments because

Table 8.28. Percentage leaf and ear area affected by aphids, leaf rust, powdery mildew, *S. nodorum (S. nod)*, *S. tritici (S. trit)*, *C. sativus (C. sat)* and necrotic leaf area (NLA) and stem rust of the Composite 1, G1, Unselected population (C-1, G1, UP), Composite 1, G6 after two Single Plant Selections (C-1, G6, SPS) and one cultivar in 1979.

| | Growth stage 45 DC | | | | | | | Growth stage 75 DC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number aphids per tiller | leaf rust | | | Powdery mildew + *S.nod* + *S.trit* + *C.sat* | | | NLA | | Stem rust | *S.nod*+ *C.sat* on ear |
| | | leaf1 | leaf2 | leaf3 | leaf1 | leaf2 | leaf3 | leaf1 | leaf2 | | |
| C-1,G1,UP | 9a[1]) | 0.3a | 1.4a | 2.3a | 0.1a | 2.0a | 11.8a | 47a | 95a | 2.5a | 14.5b |
| C-1,G6,SPS | 9a | 0.1a | 0.6b | 1.3a | 0.0a | 0.7a | 4.6b | 14b | 91b | 1.7a | 0.2c |
| Nobre | 11a | 0.4a | 0.7b | 1.5a | 0.0a | 1.1a | 2.7b | 49a | 97a | 0.0b | 31.7a |

[1]) Data are significantly different (P=0.05) according to Duncan's multiple range test if they carry no similar letters.

it reflects the total effect of various pathogens together which are not easy to distinguish in the field, competing with each other for the green leaf area to parasitize (table 8.28).

### *8.9.4 Response to selection by Single Plant Selection and Bulk Seed Selection on two Composites*

In 1981 a crop loss experiment was performed assessing the selection response after four selection cycles of the SPS and BSS procedures on composites 1 and three selection cycles of SPS and BSS procedure on composite 2. These SPS and BSS Latest selections were compared with the Unselected populations. The experiment has been performed as follows: The plot size was 4.0 x 9.0 m, the seed rate was 60 germinating seeds per m row. Borders of rye with a width of 2.0 m were sown to reduce interplot interference. Four different treatments were applied: i) no plant protection, ii) protection with fungicides, iii) protection with insecticides, iv) protection with fungicides + insecticides. The pesticide treatments were: 1. At tillering stage (25 DC): Bayleton, 1 kg/ha. 2. At mid boot stage (45 DC): Bayleton, 1 kg/ha. 3. At late boot stage (49 DC): Pyrimor 50%, 300 g/ha. All the applications were done with 250 l water per ha.

At medium ripe milk stage (75 DC) thirty plants per plot were assessed for leaf rust and necrotic leaf area on the flag and second leaf, stem rust on the stem and *F. graminearum* + *S. nodorum* + *C. sativus* (=necrotic area) on the ear.

The yield level has been rather high from which it can be concluded that the disease/insect pressure has been rather low (table 8.29). The SPS procedure has been effective in both composites. However it has been more effective in Composite 1 than Composite 2 what also was expected as this Composite 1 had passed one more SPS procedure cycle in 1977. That year was a very effective year for

Table 8.29. Yield in kg/ha of the Composite 1, G2, unselected population (C-1, G2, UP), Composite 1 after four Single Plant Selections (C-1, G10, SPS), Composite 1 after four Bulk Seed Selections (C-1, G10, BSS), Composite 2, G2, unselected population (C-2, G2, UP), Composite 2 after three Single Plant Selections (C-2, G8, SPS), Composite 2 after three Bulk Seed Selections (C-2, G8, BSS) with and without fungicides and insecticides in 1981.

| | No plant protection | Fungicides | Insecticides | Fungicides + Insecticides | Average |
|---|---|---|---|---|---|
| C-1, G2, UP | 1,949 b[1] | 2,816 ab | 1,905 b | 2,754 b | 2,356 |
| C-1, G10,SPS | 2,485 a | 3,050 a | 2,386 a | 3,182 a | 2,776 |
| C-1, G10,BSS | 1,941 b | 2,588 b | 2,170 ab | 2,470 b | 2,292 |
| | | | | | |
| C-2, G2, UP | 2,220 b | 2,696 b | 2,019 b | 2,641 b | 2,394 |
| C-2, G8, SPS | 2,664 a | 2,811 ab | 2,619 a | 2,937 a | 2,757 |
| C-2, G8, BSS | 2,497 a | 2,983 a | 2,400 a | 3,137 a | 2,754 |
| | | | | | |
| Average | 2,293 | 2,824 | 2,250 | 2,853 | |

[1]) For each composite the treatments carrying different letters are significantly different according to Duncan's multiple range test (P=0.05).

Table 8.30. Percentage leaf rust (LR), necrotic leaf area (NA) and stem rust (SR) assessments of the Composite 1, G2, unselected population (C-1,G2,UP), Composite 1 after four Single Plant Selections (C-1,G10,SPS), Composite 1 after four Bulk Seed Selections (C-1,G10,BSS), Composite 2, G2, unselected population (C-2,G2,UP), Composite 2 after three Single Plant Selections (C-2,G8,SPS), Composite 2 after three Bulk Seed Selections (C-2,G8,BSS) with (+F) and without (-F) fungicides in 1981.

| | | Flag leaf | | Second leaf | | Leaf Sheath | Ear |
|---|---|---|---|---|---|---|---|
| | | LR | NA | LR | NA | SR | NA |
| C-1,G2, UP | −F | 15.9 a[1]) | 28.3 a | 25.5 a | 58.3 a | 1.5 a | 5.0 a |
| C-1,G10,SPS | −F | 4.2 b | 6.5 b | 8.0 b | 30.8 b | 1.2 a | 1.5 b |
| C-1,G10,BSS | −F | 2.9 b | 5.2 b | 3.7 b | 29.8 b | 1.0 a | 1.8 b |
| | | | | | | | |
| C-1,G2, UP | +F | 2.3 a | 2.8 a | 4.0 a | 11.3 a | 1.0 a | 1.0 a |
| C-1,G8, SPS | +F | 1.4 a | 3.0 a | 2.8 a | 12.6 a | 0.0 a | 0.5 a |
| C-1,G8, BSS | +F | 1.5 a | 2.7 a | 2.2 a | 10.2 a | 0.1 a | 0.7 a |
| | | | | | | | |
| C-2,G2, UP | −F | 9.3 b | 16.0 a | 13.8 a | 33.5 a | 1.0 a | 4.0 a |
| C-2,G10,SPS | −F | 3.5 a | 4.8 b | 5.8 b | 11.1 b | 0.0 b | 1.4 b |
| C-2,G10,BSS | −F | 3.4 a | 5.3 b | 4.2 b | 13.5 b | 0.0 b | 2.1 b |
| | | | | | | | |
| C-2,G2, UP | +F | 3.0 a | 5.3 a | 4.0 a | 8.2 a | 0.1 a | 0.9 a |
| C-2,G8, SPS | +F | 2.0 a | 2.9 a | 1.9 b | 4.7 a | 0.0 a | 0.2 a |
| C-2,G8, BSS | +F | 2.1 a | 3.3 a | 1.7 b | 5.0 a | 0.0 a | 1.2 a |

