Doctoral Thesis

Resistance to leaf and glume bloch (Septoria nodorum Berk.) and sensitivity in vitro to pathogen metabolites in winter wheat (Triticum aestivum L.)

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Resistance to leaf and glume blotch (*Septoria nodorum* Berk.) and sensitivity *in vitro* to pathogen metabolites in winter wheat (*Triticum aestivum* L.)

A dissertation submitted to the
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1 Summary

*Septoria nodorum* (Berk. in Berk and Broome) is a pathogen of wheat (*Triticum aestivum* L.) causing leaf and glume blotch (SNB). It is a serious pathogen in many wheat growing areas throughout the world and may reduce yields up to 50%. Resistance tests with adult plants in the field are very time consuming and also often inaccurate as environmental factors have a strong influence on infection frequency and disease progress. A project was therefore carried out with the aim to develop an *in vitro* test for the identification of genotypes resistant to SNB. The basis for this project was a study with a crude extract of a *Septoria nodorum* culture as a selective agent. It was possible to distinguish resistant and susceptible cultivars in an *in vitro* test with zygotic embryos. In our project, we wanted to test whether this *in vitro* test can also be used to detect resistant and susceptible genotypes in early segregating populations. Specific crosses between eight winter wheat lines showing contrasting resistance reactions for septoria nodorum blotch on leaves and ears were made. The resistance level of both leaf and ear was evaluated after artificial inoculation in the field for the parental lines, the F1 progenies and for F3 and F4 lines. In addition, this plant material was tested *in vitro* using a toxin-containing crude extract of *Septoria nodorum* as selective agent and culturing zygotic embryos or mature seeds. No significant correlations were found between the *in vitro* screening and septoria nodorum blotch on the leaves after artificial infection in the field. However, a good agreement between the sensitivity to the toxins *in vitro* and resistance in the field on the ear was found for the parental lines, the F1 and F5 generation but not for the F3 generation. The high correlation between these traits and the ease of the method especially for culturing mature seeds leads to the conclusion, that the *in vitro* screening could be integrated into wheat breeding programs in later generations. Populations showing a high susceptibility to the pathogen metabolites *in vitro* could be discarded. Another promising implementation for wheat breeding would be the screening of advanced breeding material or candidate partners in a crossing program for resistance on the ear. However,
the *in vitro* screening is not precise enough to select single plants in early segregating populations.

The data of the field assessment allowed to study the inheritance of resistance against *Septoria nodorum* on the leaves and the ears. It was possible to determine the genetic variation between as well as within segregating populations and therefore to estimate the probability to detect new sources of resistance. The variation observed in this study within and among the segregating populations suggests a quantitative inheritance pattern influencing the expression of the two traits. The components of variance between F2 families within a population were as high as for SNB on the ear or even higher for SNB on the leaf than those between populations. Therefore, a strong selection within a few populations may be as effective to find new resistant genotypes as selection in a large number of populations. In almost all crosses progenies were found that were more resistant than the better parent. Thus transgression breeding may also be a tool to breed for higher levels of resistance to septoria nodorum blotch.
Zusammenfassung


2 General introduction

2.1 Relevance of the pathogen

Septoria nodorum leaf and glume blotch (SNB) is caused by the fungus *Leptosphaeria nodorum* E. Müller (=*Phaeosphaeria nodorum* (E. Müller) Hedjaroude), anamorph = *Septoria nodorum* (Berk.) Berk. in Berk. & Broome (= *Stagonospora nodorum* (Berk.) Castellani & Germano). In Switzerland SNB plays a major role as damaging disease, together with powdery mildew (*Erysiphe graminis*), eyespot (*Pseudocercosporella herpotrichoides*), scab (*Fusarium* spp.), leaf rust (*Puccinia recondita*) stripe rust (*Puccinia striiformis*) and *Septoria tritici* blotch. It causes economic loss in most wheat growing areas all over the world. Yield losses of 50% and considerable damage in crop quality are possible, when conditions for disease development are favourable (Eyal et al., 1987, Karjalainen et al., 1983). These yield losses are mainly due to the reduction of the thousand kernel weight which can be attributed to shrivelled kernels (Brönnimann, 1968). With increasing latitude and increasing precipitation during the grain filling period, incidence and severity of the pathogen increase as well (Leath et al., 1993). Therefore, SNB is a widespread disease in Switzerland, where humid conditions during the vegetation period often occur.

2.1.1 Taxonomy and epidemiology

After Ainsworth et al. (1971), *Septoria nodorum* belongs to the Deuteromycotina or fungi imperfecti where the sexual stage of most of the fungi is absent or not known. Fungi of the genus *Septoria* are classified among the order Sphaeropsidales, characterised by the production of conidia, termed pycnidiospores, which are produced in variously shaped, semiclosed fruiting bodies known as pycnidia. However, for many fungi, the sexual stage is known and it is assumed that the sexual state of *Septoria nodorum* is associated with the class Ascomycetes (Eyal et al., 1987). The identification of *Septoria*
*nodorum* is most reliably done according to the conidial morphology. The conidia (pycnidiospores) have 1-3 septa, and they are usually no longer than 25 μm, however, the variability in conidia size and shape as well as colony morphology is large and may be affected by environment (King et al., 1983). Although the sexual state has been reported in several countries and will most likely be found elsewhere, it is the asexual state that causes most disease symptoms and associated yield losses. However, Keller et al. (1997) suggested that gene flow among populations is not restricted and that the regular occurrence of sexual reproduction contribute to the high gene and genotype diversity within populations and similarity between populations found in his study.

Primary sources of infection are infected seeds, plant debris and in a lesser extent alternative hosts such as grasses or other cereals on which *Septoria nodorum* can overwinter (Harrower, 1977). Coleoptiles are first infected by seedborn spores of *Septoria nodorum* (Shah et al., 1995) and pycnidia on the coleoptile may provide secondary inoculum for infection of the leaves. Inoculum on infected seeds stored at 5°C may survive for more than 10 years (Cunfer, 1991). Induced seed shrivelling didn't affect wheat emergence and resulted in plants that performed similarly to those produced from unshrivelled seed, but in conditions favourable for leaf and glume blotch development, epidemics could be initiated from infected seeds (Gilbert et al., 1995). Environmental conditions thus contribute more to later disease symptoms than the degree of seed infection (Cunfer and Johnson, 1981, Djurle and Yuen, 1991). On debris from previous wheat crops, *Septoria nodorum* is able to overwinter and to form new pycnidia. Spores are released from pycnidia and dispersed by splash (Faulkner and Colhoun, 1976). Primary sources of infection may also be Ascopores released from Pseudothecia, which play an active role in over-seasoning (Eyal et al., 1987).

The infection of *Septoria nodorum* begins with the penetration of a spore on a leaf. First no symptoms are visible and the disease is latent. First symptoms
are visible 10 to 20 days after infection, but the lesions are not yet sporulating. An infectious period is following, during which new spores can be released from pycnidia and disperse by water splash. These spores can then infect healthy parts of the leaves. Generally the spores are dispersed from the lower leaves to the upper ones and the ear. The infectious period is over, when the fungus no longer produces spores (Djurle and Yuen, 1991).

Conditions favourable for disease development are frequent precipitations and high humidity with temperatures between 18 and 25°C during the grain filling period (King et al., 1983). Moreover the growth stage of the host influences symptom development. Plants at the seedling stage are usually less susceptible than during stem elongation (Fried and Brönnimann, 1982, Hansen et al., 1994). The pollutant ozone seems to increase susceptibility to *Septoria nodorum* at tillering stage or after ear emergence but not during stem elongation. Symplastic leaf permeability probably play a role in ozone-induced leakage of nutrients from leaves, which could be a reason for improved growth of *Septoria nodorum* on and in its host (Tiedemann and Pfähler, 1994).

### 2.1.2 Symptoms and mode of infection

First symptoms are little brown spots, which spread out to irregularly lensshaped necrotic lesions, surrounded by chlorotic area. An accurate diagnosis is possible on the basis of the pycnidia, which are arranged in small groups. They are circular, yellowish to brownish and have an opening with first a pale, later a dark margin.

Through electronmicroscopic investigations more details are known about the infection structures of the pathogen in leaves of wheat (Zinkernagel et al., 1988, Karjalainen and Lounatmaa, 1986). Ten hours after inoculation, first penetration through lysis of the cuticle can be observed either by the hypha or the conidia, probably due to enzymatic activity. Lehtinen (1993) described a series of cell wall degrading enzymes that were secreted by *Septoria nodorum*.
grown in media containing wheat cell walls as the sole carbon source. Formation of appressoria is rare and so is the penetration through stomata. Direct penetration of epidermic cells as observed by Karjalainen and Lounatmaa (1986) is rare as well and occurs only in leaves that have been infected since a long time (Zinkernagel et al., 1988). Lysis of epidermic cells can be observed although the parasite didn’t yet penetrate the cell. This leads to the assumption, that again there are toxins involved. Chemically characterised toxins produced by _Septoria nodorum_ belong to the mellein (Devys et al., 1994) and septorin (Barbier et al., 1994) series. However their mode of action is not fully clear. A further intercellular spreading to deeper leaf cell layers can be observed. Instead to grow intensively the parasite starts to produce pycnidia. Therefore necrotic lesions stop to spread out after a certain time.

### 2.2 Disease control

There are several approaches to control leaf and glume blotch. Long term crop rotations (Eyal, 1981) have a potential to reduce inoculum. Ploughing in the infected stubble may also be a means to reduce inoculum potential (Harrower, 1974). However, when seed infection is high and weather conditions are favourable, epidemics can arise from this source of inoculum (Cunfer and Johnson, 1981). Fertilisers (nitrogen, potassium and phosphorus) can affect susceptibility directly or indirectly and an adequate fertilisation is therefore a further measure to disease control.

Sereological methods such as ELISA (Enzyme linked immunosorbent assay) may be interesting for seed or seedling testing in the field (Lagerberg et al., 1995). These methods allow to identify the presence of the pathogen and to quantify it before symptoms on the plants are visible. Such methods would be useful to predict a later infection pressure and therefore the need of fungicide applications.
However, in years with high infection pressure and high precipitation during the vegetation period, fungicide applications may be necessary. The active substance of the fungicides applied belongs to the group of Triazole (Propiconazole, Epoxiconazole, Metconazole, Cyproconazole). Although propiconazole used as fungicide against *Septoria nodorum* from 1985 - 1989 in the New York state, this didn't lead to populations of *Septoria nodorum* with decreased sensitivity to it, due to a low level of phenotypic variability in propiconazole sensitivity (Peever et al., 1994). This risk of adaptation of the pathogen to fungicides seems to be low, as Schmidt (1995) couldn't detect a continual building of resistance in the population in the field with the highest selection pressure in a long term field monitoring.

### 2.2.1 Resistance of wheat to *Septoria nodorum*

The cultivation of varieties showing a high level of partial resistance and breeding for such varieties have the highest potential to control disease and therefore to reduce costs and fungicide applications. This leads to a lower risk to pollute the environment. Among the wheat germplasm as well as among the related *Triticum* species, no immune reaction to SNB was identified until now. Nevertheless, some wheat cultivars and lines show a high level of partial resistance to SNB (Jeger et al., 1983, Tomerlin et al., 1984). Ma and Hughes (1993) found a high frequency of resistant genotypes in *T. monococcum*, *T. tauschii* and *T. timopheevii*, but not in *T. dicoccum* and *T. durum*. Another source of resistance may be the use of *Hordeum chilense* in wide crosses with wheat, named *tritordeum* (Rubiales et al, 1996). Some sibs of diploid and tetraploid *Aegilops* species showed as well a high level of partial resistance and could therefore be used as source of resistance (Frauenstein and Hammer, 1985, Ecker et al., 1990). Up to now it is known that the partial resistance of the ear is inherited independently from the partial resistance of the leaf (Fried and Meister, 1987, Bostwick et al, 1993) and that both are controlled polygenically and quantitatively (Wilkinson, 1990, Bostwick et al., 1993). The reaction of a number of monosomic lines to two strains of *Septoria*
nodosum showed, that resistance genes are located on different chromosomes (Rapilly et al., 1988). An exception to the polygenic nature of resistance are the monogenically controlled seedling resistances of Variety "Atlas 66" (Frecha, 1973) on chromosome 1B (Kleijer, 1977) and of T. timopheevii-derived durum lines on chromosome 3A (Ma and Hughes, 1995). Resistance to SNB has been determined at the seedling stage (Mullaney et al., 1982, Krupinsky et al., 1972, Krupinsky et al., 1977), at detached leaves (Ecker et al., 1989, Karjalainen, 1984) and at adult plants (Bostwick et al., 1993, Arseniuk et al., 1991, Loughman et al., 1994). Partial resistance has been determined measuring disease severity in terms of disease progress and calculating an area under disease progress curve (Bostwick et al., 1993) or at measuring different components of resistance such as infection efficiency, lesion size and latent period (Ecker et al., 1989). Detection of resistance at the seedling stage or at detached leaves has the advantage, that environmental influences or interactions with the growth stage of the host can be excluded, i.e. there are controlled conditions. However, resistance reactions observed at the seedling stage or at detached leaves were not always expressed at the adult plant stage under field conditions (Arseniuk et al., 1991, Trottet and Benacef, 1989, Nelson and Marshall, 1990). Moreover, resistance on the ear can not be detected by such methods. Screening of adult plants in the field on the other hand causes often problems to detect quantitative differences. Moreover interactions with disease development and the environment or the growth stage may cover the effective resistance characteristics of the investigated genotypes. However, artificial infections under field conditions proved to be more reliable than artificial inoculations under greenhouse conditions (Bruno and Nelson, 1990).

2.2.2 Breeding for resistance

Together with breeding for quality, breeding for resistance against fungal diseases is a major goal of the wheat breeding programs at the Swiss Federal Research Station for Agroecology and Agriculture (FAL) Zürich-Reckenholz. Each year, a large number of advanced winter wheat, spring wheat and spelt
lines are screened for resistance against the major diseases including SNB. Plots are infected artificially and individually according to their growth stage. Resistance on the leaves and the ears are recorded 5 to 8 times separately. In order to compare the resistance reactions over several years, area under the disease progress curves are calculated. Assessment over several years are necessary to provide valuable information about the effective resistance characteristic of a line. However, the screening for SNB resistance starts only in later generations. A selection of single plants or head rows in early generations would be too time consuming and inaccurate. Alternative methods to screen a large number of genotypes with limited need of space don't yet exist. However, in recent years biotechnological methods to detect resistance or to transfer resistance through gene transfer have been a field of research. The detection of single genes which are responsible for a vertical resistance is usually not of interest to breeders, because a single mutation of the pathogen could lead to a break down of the resistance. Detecting and combining several of such genes in a variety (pyramidisation) on the other hand is likely to express a durable resistance, because a pathogen has to overcome all resistance genes. At the FAL, molecular markers for several of such leaf rust resistance genes have been developed (Schachermayr et al., 1994), and an application of this method in the breeding program will soon be realised. For quantitative traits such as the resistance against Septoria nodorum, the development of molecular markers is more difficult. Molecular markers that detect quantitative traits are called QTL markers, and the development of such markers for septoria nodorum resistance at the FAL is in a final stage now.