[1]) Per composite and treatment, data are significantly different (P=0.05) according to Duncan's multiple range test if they carry no similar letters.

selection for leaf rust (Table 8.1), *S. nodorum* (Table 8.16) and C. sativus (Table 8.18) resistance what in this experiment together with other pathogens was assessed as the necrotic area (the percentage affected leaf area of all pathogens combined). The SPS populations of Composite 1 and 2 yielded 27.5 and 20% more than the unselected populations respectively. Also with fungicides these SPS populations yielded 4.8 and 4.3% respectively more than the unselected populations. The chemical treatments were mainly active against fungi. As the fungicides did not give a full control of the pathogens the yield increase can be explained by both an increased resistance level and a higher yield potential of the selected population (table 8.30). In the BSS procedure seeds were selected regardless the characters that influence the yield such as plant habit, number of grains per ear and number of ears per plant. The BSS procedure was effective for a yield improvement in Composite 2 but not in Composite 1. A reduction of the yield potential of composite 1 itself, which was observed in the fungicide and fungicide + Insecticide treatments, seems to be the cause that the yield failed to increase after the BSS procedure in Composite 1 in spite of the higher resistance level. This BSS is besides a selection for resistance also a selection procedure for genotypes adapted to the environmental conditions of Passo Fundo (acid soils with aluminum toxicity and adverse climatic conditions), which are far from ideal. A selection in the direction of a plant type producing fewer grains per plant can be expected as a result (see also chapter 8.9.6; table 8.32)

### *8.9.5 Response to selection by four different selection procedures on three composites*

In 1982, the indirect selection response for yield obtained by selection for partial resistance through four different selection procedures SPS, BSS, LS, and NS has been investigated. All the composite populations described in chapter 8.1., which had passed the number of selection cycles described in table 7.3 were tested. As in the previous experiments yield loss comparisons were made between unprotected plots and plots protected with fungicides and/or insecticides.

The experiment was performed as a combined latin square and split-plot design, in which the chemical treatments formed the main plots and the various composite populations and selection procedures the subplots (Schuster et al.,1978). The plot size was 4.0 x 9.0 m, the seed rate was 60 germinating seeds per m row. Borders of oats of three rows were sown to reduce interplot interference. Four different treatments were applied: i) no plant protection, ii) protection with fungicides, iii) protection with insecticides, iv) protection with fungicides + insecticides. The pesticide treatments were: 1. At tillering stage (25 DC): Bayleton, 0.5 kg/ha + Dithane 2.5 kg/ha. 2. At flag leaf just visible stage (39 DC): a) Bayleton, 0.5 kg/ha + Dithane 2.5 kg/ha. b) Pyrimor 50%, 150 g/ha. 3. At medium milk stage (75 DC): a) Bayleton, 0.5 kg/ha + Dithane 2.5 kg/ha + Benlate 0.5 kg/ha. b) Pyrimor 50%, 150 g/ha + Carbaryl 0.5 kg/ha. All the applications were done with 250 l water per ha.

At the growth stages 49 and 73 DC twenty plants per plot were assessed for leaf rust and necrotic leaf area on the flag and second leaf and necrotic area on the ear.

The poor weather affected this experiment seriously. Two days after sowing a continuous and heavy rainfall washed away soil and seed of many plots from the hill where the experiment was planted. To obtain as much usable information as possible in this situation, observations were not done on a per plot basis but on a square meter basis on areas within the plots were not too much damage occurred. From these data yields per ha were estimated. During the harvest period continuous rain made a uniform harvest difficult, as the experiment could only be harvested with intervals of four to five days, the grain containing 13 to 19% moist. In general the delayed harvest had a stronger negative effect on the plots without fungicide than on those with fungicides because they ripened earlier due to the leaf rust infection. Moreover the results of this experiment in 1982 reflected very well the main problem of wheat production in Rio Grande do Sul in 1982 with its lowest productivity since 1972 (383 kg/ha). The break down of race-specific resistance to leaf rust of the main wheat cultivar CNT-10 caused a leaf rust epidemic in Rio Grande do Sul as was never experienced before. Several hectares of CNT-10 had been sown one month earlier than the experiment on neighboring fields causing an extremely heavy inoculum pressure which strongly reduced the effect (interplot interference reduction) of the three-row-wide strips of oats around the plots. Another problem was that the field appeared to be infested with Soil Born Mosaic Virus. It was obvious that the tested populations had not accumulated much SBMV resistance as also was seen in the SBMV resistance test (chapter 8.8.1). A third problem was an attack of caterpillars shortly before harvest time.

At growth stage 13 DC, the number of plants per $m^2$ was determined. It varied between 214 and 260 (on average 243) which is about 30% less than under more normal growing conditions is expected. The average number of ears per $m^2$ was 311, with no significant differences between the chemical treatments.