2.3 In vitro selection

Another alternative method to select or detect resistance is the use of in vitro selection methods. In comparison with in vivo selection, which is commonly applied in plant breeding programs, in vitro selection methods have several advantages. It is possible to screen a large number of individuals within a short time under controlled conditions in the lab and, therefore help to advance
breeding progress. It is possible to exclude interactions with the environment, which might cover certain traits. Instead of whole plants, organs, small amounts of tissues or even single cells can be tested (Ahmed and Sagi, 1993). In vitro selection allows the specific use of genetic variability which is induced from the in vitro culture of plant cells or tissue. Genetic variability induced by cell or tissue culture conditions was described as somaclonal variation in the beginning of 1980 (Larkin and Scworoft, 1981). However, this somaclonal variation is not directed and mutants with positive traits have to be identified. Therefore, addition of toxic compounds to the culture media or the application of abiotic stress results in a defined selection pressure. Together with the in vitro selection for disease resistance, there are a number of other possible applications for in vitro selections such as selection for herbicide tolerance, selection for salt tolerance, selection for tolerance to metals, selection for tolerance to high or low temperatures and selection for tolerance to water stress (Haines, 1993). In addition to this, plant cell culture systems represent a potential renewable source of valuable medicinals, flavours, essences and colorants that cannot be produced by microbial cells or chemical synthesis (DiCosmo and Misawa, 1995). This report will focus on the in vitro selection for disease resistance. In vitro selection for resistance to a pathogen can be realised when in vitro cultures are exposed to toxins produced by the pathogen, synthetic toxin analogues, to a pathogen filtrate, to extracts of the pathogen or to the pathogen itself (Daub, 1986).

2.3.1 The use of pathogens as selecting agents

The use of the pathogen as the selective agent is a rare approach, particularly due to difficulties in growing or controlling the growth of the pathogen in culture. Methods for the coculture of protoplasts and viruses have been developed in order to get virus-free regenerates. For example, Toyoda et al. (1989) used tobacco axillary buds infected with tobacco mosaic virus to induce callus, and after 6 month subculture, healthy shoots were regenerated, from which 3% were highly resistant and 33% moderately resistant to tobacco.
mosaic virus. Pathogens other than viruses, under the right conditions, can be used as the selecting agent to select for disease resistance. By coculturing callus derived cells of celery with isolates of *Septoria apiicola*, Evenor et al. (1994) developed resistant cells. Plants regenerated from these cells showed different degrees of tolerance to *Septoria apiicola*. Selfing the most tolerant plants yielded tolerant progenies. The ranking of poplar clones for field resistance to *Septoria musiva* was similar to that derived by inoculation of cultured leaf disks with spores of this pathogen (Ostry et al., 1988). On the other hand, Sacristan (1982) was unable to select callus of *Brassica* species resistant to *Phoma lingam* and *Plasmodiophora brassicae* by inoculation cultures with spores of the pathogens.

2.3.2 The use of pathogen metabolites as selecting agents

2.3.2.1 Examples for crops others than wheat

A more common approach to select for disease resistance is the use of toxic metabolites produced by pathogens. In comparison with the use of the pathogen itself, this system has the advantage that cultured cells or tissues can be exposed easily and uniformly to toxic compounds by dispersing cells in toxin solutions or plating them on media containing pathogen metabolites that are toxic (Daub, 1986).

An assay based on electrolyte leakage from callus tissue of eggplant treated with culture filtrate of *Verticillium dahliae* was useful for screening genotypes for resistance to *Verticillium* wilt (Cristinzio et al., 1994). Loss of electrolytes was significantly lower for resistant genotypes than that for susceptible genotypes. Investigations about the possibility of *in vitro* selection for resistance to *Verticillium* wilt in *Medicago sativa* were carried out using a filtrate from mycelial cultures of *Verticillium albo-atrum* (Frame et al., 1991). Plants regenerated from filtrate-containing *Medicago sativa* cultures initiated from two genotypes had significantly lower average disease severity indices than plants regenerated
from control plants and the donor plants. Culture filtrates of *Fusarium oxysporum* f. sp. *medicaginis* were useful to select resistant calli of *Medicago sativa* (Arcioni et al., 1987). Regenerated plants have been evaluated for in vivo resistance to the pathogen and three out of eight plants were resistant to the fungus and a high correlation between resistance to culture filtrate and in vivo resistance was observed. Using filtrates of *Fusarium* spp. in embryogenic cell suspension culture of alfalfa, Binarova et al. (1990) found 12-20% more plants with increased resistance to pathogens than in the group of plants regenerated from a control cell line. *Fusarium* culture filtrates (*Fusarium oxysporum*) have been further used to select resistant plants in potato (Behnke, 1980). On the other hand, fungal culture filtrates of *Fusarium oxysporum* f. sp. *melonis* to tissue from susceptible and resistant genotypes of muskmelon didn’t differentiate susceptible and resistant genotypes (Megnegneau and Branchard, 1991). The use of *Drechslera teres* culture filtrates showed an agreement between toxin tolerance *in vitro* and resistance of barley against the pathogen (Hunold et al., 1992). Partially purified culture filtrates of *Colletotrichum kahawae*, the causal agent of coffee berry disease, had selective effects on calli derived from susceptible and resistant genotypes (Nenyange et al., 1995). Normal plants were regenerated through somatic embryogenesis of callus lines that survived the phytotoxin treatment. *In vitro* and in vivo testing of these plants against the partially purified culture filtrates showed that increased resistance to the toxin had been obtained. Soybeans resistant to *Septoria glycines* were selected from cultured cells using a hostspecific pathotoxic culture filtrate of *Septoria glycines* (Song et al., 1994). However, Lee et al. (1996) showed, that some regenerated lines resistant to *Septoria glycines* lost their resistance reaction in later generations. Maize callus was treated with partially purified toxin from *Helminthosporium maydis* race T (pathogen of southern corn leaf blight) and all plants regenerated were resistant (Gengenbach et al., 1975, Brettel et al., 1980). In rice, Ling et al. (1985) and Vidhyasekaran et al. (1990) selected calli resistant to the crude toxin of *Helminthosporium* spp., and obtained an increased resistance level to *H. oryzae* in the regenerated plants and their progenies. For obtaining oats
resistant to *Helminthosporium victoriae*, Rines and Luke (1985) used the pathotoxin victorin to select resistant calli, from which resistant plants were recovered. The resistance has been transmitted to the later generations. A host specific phytotoxin isolated from *Alternaria solani*, which causes early blight disease of tomato, was used for *in vitro* gametophyte selection. A high correlation was found between the resistance of the pollen to the toxin and the blighting of tomato plants (Darakov, 1995). A partially purified toxin from *Phytophthora infestans* was not useful as an *in vitro* selection agent, when potato protoplasts and microspores were grown *in vitro* in toxin containing media. No correlation was found between *in vitro* toxin resistance of the protoplasts or microspores and resistance of the plants (Möllers et al., 1992).

### 2.3.2.2 Examples for wheat

In wheat, a number of *in vitro* selection attempts were made using pathogen metabolites as selecting agent. Barely and wheat calli were screened for resistance to purified culture filtrates of *Helminthosporium sativum* P.K. and B. (Chawla and Wenzel, 1987). The selection resulted in 6% to 17% surviving calli, from which less sensitive barley and wheat plants were regenerated. However, this decreased sensitivity to the pathogen filtrates was not transmitted to the progenies. In the case of bacterial blight, caused by *Pseudomonas syringae* pv. *syringae*, syringomycin was applied *in vitro* for induction of resistance (Pauly et al., 1987). The inhibition of callus growth provided a means to select for resistance. Coleoptile tissue segments from 14 spring wheat cultivars were exposed to metabolites of *Fusarium graminearum* (Wang and Miller, 1988). A comparison of the susceptibility to these metabolites *in vitro* and the resistance to fusarium head blight indicated, that resistant cultivars could tolerate much higher concentrations of the metabolites than susceptible cultivars. Ahmed et al. (1991) selected calli of spring and winter wheat for *Fusarium* resistance *in vitro*. They used the double-layer technique, where media were first inoculated with mycelia of *Fusarium graminearum* and *F. culmorum*. The fungal cells were killed by autoclaving and
the agar medium containing the thermostable toxic metabolites was overlayed with callus growing medium. Surviving calli on these media were regenerated to plants. After screening for resistance, by artificial infections in the greenhouse, 3% of the regenerated F2 plants have been found to be more resistant than the original cultivars. Ahmed et al. (1996) compared the double layer technique with the culture filtrate technique. Culture filtrates of *Fusarium graminearum* and *F. culmorum* were added to callus growing medium. Screening the regenerants proved, that the culture filtrate technique gave better results than the double-layer procedure. Fadel and Wenzel, (1993) used F1 microspore populations of wheat to screen for tolerance to *Fusarium in vitro*. Microspores from donor hybrids which were produced from very susceptible cultivars were killed by lower toxin concentrations than microspores from hybrids of less susceptible parents. It was possible to enrich the fraction of regenerating microspores which contain the gene complex containing increased resistance to *Fusarium* by the use of a pathotoxin. Bruins et al. (1993) used four types of wheat plant material, seedlings, coleoptile segments, anther-derived callus and anther-derived embryos in a *in vitro* screening. As selecting agent they used deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3-ADON) which are produced by *Fusarium graminearum* and *F. culmorum*. Growth of all types of plant material was reduced by DON, but growth analysis of 40 callus clones did not show any correlation with the known resistance level in the field.

*In vitro* selection for resistance against *Septoria nodorum* in wheat is an approach that have been followed by only two authors until now. Leath and Papke (1989) used different fractions of the pathogen extracts for selection of resistant calli derived from immature embryos. These calli were regenerated but it was not reported, if the regenerated plants did show a higher level of resistance. Keller et al. (1994) used extracts from wheat grains inoculated with *Septoria nodorum* and screened wheat embryos of nine wheat lines with known field resistance. They found that the extracts had a selective action and there was a good agreement between field resistance on the ear and the sensitivity
of the wheat embryos against the selective extracts. On the other hand, pure mellein, which is a characterised toxin produced by *Septoria nodorum*, had no selective action and was toxic for all lines tested.

### 2.3.3 Problems encountered when dealing with *in vitro* selection

Crude extracts usually contain partially characterised toxins but also other metabolites may be present. On the other hand, the use of host-specific toxins can result in plants possessing qualitative, usually monogenic disease resistance (Ahmed and Sagi, 1993). Plants with such major resistance genes generally show complete resistance to a specific pathogen. However, this resistance might be overcome within a few years by mutations of the pathogen populations. Therefore, the use of non specific toxins, which usually are present in crude extracts, can be valuable as selective agents, because selection of plants exhibiting only partial resistance are of great interest for breeding for durable resistance.

In many *in vitro* selection experiments, reactions at the cellular level were not correlated with the reactions on the regenerated plants (Möllers et al, 1992). Moreover the resistance at the plant level due to *in vitro* selection was not always transmitted to the progenies (Chawla and Wenzel, 1987). For the selection and screening, different plant parts can be used, such as leaf discs, root pieces, immature inflorescences, florets, caryopses, mature or immature embryos, seeds or callus cultures of various somatic parts, as well as single cells in cell suspensions, protoplasts and pollen grains. The physiological state, age and form of the organs used in the *in vitro* selection strongly affect the efficiency of selection (Ahmed and Sagi, 1993).

Despite many problems encountered with the *in vitro* selection for disease resistance, the usefulness of this approach have been proved in many studies (Van den Bulk, 1991). One of the best ways to test whether resistance can be
expressed at the *in vitro* level is to compare the response of tissue cultures of resistant and susceptible varieties to pathogen inoculation (Daub, 1986).

2.4 Description and objectives of the present study

Since the results of the *in vitro* screening of immature wheat embryos for Septoria resistance of Keller et al. (1994) were very promising, we tried to optimise this test and investigate its usefulness for selection in early generations. From eight winter wheat varieties and lines whose resistance reactions against *Septoria nodorum* on ear and leaf was evaluated in the field over three years, the culture of zygotic embryos was induced. The sensitivity of embryos to extracts of *Septoria nodorum* was compared to the known field resistance reaction of the tested varieties and lines, which differed significantly in this trait. Encouraged by the high correlation of the two traits, the *in vitro* screening was applied to detect resistant germplasm out of segregating populations in early generations. The eight winter wheat lines were therefore crossed in order to get a broad spectrum of resistance reactions within and between the resulting 16 populations. In the generation F2, individual genotypes out of each population were selected and the culture of zygotic embryos (F3 embryos) was induced, and an extract of *Septoria nodorum* was used as selective agent. From each F2 individuum, one ear was left to produce seeds which were sown as a F3 head row in the field. The resistance reaction on ear and leaf of the head row was recorded in a field trial under artificial infection. This provided reliable data to compare the sensitivity of wheat embryos (F3) *in vitro* to extracts containing toxins from *Septoria nodorum* to the resistance reaction of the F3 plants in the field (Comparison of F3 embryos with F3 plants which both went back to the same F2 plant). F1 embryos from all 16 crosses were screened *in vitro* as described for the F3 embryos, and compared to the resistance reaction in the field.

From the single F3 head rows, 15 plants were selected and seeds from a single ear were sown again as a head row, giving 15 F4 head rows which went
back to a single F2. The resistance reaction of ten head rows was evaluated again in a field trial under artificial infection. This provided reliable data to compare the resistance reaction of F4 plants to an *in vitro* screening which was carried out on F5 seeds derived from the F4 head row. These seeds were cultivated *in vitro* on media containing the extract of *Septoria nodorum* and the sensitivity to the extract was compared to the resistance reaction in the field (Comparison of the resistance reaction of F4 plants in the field with the sensitivity of F5 seeds of the same plants to extracts of *Septoria nodorum in vitro*).

The data set obtained by the field assessment for a large number of wheat genotypes derived from 16 crosses in 3 generations allowed to study resistance mechanisms responsible for the inheritance of resistance against *Septoria nodorum* on the leaves and the ears. It was possible to determine the genetic variation between as well as within segregating populations and therefore to estimate the probability to detect new sources of resistance. On the basis of a wide range of crosses, possible mechanisms responsible for the resistance reaction to *Septoria nodorum* and crossing strategies in wheat breeding programs are discussed in a **first part**.