The yield losses were mainly due to leaf rust, SBMV and the caterpillars *Pseudaletia sequax* and *Pseudaletia adultera* and to a smaller extend to powdery mildew, *C. sativus* and *F. graminearum*. The yield loss estimated by the treatment without chemical plant protection of the unselected composites 1, 2 and 3 was 68.9, 65.7 and 61.0% respectively. In table 8.31 this yield loss is expressed as an average for the three unselected composites 1, 2 and 3 showing clearly how

Table 8.31. Yield in kg/ha, thousand grain weight (TGW) and number of grains per ear (NGE) averaged over the three unselected composites 1 ,2 and 3.

| | No plant protection | Fungicides | Insecticides | Fungicides + Insecticides |
|---|---|---|---|---|
| Yield | 903 | 2450 | 1119 | 2609 |
| TGW | 26.9 | 32.1 | 27.8 | 33.3 |
| NGE | 26.5 | 32.2 | 27.4 | 31.5 |

Table 8.32. Number of selection cycles (NSC), percentage yield increase (YI), percentage yield increase per selection cycle (YI/SC), percentage thousand grain weight increase per selection cycle (TGW/SC) and percentage number of grains per ear increase per selection cycle (NGE/SC) based on yield experiment in 1982.

| Procedure | Composite | NSC | YI | YI/SC | TGW/SC | NGE/SC |
|---|---|---|---|---|---|---|
| SPS | 1 | 5 | 39.28 | 7.86 | 1.12 | 2.04 |
| SPS | 2 | 4 | 14.35 | 3.59 | -0.22 | 5.56 |
| SPS [1]) | 3 | 4 | 12.09 | 3.02 | 0.79 | 0.82 |
| SPS [2]) | 3 | 4 | 13.38 | 3.34 | 0.97 | -1.32 |
| Mean SPS | | | | 4.45 | 0.66 | 1.79 |
| BSS | 1 | 5 | -3.27 | -0.65 | 0.64 | -1.00 |
| BSS | 2 | 4 | 13.97 | 3.49 | 1.28 | 2.94 |
| BSS | 3 | 4 | 22.66 | 5.66 | 0.23 | -0.13 |
| Mean BSS | | | | 2.83 | 0.72 | 0.60 |
| LS | 1 | 1 | 11.14 | 11.14 | -4.08 | 19.21 |
| LS | 2 | 1 | 24.92 | 24.92 | 12.77 | 12.18 |
| LS | 3 | 1 | 19.73 | 19.73 | 2.95 | 2.57 |
| Mean LS | | | 18.60 | 18.60 | 3.88 | 11.32 |
| NS | 1 | 5 | 18.73 | 3.74 | 1.01 | 0.65 |

[1]) = Passo Fundo
[2]) = Londrina

this yield loss due to parasites besides in grain yield also is expressed by the yield components thousand grain weight and number of grains per ear.

In spite of the interplot interference caused by the severe leaf rust epidemic, the yield of the populations obtained by the SPS, BSS and LS procedures increased significantly. In the ANOVA, only the main effects of chemical treatments and selection procedures were significant. The interactions were not significant for all three composites 1, 2 and 3.

The fungicide treatments were very effective, while the effect of the insecticide was small with only some impact on the caterpillar attack a few weeks before harvest. The effects of fungicides and insecticides appeared to be additive. From this it has been concluded that the response to selection for partial resistance to fungi can be deducted from both treatments 'No plant protection' and 'Insecticides'. Considering these treatments together one has six estimates of the percentage yield increase for the LS, SPS (of Passo Fundo), BSS procedures on composites 1, 2 and 3 and 2 estimates of the percentage yield increase for the SPS of Londrina and NS procedure of composite 1 and 3 respectively. From this yield increase also the percentage yield increase per selection cycle can be deducted. These results also have been calculated for the yield components thousand grain weight and number of grains per ear and are presented together in table 8.32.

The results show clearly that the LS with 18.6% yield increase, 3.88% thousand grain weight and 11.32% number of grains per ear increase per selection cycle is superior to the SPS, BSS and NS procedures. The SPS procedure was most

Table 8.33. Yield in kg/ha of three Composites, the unselected populations and latest selections of Single Plant Selection (SPS), Bulk Seed Selection (BSS), Line Selection (LS) and Natural Selection (NS) without plant protection (NoPP), with fungicides (Fung), with insecticides (Insec) and fungicides + insecticides (Fung/Insec) in 1982.

| | NoPP | Fung | Insec | Fung/Insec | Average |
|---|---|---|---|---|---|
| C-1,G2 ,UP | 815 | 2,418 | 1,007 | 2,619 | 1,715 de[1]) |
| C-1,G4 ,SPS | 1,066 | 2,652 | 1,367 | 2,834 | 1,980 abc |
| C-1,G12,SPS | 1,117 | 2,759 | 1,425 | 3,175 | 2,119 ab |
| C-1,G12,BSS | 1,043 | 2,405 | 1,307 | 2,814 | 1,892 cde |
| C-1,G12,LS | 1,178 | 2,816 | 1,528 | 3,076 | 2,150 a |
| C-1,G5 ,NS | 994 | 2,712 | 1,163 | 3,180 | 1,987 abc |
| C-2,G2 ,UP | 983 | 2,545 | 1,147 | 2,865 | 1,885 bc |
| C-2,G4 ,SPS | 967 | 2,748 | 1,090 | 2,772 | 1,894 bc |
| C-2,G10,SPS | 1,086 | 2,909 | 1,356 | 2,840 | 2,048 ab |
| C-2,G10,BSS | 1,122 | 2,535 | 1,220 | 3,020 | 1,974 abc |
| C-2,G10,LS | 1,151 | 2,702 | 1,426 | 3,057 | 2,084 a |
| C-3,G2 ,UP | 912 | 2,386 | 1,203 | 2,342 | 1,711 b |
| C-3,G9 ,SPS PF[2]) | 1,056 | 2,525 | 1,304 | 2,913 | 1,950 a |
| C-3,G9 ,SPS Lo[3]) | 1,131 | 2,635 | 1,236 | 2,831 | 1,958 a |
| C-3,G9 ,BSS | 1,179 | 2,565 | 1,396 | 2,720 | 1,965 a |
| C-3,G9 ,LS | 1,149 | 2,606 | 1,365 | 3,073 | 2,048 a |
| C-4,-- ,SPS | 1,006 | 1,839 | 1,304 | 2,613 | 1,690 |
| CNT-10 | 677 | 2,267 | 544 | 2,318 | 1,451 |
| Average | 1,036 a | 2,557 c | 1,244 b | 2,837 d | |

[1]) For each composite and treatment carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).
[2]) PF = Passo Fundo
[3]) Lo = Londrina.

effective in composite 1 what was expected considering the results of the previous chapters 8.2 – 8.9.4 where the beneficial effect of the SPS in 1977 under very severe epidemic conditions often has been found. A small yield gain of 4.45% per selection cycle was obtained. Also the thousand grain weight and number of grains per ear increased with 0.66 and 1.79% per selection cycle respectively, what has been significantly less than has been observed for the LS procedure. The results of the BSS are not very constant for the three composites, however it is a procedure with the advantage that it is very easy to carry out. In the experiment of chapter 8.9.4 it was seen that the BSS procedure on composite 1 resulted in a lower yield in spite of an increased resistance level. Table 8.32 confirms the suggestion made in chapter 8.9.4 that besides an increased level of resistance the BSS procedure favoured plants with a reduced number of grains per ear. Also the increase of the thousand grain weight was almost nil. For composite 2 the number of grains per ear and thousand grain weight increased resulting also in an higher yield in kg/ha.