In a **second part**, the results from the *in vitro* screening using an extract of *Septoria nodorum* will be presented. The reactions to the pathogen extract observed *in vitro* was compared with the resistance reactions in the field for the 16 populations. Reasons for the inconsistent correlations found in different generations will be discussed as well as problems that arise from such a screening procedure. There will be an outlook about further investigations that have to be done to solve these problems and there will be a discussion about a possible and promising implementation of the *in vitro* methods into wheat breeding programs.
3 Part 1: Inheritance of resistance to leaf and glume blotch caused by *Septoria nodorum* Berk. in winter wheat

### 3.1 Abstract

16 crosses between eight winter wheat cultivars were screened for resistance to *Septoria nodorum* leaf and glume blotch in the generations F1, F3 and F4 using artificial inoculation in the field. The F1 of most crosses showed dominance for susceptibility on ear and leaf. Effects of general combining ability were of similar magnitude as effects for specific combining ability. On the basis of the phenotypic difference of the parents, no prediction was possible about the amount and the direction of genetic variance in the segregating populations. The variation observed in this study within and among the segregating populations suggests a quantitative inheritance pattern influencing the expression of the two traits. The components of variance between F2 families within a population were as high as (for *Septoria nodorum* blotch on the ear) or higher (for *Septoria nodorum* blotch on the leaf) than those between populations. Therefore, a strong selection within a few populations may be as effective to find new resistant genotypes as selection in a large number of populations. In almost all crosses, progenies were found that were more resistant than the better parent. Thus transgression breeding may be a tool to breed for higher levels of resistance to *Septoria nodorum* blotch. Highly resistant genotypes were found even in combination with two susceptible parents. The genetic source for septoria resistance is probably broader than it is generally assumed and could be used to improve *Septoria nodorum* resistance by combination breeding followed by a strong selection in large populations.
3.2 Introduction

Leaf and glume blotch of wheat play a major role as damaging diseases. They are caused by the fungus *Leptosphaeria nodorum* E. Müller (=*Phaeosphaeria nodorum* (E. Müller) Hedjaroude), anamorph = *Septoria nodorum* (Berk.) Berk. in Berk. & Broome (=*Stagonospora nodorum* (Berk.) Castellani & Germano). To simplify matters, in the frame of this report the disease will be called septrioa nodorum blotch (SNB). With increasing latitude and increasing precipitation during the grain filling period, incidence and severity of the pathogen increase as well (Leath et al., 1993). Therefore, SNB is a widespread disease in Switzerland, where humid conditions during the vegetation period often occur. Attacking ears and leaves of wheat it can reduce yield up to 50% and may also reduce milling and baking quality (Eyal et al., 1987, Rosielle and Brown, 1980).

Breeding for resistance is the most ecological and economical approach to disease control and is a prerequisite for a sustainable agriculture. A number of lines and cultivars of wheat show a minimal yield reduction after a severe infection with SNB, i.e. they are tolerant (Brönnimann, 1975, Tvaruzek and Klem, 1994). Even though this is a desirable trait, in practice farmers apply fungicides according to visible disease symptoms. This underlines the necessity of resistance breeding for less symptoms and reduced pathogen development, as an effective way to reduce fungicide application and costs.

Among the wheat germplasm as well as among the related *Triticum* species, no immune reaction to SNB was identified until now. Nevertheless, some wheat cultivars and lines showed a high level of partial resistance to SNB (Jeger et al., 1983, Tomerlin et al., 1984). Ma and Hughes (1993) found a high frequency of resistant genotypes in *T. monococcum*, *T. tauschii* and *T. timopheevii*, but not in *T. dicoccum* and *T. durum*. Partial resistance of the ear is not or only moderately correlated with the partial resistance of the leaf (Fried and Meister, 1987, Bostwick et al, 1993) and both are controlled polygenically (Wilkinson et
al., 1990, Bostwick et al., 1993). Exceptions to this are the monogenically controlled seedling resistances of variety "Atlas 66" (Frecha, 1973) on chromosome 1B (Kleijer, 1977) and of T. timopheevii-derived durum lines on chromosome 3A (Ma and Hughes, 1995). Resistance to SNB has been determined at the seedling stage (Mulaney et al., 1982, Krupinsky et al., 1972, Krupinsky et al., 1977), at detached leaves (Ecker et al., 1989) and at adult plants (Bostwick et al., 1993, Arseniuk et al., 1991, Loughman et al., 1994, Fried and Meister, 1987). However, resistance reactions observed at the seedling stage or at detached leaves were not always expressed at the adult plant stage under field conditions (Arseniuk et al., 1991, Trottet and Benacef, 1989, Nelson and Marshall, 1990). Moreover a screening at the seedling stage gives no indication about resistance reactions on the ear. Therefore, a field screening is essential to determine the level of resistance on leaves and ears and to study inheritance mechanisms on both organs. Information about the genetic basis responsible for the expression of partial resistance in the field is of great importance to wheat breeders. This knowledge might lead to specific germplasm combinations showing a higher level of resistance.

Specific crosses between eight winter wheat lines showing different resistance reactions on the ear and the leaves were made. These lines originate from the actual Swiss or European breeding material. The resistance level of both leaf and ear was evaluated after artificial infection in the field at the adult plant stage for the parental lines, the F1, F3 and F4 generation of each cross. Moreover, the influence of morphological traits to the expression of partial resistance was estimated over all populations and for each population separately.

Based on the study of Keller et al. (1994), who were able to distinguish resistant and susceptible wheat cultivars by means of in vitro screening, we wanted to test if this method is useful for resistance screening in early segregating populations. Such a selection would be very useful to wheat breeders. Therefore a main objective of this study was to obtain reliable
phenotypic data of the level of resistance against SNB in early segregating generations in the field. Results of the comparison of the field data with the *in vitro* screening for resistance against *Septoria nodorum* are discussed in Part 2.

The data set obtained by this field assessment over a large number of wheat genotypes allowed to study resistance mechanisms responsible for the inheritance of resistance against *Septoria nodorum* on the leaves and the ears. It was possible to determine the genetic variation between as well as within segregating populations and therefore to estimate the probability to detect new sources of resistance. On the basis of 16 different populations, crossing strategies in wheat breeding programs are discussed.

### 3.3 Materials and methods

#### 3.3.1 Parental lines and crosses

Eight winter wheat lines and varieties with contrasting resistance against SNB on ear or leaf, respectively, were selected as parents (Table 1). They were chosen on the basis of the results of the official variety tests of the Swiss Federal Research Station for Agroecology and Agriculture (FAL) Zürich-Reckenholz.

The parental lines were divided into two groups (group ear and group leaf), with two lines showing a high level of resistance and two lines showing a low level of resistance on the corresponding organ in these groups. Within each group, a diallel cross without reciprocal crosses was carried out (Table 2). Four additional crosses were made with entries that at the same time had either a low or a high resistance level on both organs (i.e. SN- x SN+, SN- x Zenith, SN- x Greif, Zenith x Greif).
Table 1: Chosen parental lines with their pedigrees, origins and resistance reactions.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Pedigree</th>
<th>Origin</th>
<th>Resistance to ear blotch</th>
<th>Resistance to leaf blotch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arina</td>
<td>Moisson / (Can3842 / Heine VII)</td>
<td>CH</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Iena</td>
<td>Champlein / Courtôt</td>
<td>F</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fomo</td>
<td>72837 / Kormoran</td>
<td>CH</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>SN-</td>
<td>Major / Hoeser52</td>
<td>CH</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SN+</td>
<td>Arminda / Roazon</td>
<td>CH</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Boval</td>
<td>(Caribo / Hoeser48) / (Can3842 / Tano)</td>
<td>CH</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Zenith</td>
<td>Can3842 / Heine VII</td>
<td>CH</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Greif</td>
<td>(Maris Hobbit / Carimulti) / Carimulti</td>
<td>D</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ High level of resistance
- Low level of resistance
0 Intermediate

Table 2: Crossing scheme

<table>
<thead>
<tr>
<th>Group ear (females)</th>
<th>Males</th>
<th>Arina</th>
<th>Iena</th>
<th>Fomo</th>
<th>SN-</th>
<th>SN+</th>
<th>Boval</th>
<th>Iena</th>
<th>Zenith</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iena</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fomo</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SN-</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SN+</td>
<td></td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boval</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Iena</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zenith</td>
<td></td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Greif</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

3.3.2 Field trials

In order to detect quantitative differences in the resistance level to septoria nodorum leaf and glume blotch, progenies of the 16 crosses in the generations F1, F3 and F4 as well as the parental lines were screened in field trials in Zürich-Reckenholz, Switzerland, in 1995 and 1996, respectively, using artificial inoculation as described below.
3.3.2.1 Field trial F1 crosses in 1996

F1 progenies of the 16 crosses were sown in 1995 as a 1-row plot together with the parental lines. In order to avoid interactions with other diseases (powdery mildew, scab, leaf- and stripe rust), the trial was sprayed with 1 l/ha Tiptor (Maag, Switzerland) six weeks before inoculation with Septoria nodorum. Oat slugworms were controlled by spraying 1.5 l/ha Zolone (Maag, Switzerland) at growth stage 50 to 55 (Zadoks et al., 1974). The same protection measures were applied in the field trials with the F3 and F4 populations.

3.3.2.2 Field trial F3 populations in 1995

18 to 28 individual plants out of each F2 population were selected randomly (352 in total). From these selected F2 plants, seeds from one ear were sown as single F3 head rows (1.2 m long) in autumn 1994 together with the parental lines (1 row per parent with four replications). The remaining ears of each F2 plant were used for an in vitro screening (Part 2).

3.3.2.3 Field trial F4 populations in 1996

From each F3 line (head row), 15 ears were harvested and threshed separately. The F4 seeds were sown in autumn 1995 as 5-row plots, where each row represented a single head row. The design was a rectangular lattice with three replications, including the 352 5-row plots of the progenies of the 16 crosses and the eight parents as six replicated entries per replication (400 plots per replication).

3.3.2.4 Inoculation procedure

Inoculum for the artificial inoculations was prepared as described by Fried (1989). For the inoculation, a mixture of a broad spectrum of isolates collected in Switzerland was used. The trials were inoculated four times with a spore
suspension of 1 million viable spores per ml (400 l/ha, 20 ml per row). The first inoculation was applied at booting stage (stage 47 to 49). The second to fourth inoculation was applied to the heads. Taking into account the differences in earliness of the different genotypes, the second inoculation was carried out, when the first third of the plants was in stage 59 to 61, the third when the second third was in this stage (one week after the first inoculation) and the last when the remaining plants reached this stage (two weeks after the first inoculation). In the field trial with the F4, only two of the three replications were inoculated.

3.3.2.5 Scoring of data

The following traits were recorded on single row basis in the field trials F1, F3 and F4 at FAL, Zürich in 1995 and 1996: days till ear emergence (DEA) and flowering (DFL) from January first, plant height in cm (HCM), percentage of necrosis on each of the top three leaf layers (5 to 7 times within four weeks, between growth stage 50 and 80), percentage of necrosis on the ear (4 to 7 times within four weeks, between growth stage 60 and 85). In order to obtain a normal distribution of the percentage of diseased area on ear and leaf, each recording was transformed with log(x+1). For each line, transformed scores of all scoring dates were added up. The resulting value was used to determine quantitative differences in the resistance reaction and it will be used further on as SNEA for the severity index on the ear and SNLF for the severity index on the leaf. The colour (wax layer) of the ears (CEA) and the leaves (CLF), which might influence the resistance reaction, were recorded as well. A score of 1 represented a genotype with a strong wax layer, a score of 9 a genotype with no wax layer.

3.3.3 Determination of thousand kernel weight reduction

The reduction of the thousand kernel weigh (TKW) of the eight parental lines due to the artificial inoculation was determined. From the first inoculated
replication and the inoculated replication in the field trial F4, two of the six replicated entries of the parental lines were harvested. For each parental line harvested, the TKW was determined by weighting 3 times 200 kernels. The differences in TKW between the two treatments (inoculated and not inoculated) was taken as reduction due to the artificial inoculation.

3.3.4 Statistical analysis

Data analysis included simple correlation analysis between the severity indices (SNEA, SNLF), plant height (HCM), wax layer (CEA, CLF) and earliness (DEA, DFL). SNEA and SNLF of the field trial F3 in 1995 were correlated to SNEA and SNLF of the field trial F4 in 1996. Heterosis for SNEA and SNLF in the F1 was determined by comparing the F1 value to the mid parent value. On the basis of the crossing scheme, general combining ability (GCA) and specific combining ability (SCA) were calculated for SNEA and SNLF within each group. Analysis of lattice design as well as analysis of variance (ANOVA) with complete block design were carried out for the field trial F4 on the basis of the mean value of the 5 row plots (n = 400 per replication) using PLABSTAT (H.F. Utz, Institute of Plant Breeding, University of Hohenheim, Germany). To test the genetic variance between and within populations, the following factors were included in the ANOVA model: Blocks (B, the two replications), populations (P) and F2 derived families within populations (F:P). The following mixed model (Model I) with random factors was used:

B + P + F:P + BF:P

ANOVA was further calculated for each population separately, using the following model (Model II):

B + F + BF
In order to quantify the variance between the F4 headrows derived from different F3 plants but the same F2 plant, a hierarchical model (Model III) was applied by adding the factor headrows (R) nested within family and block:

\[ B + P + F:P + BP + BF:P + R:BFP \]

The data set for this calculation consisted therefore on 4000 values (5 rows x 400 families x 2 replications). Due to the lack of true replications, the variance components caused by the segregation between the F4 headrows derived from different F3 plants but the same F2 plant \( (\sigma^2_{R:BFP}) \) could not be tested for significance.