The NS selection procedure resulted in a small but significant increase in yield, probably mainly due to the higher level of SBMV resistance as was observed in chapter 8.8.2. and also seen in this experiment. Moreover, considering the very high yield under full chemical control conditions, the high level of heterozygousity might have resulted in a heterosis effect.

Taking the averages of the four chemical treatments for yield, thousand grain weight and number of grains per ear, one gets almost the same result as discussed for the without fungicide condition. The LS is again the best selection procedure followed by SPS and BSS (Table 8.33, 8.34).

Table 8.34. Thousand grain weight in g of three Composites, the unselected populations and latest selections of Single Plant Selection (SPS), Bulk Seed Selection (BSS), Line Selection (LS) and Natural Selection (NS) without plant protection (NoPP), with fungicides (Fung), with insecticides (Insec) and fungicides + insecticides (Fung/Insec) in 1982.

| | NoPP | Fung | Insec | Fung/Insec | Average |
|---|---|---|---|---|---|
| C-1,G2 ,UP | 27.3 | 31.3 | 27.8 | 32.7 | 29.8 e[1]) |
| C-1,G4 ,SPS | 26.9 | 34.4 | 28.8 | 34.6 | 31.2 bc |
| C-1,G12,SPS | 28.5 | 34.4 | 29.7 | 35.7 | 32.1 a |
| C-1,G12,BSS | 27.8 | 34.4 | 29.7 | 35.9 | 31.9 ab |
| C-1,G12,LS | 26.2 | 33.2 | 27.2 | 34.4 | 30.3 de |
| C-1,G5 ,NS | 28.9 | 34.7 | 29.3 | 36.1 | 32.3 a |
| C-2,G2 ,UP | 27.2 | 32.6 | 28.0 | 35.0 | 30.7 c |
| C-2,G4 ,SPS | 26.0 | 34.4 | 27.1 | 36.1 | 30.9 bc |
| C-2,G10,SPS | 27.0 | 33.2 | 27.7 | 34.9 | 30.7 c |
| C-2,G10,BSS | 27.6 | 32.5 | 28.2 | 34.4 | 30.7 c |
| C-2,G10,LS | 28.9 | 34.4 | 31.0 | 34.3 | 32.1 d |
| C-3,G2 ,UP | 26.3 | 32.3 | 27.6 | 32.2 | 29.6 c |
| C-3,G9 ,SPS PF[2]) | 27.1 | 33.7 | 28.5 | 34.1 | 30.8 ab |
| C-3,G9 ,SPS Lo[3]) | 27.0 | 34.7 | 29.0 | 35.1 | 31.4 a |
| C-3,G9 ,BSS | 26.5 | 33.4 | 27.9 | 33.1 | 30.2 bc |
| C-3,G9 ,LS | 26.9 | 33.6 | 28.6 | 34.8 | 31.0 ab |
| C-4,-- ,SPS | 27.6 | 32.9 | 29.6 | 34.6 | 31.2 |
| CNT-10 | 21.8 | 29.4 | 20.0 | 30.1 | 25.8 |
| Average | 27.0 d | 33.3 b | 28.1 c | 34.3 a | 30.7 |

[1]) For each composite and treatment carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).
[2]) PF = Passo Fundo
[3]) Lo = Londrina

Table 8.35. Percentage necrotic area on the ear of three Composites (Comp), the unselected populations and latest selections of Single Plant Selection (SPS), Bulk Seed Selection (BSS), Line Selection (LS) and Natural Selection (NS).

| Population | Comp 1 | Comp 2 | Comp 3 |
|---|---|---|---|
| Unselected population, G1/2 | 4.3 a[1]) | 4.8 a | 8.0 a |
| SPS intermediate population, G4 | 2.5 b | 2.5 b | – |
| SPS latest selection, G12/10/9 | 1.3 b | 1.5 b | 3.8 b |
| BSS latest selection, G12/10/9 | 1.8 b | 2.0 b | 2.5 b |
| LS latest selection, G12/10/9 | 1.3 b | 2.3 b | 2.8 b |
| NS latest selection, G5 | 4.0 a | – | – |

[1]) The necrotic area assessments carrying different letters are significantly different according to Duncan's multiple range test (P = 0.05).

Composite 4 did not yield differently from the unselected composite 1 for all treatments. In the field it was observed that this population suffered most from SBMV. There appeared to be a segregation of SBMV resistance. Many plants failed to produce an ear. The thousand grain weight became slightly higher than the unselected composite 1. However the number of grains per ear was increased drastically showing the higher yield potential of this population under SBMV free conditions. Without plant protection, with fungicides, with insecticides and fungicides + insecticides the composite 4 produced 19.8, 13.3, 14.8 and 33.7% more grains per ear respectively than the unselected composite 1.

The disease assessments were made for the leaf rust on the flag leaf and second leaf at growth stage 49 DC. which has been discussed in chapter 8.2 experiment II (Table 8.2). At growth stage 73 DC the necrotic area of the ear has been assessed and the average of both treatments 'No plant protection' and 'Insecticides' being not significantly different are presented in Table 8.35.. *C. sativus* and *F. graminearum* on the grains were discussed in chapter 8.5.2 and 8.6 (Table 8.18 and 8.19). Observations showed that the SPS, BSS and LS procedures resulted in a significant increase of partial resistance to these pathogens however it is only partly reflected in the yield increase due to severe leaf rust epidemic and strong effects of interplot interference.