Heritability \( (h^2) \) of the 400 entries based on the lattice design was calculated according to the following formula.

\[
h^2 = \frac{\sigma_F^2}{\sigma^2_F + \sigma^2_{\text{ems}} / 2}
\]

\( \sigma^2_F \) = variance of different F2 families over 16 populations

\( \sigma^2_{\text{ems}} \) = effective error of the mean square

Broad sense heritability based on the mean values of 5-row-plots (Model II) was calculated for each population according to Hallauer and Miranda (1981).

\[
h^2 = \frac{\sigma_F^2}{\sigma^2_F + \sigma^2_B / 2}
\]
3.4 Results

3.4.1 Parental lines in field trial F3 and field trial F4

The lowest level of disease severity on the ear showed Arina with only 20% necrosis at the last recording date in field trial F3 in 1995, whereas Boval was severely attacked and 90% of the ear tissue was necrotic due to the artificial inoculation with SNB. In the field trial F4 in 1996, Arina was again the most resistant and Boval the most susceptible parent on the ear (15% and 75% necrosis at the last recording date, respectively). The most resistant parent on the leaf was SN+ in both trials (15% and 35% necrosis on the flag leaf at the last recording date in 1995 and 1996, respectively), and the most susceptible parent on the leaf was SN- in both trials (70% and 90% necrosis on the flag leaf at the last recording date in 1995 and 1996, respectively). Table 3 shows the values for SNEA and SNLF of the eight parental lines in the order of increasing SNEA values of the field trial F4 in 1996. Plant height showed a variation between 95 cm of the short strayed varieties Lena and Greif and 125 cm of the tallest variety Arina (Table 3). Arina, Greif and Zenith, the three most resistant varieties on the ear, had a strong wax layer on the ear (score 2 for colour of ear). Boval with the highest SNEA value of the eight parents had the earliest heading date. Reduction of thousand kernel weight (TKW, Table 3) due to the artificial infection with SNB was not significantly correlated with SNEA ($r = 0.30$) or SNLF ($r = 0.13$).
Table 3: Parental lines and their resistance reaction on ear and leaf to artificial inoculation with \textit{Septoria nodorum} from the F4 field trial in 1996. Reductions of thousand kernel weight in \% (TKW) due to the artificial inoculation are given as well as days till ear emergence from january first (DEA), plant height in cm (HCM), colour of leaf (CLF, 1 strong wax layer, 9 no wax layer) and colour of ear (CEA, 1 strong wax layer, 9 no wax layer).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>SNEA</th>
<th>SNLF</th>
<th>TKW decrease</th>
<th>DEA</th>
<th>HCM</th>
<th>CLF</th>
<th>CEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARINA</td>
<td>3.06</td>
<td>23.51</td>
<td>6%</td>
<td>155</td>
<td>125</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>GREIF</td>
<td>3.14</td>
<td>18.34</td>
<td>19%</td>
<td>157</td>
<td>95</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>ZENITH</td>
<td>3.38</td>
<td>22.3</td>
<td>9%</td>
<td>159</td>
<td>120</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>IENA</td>
<td>3.54</td>
<td>21.94</td>
<td>15%</td>
<td>155</td>
<td>95</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>SN+</td>
<td>3.64</td>
<td>20.14</td>
<td>0%</td>
<td>158</td>
<td>110</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>SN-</td>
<td>4.36</td>
<td>25.23</td>
<td>21%</td>
<td>160</td>
<td>100</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>FORNO</td>
<td>4.78</td>
<td>22.31</td>
<td>14%</td>
<td>155</td>
<td>102</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>BOVAL</td>
<td>5.59</td>
<td>20.53</td>
<td>14%</td>
<td>151</td>
<td>110</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>3.94</td>
<td>21.79</td>
<td>12%</td>
<td>156.25</td>
<td>107.13</td>
<td>5.38</td>
<td>4.25</td>
</tr>
</tbody>
</table>

\(s\): Standard deviation

\(SNEA\): \textit{Septoria nodorum} severity index on ear (log\(x+1\) of the sum of the estimated diseased area from 4 recording dates)

\(SNLF\): \textit{Septoria nodorum} severity index on leaf (log\(x+1\) of the sum of the estimated diseased area from 4 recording dates)

Disease severity indices of the parental lines on ear and leaf in field trial in 1995 based on 1-row plots were highly correlated to the disease severities in field trial in 1996 based on 5 row plots (\(r = 0.90\) for SNEA and \(r = 0.92\) for SNLF, \(P<0.01\)). Correlations between disease indices described by Keller et al. (1994) and SNEA and SNLF in field trial in 1996 were highly correlated as well (0.95 for SNEA and 0.88 for SNLF, \(P<0.01\)). A very low variation between the replicated entries of the parental lines in field trial in 1995 as well as in field trial in 1996 was observed (data not shown).

SNEA was not significantly correlated with SNLF for the eight parental lines (\(r = 0.34\) in field trial in 1996, \(P>0.05\)). Correlations of SNEA and SNLF with other traits (data from field trial in 1996) are shown in Table 4. Although moderately high (e.g. \(r = 0.60\) between colour of ear and SNEA), these correlations are not significant due to the small number of parental lines.
Table 4: Correlations of different traits to SNAE and SNLF for the eight parental lines in field trial F4 in 1996

<table>
<thead>
<tr>
<th>Trait 1</th>
<th>Trait 2</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to ear emergence</td>
<td>SNAE</td>
<td>-0.44 n.s</td>
</tr>
<tr>
<td>Days to flowering</td>
<td>SNAE</td>
<td>-0.34 n.s</td>
</tr>
<tr>
<td>Plant height</td>
<td>SNAE</td>
<td>-0.07 n.s</td>
</tr>
<tr>
<td>Colour of leaves</td>
<td>SNAE</td>
<td>0.51 n.s</td>
</tr>
<tr>
<td>Colour of ears</td>
<td>SNAE</td>
<td>0.60 n.s</td>
</tr>
<tr>
<td>Colour of leaves</td>
<td>SNLF</td>
<td>0.28 n.s</td>
</tr>
<tr>
<td>Colour of ears</td>
<td>SNLF</td>
<td>0.24 n.s</td>
</tr>
<tr>
<td>Days to ear emergence</td>
<td>SNLF</td>
<td>0.21 n.s</td>
</tr>
<tr>
<td>Days to flowering</td>
<td>SNLF</td>
<td>0.26 n.s</td>
</tr>
<tr>
<td>Plant height</td>
<td>SNLF</td>
<td>-0.02 n.s</td>
</tr>
</tbody>
</table>

n.s. P>0.05

3.4.2 Field trial F1

The two most resistant F1 progenies against ear blotch resulted from the cross Arina x SN- and Arina x Iena (Table 5). Arina x SN- showed a slightly negative heterosis for severity index on the ear (SNEA), i.e. the F1 was more resistant than the mean value of the two parents. On the other hand, Arina x Iena showed a high positive heterosis, i.e. the F1 had about a 15% higher disease severity than the mean of the two parents (Table 5). The three most susceptible F1 progenies for ear blotch were all derived from crosses with the susceptible parent Boval, with a slightly negative heterosis for SN+ x Boval and positive heterosis for Boval x Zenith and Boval x Iena (Table 5). SN+ x Iena and SN+ x Boval produced the most resistant progenies on the leaf with negative heterosis (more resistant than the mean of the two parents) both (Table 5). F1 progenies from the crosses Boval x Zenith and Forno x SN- showed the highest SNLF values, with a high positive heterosis of 13% (more susceptible than the mean of the two parents) for Boval x Zenith. On average the heterosis were positive for both, SNEA and SNLF, with higher values for SNEA. This indicates dominance for susceptibility to SNB, especially on the ear. In the case of SN+ x Iena, a high positive heterosis for SNEA was recorded, together with a high negative heterosis for SNLF. There were more such cases, were heterosis
went in different directions for SNEA and SNLF (for example lena x Forno or SN- x Greif, Table 5). General combining ability (GCA) and specific combining ability (SCA) for SNEA and SNLF are summarised in Table 6. Arina, the parent with the highest level of resistance on the ear had the highest negative GCA for SNEA (-0.24, reduction of necrosis), whereas Forno, with a high level of susceptibility on the ear was the parent with the highest positive GCA for SNEA (+0.23, increasing necrosis). Lena, with a high level of resistance on the ear showed only a slightly negative GCA. The highest positive SCA for SNEA (-0.20, reduction of necrosis) showed Arina in the cross with SN- and the highest negative SCA (+0.12, increasing necrosis) showed Forno in the cross with SN-. For SNLF, GCA ranged from -1.21 for SN+ (reducing necrosis) to +1.86 for Zenith (increasing necrosis). The highest positive SCA was found in the cross SN+ x Boval (-0.95), whereas a very high negative SCA was found in the cross SN+ x Zenith (+1.97).