### *8.9.6 Yield of F6 lines from the Line Selection procedure*

A yield experiment was done on 200 F6 lines of each composite 1 and 2 and 100 F6 lines of composite 3 derived from selected F4 lines of the LS procedure. These 500 lines were tested in a field experiment with plots of three rows of 5 m length. Lines were evaluated per composite in randomized blocks with three replicates. The cultivar Nobre was used as a control. It occurred after each 20 plots. The repeated cultivar Nobre made with its dark coloured glumes and susceptibility to most of the pathogens orientation in the field and selection for disease resistance easier. It was not an appropriate scheme for correction through a moving mean (Townley-Smith & Hurd,1973) as the distance between two plots of Nobre of 120 m is too large, giving over and under adjustments of the plot yields. A larger number of control plots was impossible for practical reasons. The replicates were sown in the downhill direction of the terrace because fertility gradients normally occur in this downward direction of the slope. No plant protection was applied.

Per composite the lines were divided in seven classes according to their yield level according to the formula:

Σ (frequency of lines x average value of class) / total number of lines

The average yield of the composites 1, 2 and 3 were 2405, 1632, 2015 kg/ha (Table 8.36). A fertility gradient as expected was found. Nobre yielded in the composite 1 blocks (planted at the upper side of the terrace) 1665 kg/ha, in the composite 2 blocks (planted in the middle part of the terrace) 1543 kg/ha and in the composite 3 blocks 1672 kg/ha (planted at the lower side of the terrace). Following the results of 1976, 1978 and 1979 (Table 8.24, 8.26 and 8.27) Nobre yields on average 91% of the unselected composite 1. Starting from this assumption the unselected composite 1 would have yielded 1955 kg/ha in composite 1 group, 1696 kg/ha in composite 2 group and 1837 kg/ha in composite 3 group. Therefore it has been estimated that the LS F6 lines of composite 1,

Table 8.36. Average yield in kg/ha of LS lines (Av. LS), Nobre, estimated yield of the unselected composite 1 (C-1 UP) and estimated percentage yield increase (% YI) of LS procedure for three composites.

| | Av. LS | Nobre | C-1 UP | % YI |
|---|---|---|---|---|
| Composite 1 | 2405 | 1779 | 1955 | 23% |
| Composite 2 | 1632 | 1543 | 1696 | –4% |
| Composite 3 | 2015 | 1672 | 1837 | 10% |

Table 8.37. Number of F6 lines from the Line Selection procedure grouped in relation to yield response in kg/ha in 1981.

| kg/ha | Composite 1 | Composite 2 | Composite 3 |
|---|---|---|---|
| 500-1000 | 1 | 8 | 4 |
| 1000-1500 | 22 | 79 | 14 |
| 1500-2000 | 24 | 70 | 24 |
| 2000-2500 | 44 | 38 | 41 |
| 2500-3000 | 86 | 5 | 17 |
| 3000-3500 | 22 | | |
| 3500-4000 | 1 | | |

2 and 3 yielded 23%, −4% and 10% more than the unselected composite 1 respectively. In table 8.29 the unselected composite 1 yielded on average almost the same as the unselected composite 2 and in table 8.33 the unselected composite 1 yielded on average the same as the unselected composite 3 and slightly less than unselected composite 2. Year and location effects make further reliable comparisons of the LS F6 lines with the unselected composites difficult. However as a general conclusion it can be said that composite 1 gave a strong response to F4 line selection for yield while the response for composite 2 and 3 was smaller. From table 8.37 it can be seen that very high yielding lines even producing more than 3000 kg/ha were produced.

# 9 Discussion

## 9.1 Population improvement procedure according to the SPS procedure

According to the suggestion of Robinson (1973,1976) the SPS procedure was started to accumulate partial resistance to various wheat pathogens. This procedure was made up of a system in which wheat was changed from a self-pollinated crop to a cross-pollinated crop with the aid of a male gametocide (chapter 9.2) and a recurrent selection was performed in the segregating population. Selected plants were multiplied in the off-season and intercrossed again in the following generation. Therefore each selection cycle consisted of one out-crossing and selection in the single plant generation (F2) and one multiplication.

## 9.2 Use of a male gametocide

In order to achieve cross-pollination the possibility of using ethrel as a male gametocide was investigated. Ethrel appeared to be fairly effective, effective enough for this type of breeding work. For research purpose or for producing hybrids it is far from satisfactory. The male gametocide has the advantage that it is easy to apply and its effects are not inherited. Large numbers of crossings can be made with a small team in one day. The disadvantage of ethrel is that it does not give full male sterility, it increases the susceptibility to pathogens and reduces the plant growth and affects the plant habit.

Considering that ethrel does not give full male sterility, it is suggested to start with a diallel scheme of hand made crossings between the selected cultivars. With the high multiplication rates of more than 2000 grains per plant in a water culture system (Table 6.2) it becomes possible to start with a relatively low number of hand made crossings and still to obtain sufficiently large F2 populations for later selection procedures. When the selected units have to be crossed again in the subsequent selection cycles, it is advisable to use a gametocide such as ethrel if large numbers of selected units have to be crossed or to use hand crossing in case much labour is available and only a restricted number of selected units have to be crossed.

## 9.3 Early and late generation selection for yield

Selection for yield or yield components can be done in early generations or in later ones, only the efficiency of selection differs. From the point of view of the population geneticist, it would theoretically be best to select as early as possible because the proportion of plants carrying most desirable genes either in heterozygous or homozygous condition is then highest. The problem in these generations however is that these desired genotypes are extremely difficult to recognize. Relatively large variances due to i) environmental variation, ii) genotype environment interactions, iii) dominance and epistasis will nearly always hinder efficient selection to a very strong extent.

In the first segregating generation (F2) one is selecting at a single plant level, whereas in later generations one has sufficient seed available to evaluate large numbers of plants in replicated plots. Therefore the experimental error in these later generations is much smaller than the one in the F2, increasing the heritability and thus the ability to recognize the desired genotypes. In general, it can be said that before F4 little selection for yield can be done. Early generation line selection (F3) is considered to be superior to bulk selection methods but is also more expensive, owing to organization, machinery for sowing and harvest, labour and land. This aspect has to be considered, especially in developing countries where one works under limited financial and technological conditions.

Visual selection applied in the early generations seems to have some effect. It cannot recognize the required high yielding genotypes but it is able to eliminate the inferior genotypes and in this way a steady but limited progress can be expected from visual selection for yield in heterogeneous populations.

Indirect selection has more possibilities for yield improvement when the characteristics selected for have a relatively high $h^2$ and are correlated well with yield. Selection for resistance against pathogens is one of the most frequently used indirect selection methods for yield improvement. Selection for qualitatively inherited resistance is normally applied, and successfully, in early generations. Selection for partial resistance, being quantitatively inherited, might be more successful in later generations than in early generations for the same reason as those related to yield. However, results on barley with partial resistance to leaf rust and powdery mildew (Parlevliet,1988; Johnson & Wilcoxson,1976) and on wheat with partial resistance to stripe rust (Krupinsky & Sharp 1978,1979) and stem rust (Knott, 1982), indicate that an improvement of partial resistance from early generation selection can be expected. A main obstacle of selection for partial resistance is interplot interference underestimating the level of partial resistance with wind spread pathogens, reducing even more the efficiency of selection in early generations where plots are very small and so interplot interference most severe.

The approach of Robinson (1973,1976) with selection for partial resistance in early generations was considered as possibly too weak in combination with too many recombinations, for which reason the accumulation of partial resistance might be slow. Therefore an other approach of Line Selection (LS) for the accumulation of partial resistance has been investigated. Besides the LS procedure also a Bulk Seed Selection (BSS) and a Natural Selection (NS) procedure were compared.

## 9.4 The selection procedures

As a general conclusion, it was found that LS is very efficient in accumulating resistance to all these pathogens with the exception of SBMV. Success of SPS depends on the heritability of the partial resistance to the pathogen. The response to SPS varied with pathogens and years. For leaf rust the latent period experiments showed very little progress obtained by SPS, although the field experiments on crop losses of 1978, 1981 and 1982 still showed a significant progress which was not detected by latent period assessments. This suggests that more genes for partial resistance than measured by the component of resistance latent period are involved or the seedling latent period parameter test is not sufficiently sensitive to measure small differences of partial resistance, which resistance is better expressed at the adult plant stage. SPS was extremely efficient for stem rust resistance and very efficient for powdery mildew resistance. It has been concluded that this selection for stem rust is so efficient because interplot interference is hardly a problem with this pathogen. With regard to *S. nodorum, C. sativus* and *F. graminearum* SPS can be efficient, but a high selection pressure is needed for accumulating the resistance and after that also maintaining the resistance level as erosion seems to occur rather easily. For Common Root Rot and BYDV the SPS also had its positive effect in the first cycle of the SPS procedure. However, after a first increase of resistance no further increase of resistance was obtained by either selection procedure possibly because of a lack of genetic variation for Common Root Rot resistance or because the selection procedures were not adequate enough.

The BSS procedure showed to be surprisingly efficient in accumulating partial resistance to leaf rust, *S. nodorum, C. sativus and F. graminearum* but the concomitant yield gain was not realized in one of the three composites. This latter aspect reduces the attractiveness of the BSS procedure.

With regards to the plant type characteristics, in the LS procedure one has the opportunity to select simultaneously for this trait and if very good lines are selected new cultivars can be produced. The SPS is a procedure of making populations with an increased level of resistance, while very little attention goes to other characteristics such as plant type. In the BSS procedure selection for plant type is nil.

The NS procedure only resulted in an increased resistance level of C. sativus and a slight improved resistance level of SBMV.

The SPS procedure of composite 4 resulted in a composite in which partial resistance was combined with an improved plant type derived from modern West European cultivars expressed in a stronger developed root system, shorter straw, better tillering and a higher yield potential. High levels of resistance to leaf rust, stem rust, powdery mildew, *S. nodorum, C. sativus*, Common Root Rot and BYDV were found. The composite 4 was compared with the G10 SPS latest selection of Composite 1 in large plots of ± 2500 m² on a farm in Palmeiras das Missoẽs, Rio Grande do Sul where it was observed that the SPS latest selection of composite 4 produced, at growth stage 32 DC, a root system with a dry weight of 0.180 gram per plant, while the SPS, G10 latest selection of composite 1 had a dry weight of only 0.031 gram per plant. Considering the combination disease resistance and plant type characteristics, composite 4 became a most interesting population for the production of future cultivars.

## 9.5 Yield improvement as a result of selection for partial resistance

Yield improvement followed the increases in partial resistance closely. It was clear that the LS procedure gave the best results (Table 8.32). The SPS procedure also resulted in an increased yield level, while the BSS composites gave an inconsistent response. Interesting has been the high yield potential of the NS procedure under plant protection condition, which was considered as a possible effect of heterosis because of the high level of heterozygousity in this composite.

## 9.6 Advised procedure

Based on the results it is concluded that the SPS procedure can be rather efficient and the LS procedure is very efficient to produce populations with a high level of partial resistance. From these populations new cultivars can be selected. A basic scheme for such a breeding programme could be first to start outcrossing between a selected number of cultivars. This can be repeated 2 or 3 generations in order to obtain a composite from which selection can start. In the G1 an early generation (± F2) SPS selection would give a first selection response. In the G3 and G4 a line selection procedure of ear to row plots can be followed and the best F4 lines intercrossed again after which moment a new cycle of line selection starts. From the best F4 lines line selection can be continued to simultaneously produce new cultivars as described in chapter 9.7. The advantage of this procedure is that everything can be done in the field and it does not require much organization structure. If one has more facilities it is possible to speed up the programme and to increase the efficiency. The water culture discussed in chapter 6.2. would make it possible to obtain larger F2 families (± 2000 plants per F1 plant) and to advance generations rapidly in 70 -75 days per generation through the SSD system. In less time F4 – F6 lines can be obtained. The higher additive genetic variance in F4 and F6 makes selection somewhat more efficient than selection in F3 and F4. However the selection procedure also asks for more attention. The advantage of the latter procedure of SSD combined with F4 and F6 line selection is that every two years almost homozygous lines are produced from which new cultivars can be selected.

## 9.7 Production of commercial cultivars

Cultivation of heterogeneous populations instead of pure lines may be considered. The traditional arguments in favour of pure lines carry relatively little weight in Brazil, where the milling quality requirements are not stringent and plant breeders rights do not exist. Heterogeneous populations with a good performance could be multiplied and sold to the farmer with an advantage that it can be improved gradually through recurrent selection. In 1981 a yield estimate has been made on the composites of the SPS procedure of composite 1, 2 and 3, which were grown in large plots and inoculated with leaf rust, stem rust, *S. nodorum, S. tritici, C. sativus* at growth stage 45 DC and *F. graminearum* at growth stage 59 DC. The plot size was 160 $m^2$ with 5 replicates. Under this condition the SPS latest selections of composite 1 G10, composite 2 G8 and composite 3 G7 yielded 1924, 2740, 2300 kg/ha respectively. This is 57%, 124% and 88% more than the average yield of Rio Grande do Sul in that year. The same composites yielded under not inoculated conditions in the small three row adjacent plots of the official EMBRAPA/CNPTrigo cultivar release trial called 'Preliminar' 2548, 2937 and 2229 kg/ha respectively.

The recurrent selection scheme discussed in chapter 9.6. has the advantage that every four years (can be 2 years if SSD methods is applied) a new, improved population can be issued if farmers accept such populations as commercial cultivars.

At the same time every four years the best F4 lines can be used as a basis to produce pure line cultivars through a continuous line selection programme. So either approach is possible within this suggested procedure. The latter approach, continued line selection, has already resulted in two lines of the LS procedure of composite 1, RH-18 and RH-54 which are in a very advanced stage

Table 9.1. Average yield in kg/ha of the wheat lines RH-18 and RH-54 and from control cultivars in EMBRAPA yield trials 'Preliminar' (1981) and 'Sul Brasileiro' (1982-1986) at twelve locations in nine regions of Rio Grande do Sul. (Moreira, EMBRAPA, CNPTrigo, personal comm.).

| Cultivar | 1981 | 1982 | 1983 | 1984 | 1985 | 1986 |
|---|---|---|---|---|---|---|
| RH-18 | – | 1937 | 2265 | 2013 | 1879 | 2176 |
| RH-54 | 4031 | 2274 | 2399 | 2120 | 1797 | 2334 |
| Maringá | 2806 | 1407 | 2023 | 1588 | 1538 | 1795 |
| Jacuí | 2547 | – | – | – | – | – |
| CNT-9 | 2783 | 624 | – | – | – | – |
| Pat-7392 | – | 2265 | 2098 | – | – | – |
| CNT-8 | – | – | 2253 | 1663 | 1568 | 1736 |
| Minuano-82 | – | – | – | 2072 | 1755 | 2014 |
| Average Rio Grande do Sul | 1223 | 383 | 1113 | 836 | 947 | 1430 |

of becoming recommended as a new cultivar in Rio Grande do Sul. In 1981 RH-54 has been for the first time tested in the EMBRAPA cultivar release trial called 'Preliminar em rede' at four locations in Rio Grande do Sul. In 1982 it was again tested in the same trial together with RH-18. Because of their good performance both lines were tested in the 'Ensaio Regional Precoce B' at 12 locations divided over nine regions in Rio Grande do Sul (Table 9.1). The control cultivars of these cultivar release trials were not the same over the years. As control cultivars those are taken that are the best at that moment. Due to breakdown of resistance, the composition of the recommended cultivar list varies regularly.

# Abstract

Beek, M. A., 1988. Selection procedures for durable resistance in wheat. Doctoral thesis. Wageningen, the Netherlands.

A wheat breeding programme for durable resistance to all locally important pathogens: leaf rust, stem rust, powdery mildew, *Septoria nodorum, Septoria tritici, Cochliobolus sativus, Fusarium graminearum*, Common Root Rot, Barley Yellow Dwarf Virus and Soil Borne Mosaic Virus was conducted in Brazil. The objective of the programme was to determine the feasibility of accumulating useful levels of partial resistance, which is quantitative resistance based on minor genes and considered to be more durable than resistance based on major race-specific genes.

Four different selection procedures of breeding for partial resistance were applied and evaluated for their efficiency: Line Selection (LS), Single Plant Selection (SPS), Bulk Seed Selection (BSS) and Natural Selection (NS). The SPS procedure was made up of a system in which wheat was changed from a self-pollinated crop to a cross-polinated crop with the aid of a male gametocide and a recurrent selection was carried out in the segregating population. Selected plants were multiplied in the off-season and intercrossed again in the following generation. The LS procedure consisted of a selection in the F4 in ear to row plots. The best plants of the best rows were multiplied in the off-season and in the F6 the best lines were selected for a new cycle of outcrossing and line selection. The BSS was also an early generation selection as the SPS procedure based on grain qualities, while the NS procedure consisted of crossing only without any artificial selection.

Crossing was done with the male gametocide ethrel. The optimum concentration for the application of ethrel in combination with the growth regulator gibberellic acid-3 under field conditions has been investigated. From these experiments one application of 2000 ppm ethrel a.i. 2-chloro-ethyl-phosphonic acid in 1000 l. water/ha at growth stage 41 – 43 DC in combination with an application of 150 ppm gibberellic acid-3, 500 l. water/ha three to four days later was most effective. Depending on environmental conditions and genotype about 60% – 80% cross pollination can be achieved. One new product R-111601 was tested but did not give the desired result.

The selection procedures were compared for partial resistance to leaf rust, stem rust, powdery mildew, *S. nodorum, C. sativus, F. graminearum*, Common Root Rot, BYDV and SBMV and yield.

In general, selection in later generations (F4 and F6) of the LS procedure showed to be more efficient than selection in early generations (F2) of the SPS procedure. The lower efficiency of SPS to be due to a relatively low heritability

for partial resistance and the effect of interplot interference in the field under such conditions. As selection in later generations requires a large amount of time in the breeding procedure, an attempt was made to reduce this delay by a Single Seed Descent method, by which system generations can be advanced in 70 – 75 days. Through BSS seed selection it also was possible to accumulate partial resistance, while progress through natural selection was almost nil.

The results convincingly showed that it is possible to simultaneously improve partial resistance to several pathogens and subsequent yield. Substantial increase in partial resistance was obtained for all pathogens with the exception of SBMV.

The yield increase of the LS with 18.6% per selection cycle is superior to those of the SPS, BSS and NS procedures with 4.4, 2.8 and 3.7% respectively.

From the results it is concluded that this new wheat breeding approach is of special interest for areas where many pathogens occur and the durability of resistance is a main concern. This applies in the first place to developing countries where stable crop yields are of extreme importance.

# Samenvatting

Een tarwe veredelingsprogramma voor duurzame resistentie tegen alle lokaal belangrijke pathogenen: bruine roest, zwarte roest, meeldauw, *Septoria nodorum, Septoria tritici, Cochliobolus sativus, Fusarium graminearum*, Common Root Rot, Barley Yellow Dwarf Virus and Soil Borne Mosaic Virus werd uitgevoerd in Zuid-Brazilië. Het doel van het programma was het onderzoeken van de mogelijkheid om partiele resitentie, welke kwantitatief in expressie is, tot een voldoende hoog niveau te accumuleren. Deze resistentie, die polygeen van aard is, wordt beschouwd als duurzamer dan de resistentie die vaak gebaseerd is op fysio-specifieke hoofd-genen.

Vier verschillende selectie procedures voor partiele resistentie werden vergeleken: Lijn Selectie (LS), Individuele Plant Selectie (SPS), Massa Zaad Selectie (BSS) en Natuurlijke Selectie (NS). Naar het voorstel van Robinson (1973,1976) werd eerst gestart met de SPS procedure om partiele resistentie tegen genoemde pathogenen op te bouwen. Deze procedure bestond uit een systeem, waarin tarwe werd veranderd van een zelfbevruchtend in een kruisbevruchtend gewas. Hiervoor werd het product ethrel gebruikt, dat mannelijke steriliteit induceert. Een recurrent selectie schema werd uitgevoerd op de uitsplitsende populaties. De geselecteerde planten werden vermeerderd in het tussen seizoen en opnieuw onderling gekruist in de volgende generatie. Op deze manier bestond iedere selectie cyclus uit één maal kruisen en selectie op de individuele plant generatie (F2) en één vermeerdering.

De efficientie van ethrel om mannelijke steriliteit en kruisbevruchting onder veldomstandigheden te bewerkstelligen werd onderzocht. De optimale concentratie voor de ethrel bespuiting was 2000 ppm (actief ingredient 2-chloorethaanfosfonzuur) per 1000 l/ha bij groeistadium 41 – 43 DC in combinatie met een bespuiting drie à vier dagen later met de groeiregulator gibberellazuur (GA3) 150 ppm, 500 l/ha. Afhankelijk van de milieu omstandigheden en het genotype kan ongeveer 60 – 80% kruisbevruchting verkregen worden. Een nieuw product R-111601 gaf niet het gewenste resultaat.

De LS procedure bestond uit een verlate selectie in de F4 in per aar gezaaide rijtjes. De beste planten van de beste rijtjes werden vermeerderd in het tussenseizoen en in de F6 werden de beste lijnen geselecteerd die dan klaar zijn voor een nieuwe cyclus van kruisbevruchting, en lijnselectie. De BSS was evenals de SPS procedure een vroege generatie selectie gebaseerd op zaad kwaliteiten als grootte, duizend korrel gewicht, specifiek gewicht en vrij zijn van pathogenen. De NS procedure bestond uitsluitend uit kruisbevruchting zonder kunstmatige selectie.

In het algemeen was selectie in latere generaties (F4 en F6) van de LS procedure efficienter dan selectie in de vroege generaties (F2) van de SPS procedure,

wat mogelijk het gevolg is van een hogere erfelijkheidsgraad voor partiele resistentie en het effect van interferentie tussen de veldjes op het proefveld. Omdat selectie in latere generaties meer tijd kost in het veredelingsprogramma, is er een 'Single Seed Descent' methode ontwikkeld, waarbij de generaties binnen 70 à 75 dagen passeren.

Ook door selectie op zaad in de BSS procedure was het mogelijk om partiële resistentie te accumuleren, terwijl de natuurlijke selektie geen enkele vooruitgang gaf.

De resultaten geven duidelijk aan dat het mogelijk is om gelijktijdig partiële resistentie tegen meerdere pathogenen te accumuleren en daarmee opbrengst te verhogen. De opbrengst verhoging van de LS procedure was met 18.6% per selectie cyclus duidelijk beter dan de SPS, BSS en NS procedures die per cyclus respectievelijk 4.4, 2.8 en 3.7% meer opbrengst gaven.

Uit de resultaten kan men concluderen dat de benadering van veredelingsmethoden gebaseerd op partiele resistentie in de tarwe zeer interessant zijn voor lokaties waar vele pathogenen voorkomen en duurzaamheid van resistentie van bijzonder belang is. Dit betreft in de eerste plaats de ontwikkelingslanden, waar een stabiele oogst een hoge prioriteit heeft.

Key words: *Triticum aestivum*, partial resistance, horizontal resistance, durable resistance, early generation selection, seed selection, natural selection, male gametocide, ethrel, *Puccinia recondita, Puccinia graminis, Erysiphe graminis, Septoria nodorum, Septoria tritici, Cochliobolus sativus, Fusarium graminearum,* common root rot, Barley Yellow Dwarf Virus, Soil Borne Mosaic Virus, Brazil.

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# Curriculum Vitae

De auteur werd op 21 december 1944 geboren in Enschede. Na zijn eindexamen HBS-b aan het Hervormd Lyceum te Amsterdam ging hij studeren aan de Landbouw Universiteit te Wageningen. In 1974 behaalde hij het ingenieursdiploma met als specialisatie plantenveredeling, erfelijkheidsleer (verzwaard) en plantensystematiek en -geografie van de tropen en subtropen met daaraan toegevoegd een volledige lesbevoegdheid biologie.

In 1970-71 heeft hij in het kader van zijn praktijktijd als cacao veredelaar gewerkt bij CEPEC / CEPLAC in Itabuna, Bahia, Brazilië. In de jaren 1973-75 was hij bij het VWO leraar biologie in Barneveld en 's Hertogenbosch. Van 1975 tot 1983 was hij FAO expert in het ziekteresistentie veredelingsprogramma in de tarwe bij EMBRAPA/CNPTrigo in Passo Fundo, RS, Brazilië. In 1983 werkte hij acht maanden als wetenschappelijk gastmedewerker bij de vakgroep Plantenveredeling van de Landbouw Universiteit te Wageningen. Van 1983 tot 1987 heeft hij gewerkt als IICA consultant in de ziekteresistentie veredelingsprogramma's voor tuinbouwgewassen en aardappel bij EMBRAPA/CNPHortaliças in Brasília, DF. Na terugkeer in Nederland werd dit proefschrift afgerond. Vanaf Juni 1988 is hij hoofd plantenveredeling bij het tuinbouw veredeling's bedrijf Nunhem's Zaden BV te Haelen in Limburg.