Table 5: SNEA and SNLF of the 16 F1 and % heterosis in relation to mid parent value in field trial F1 in 1996 (positive values: more susceptible than the mean value of the two parents, negative values: more resistant than the mean value of the two parents)

<table>
<thead>
<tr>
<th>F1</th>
<th>SNEA</th>
<th>% heterosis</th>
<th>SNLF</th>
<th>% heterosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arina x SN-</td>
<td>3.52</td>
<td>-4.55</td>
<td>23.89</td>
<td>3.52</td>
</tr>
<tr>
<td>Arina x lena</td>
<td>3.53</td>
<td>14.86</td>
<td>22.79</td>
<td>5.00</td>
</tr>
<tr>
<td>Zenith x Greif</td>
<td>3.59</td>
<td>11.94</td>
<td>21.98</td>
<td>7.34</td>
</tr>
<tr>
<td>SN- x Greif</td>
<td>3.61</td>
<td>-4.10</td>
<td>22.26</td>
<td>4.98</td>
</tr>
<tr>
<td>SN+ x Zenith</td>
<td>3.67</td>
<td>3.39</td>
<td>22.52</td>
<td>5.59</td>
</tr>
<tr>
<td>SN- x Zenith</td>
<td>3.85</td>
<td>-1.28</td>
<td>23.84</td>
<td>2.21</td>
</tr>
<tr>
<td>lena x Zenith</td>
<td>3.92</td>
<td>19.83</td>
<td>23.20</td>
<td>5.67</td>
</tr>
<tr>
<td>Arina x Fomo</td>
<td>4.02</td>
<td>9.51</td>
<td>22.72</td>
<td>4.16</td>
</tr>
<tr>
<td>lena x SN-</td>
<td>4.04</td>
<td>5.44</td>
<td>23.12</td>
<td>1.92</td>
</tr>
<tr>
<td>SN- x SN+</td>
<td>4.07</td>
<td>-0.76</td>
<td>22.64</td>
<td>2.63</td>
</tr>
<tr>
<td>SN+ x lena</td>
<td>4.11</td>
<td>17.99</td>
<td>18.94</td>
<td>-8.44</td>
</tr>
<tr>
<td>Iena x Fomo</td>
<td>4.14</td>
<td>8.46</td>
<td>20.29</td>
<td>-5.28</td>
</tr>
<tr>
<td>Fomo x SN-</td>
<td>4.31</td>
<td>-2.82</td>
<td>24.04</td>
<td>5.45</td>
</tr>
<tr>
<td>SN+ x Boval</td>
<td>4.51</td>
<td>-4.98</td>
<td>19.05</td>
<td>-4.82</td>
</tr>
<tr>
<td>Boval x Zenith</td>
<td>4.84</td>
<td>6.64</td>
<td>23.99</td>
<td>12.94</td>
</tr>
<tr>
<td>Boval x lena</td>
<td>4.84</td>
<td>8.17</td>
<td>20.59</td>
<td>-0.04</td>
</tr>
<tr>
<td>Mean</td>
<td>4.04</td>
<td>5.48</td>
<td>22.24</td>
<td>2.69</td>
</tr>
</tbody>
</table>
Table 6: General combining ability (GCA) and specific combining ability (SCA) for the diallel F1 crosses within group ear and group leaf in field trial F1 in 1996

<table>
<thead>
<tr>
<th>Group ear</th>
<th>GCA ear</th>
<th>Group leaf</th>
<th>GCA leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arina</td>
<td>-0.24</td>
<td>SN+</td>
<td>-1.21</td>
</tr>
<tr>
<td>Iena</td>
<td>-0.03</td>
<td>Boval</td>
<td>-0.17</td>
</tr>
<tr>
<td>Forno</td>
<td>0.23</td>
<td>Iena</td>
<td>-0.47</td>
</tr>
<tr>
<td>SN-</td>
<td>0.03</td>
<td>Zenith</td>
<td>1.86</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SCA ear</th>
<th>SCA leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iena x SN-</td>
<td>0.11</td>
</tr>
<tr>
<td>Iena x Forno</td>
<td>0.01</td>
</tr>
<tr>
<td>Arina x Iena</td>
<td>-0.14</td>
</tr>
<tr>
<td>Arina x SN-</td>
<td>-0.20</td>
</tr>
<tr>
<td>Arina x Forno</td>
<td>0.10</td>
</tr>
<tr>
<td>Forno x SN-</td>
<td>0.12</td>
</tr>
</tbody>
</table>

3.4.3 Field trial F3 in 1995

At the last recording date on single row basis in the field trial 1995 the F3 lines showed a large variation for resistance to SNB between and within the 16 populations. Data are not shown in detail, results are summarised as follows.

The percentage of necrosis on the ear ranged from 15% to 90% at the last recording date, the percentage of necrosis on the flag leaf ranged from 2% to 40% among the 352 lines. The severity indices SNEA and SNLF were correlated with 0.51 (P<0.01). Plant height showed also a large variation among progenies and ranged from 60 cm to 145 cm. This trait was significantly negatively correlated with SNEA (r = -0.37, P<0.01) and SNLF (r = -0.22, P<0.01). Days to ear emergence from January first were significantly negatively correlated with SNEA (r = -0.33, P<0.01). Colour of ear and SNEA were correlated with r = 0.12 (n.s. P>0.05).
3.4.4 Field trial F4 in 1996

At the last recording date on single row basis in the field trial 1996 the F4 lines ranged from 5% to 90% for percentage of necrosis on the ear and showed again a large variation between and within the 16 populations. Percentage of necrosis on the flag leaf ranged from 8% to 100% at the last recording date. In the F4 generation SNEA and SNLF were correlated with $r = 0.31$ ($P>0.01$) across the 16 populations. Plant height in cm (HCM) ranged from 70 cm to 145 cm. This trait was significantly negatively correlated with SNEA ($r = -0.32$, $P<0.01$) and SNLF ($r = -0.25$, $P<0.01$). The strongest negative correlation between HCM and SNEA was observed for F4 lines of the cross SN- x Greif ($r = -0.75$, $P<0.01$). Days to ear emergence were significantly negatively correlated with SNEA ($r = -0.36$, $P<0.01$). Colour of ear and SNEA were correlated with $r = 0.35$ ($P<0.01$). Correlations between F3 plants in 1995 on 1-row-plot basis and F4 plants in 1996 on 5-row-plot mean basis were 0.71 ($P<0.01$) for SNEA and 0.80 for SNLF ($P<0.01$) over all populations. These correlations were significant ($P<0.01$) for each of the 16 populations except for Arina x Forno for SNEA ($r = 0.42$, $P<0.05$) and SN+ x Boval for SNLF ($r = 0.36$, $P>0.05$).

Heritability based on the 400 entries of the lattice design was 0.89 ($P<0.01$) for days to ear emergence, 0.91 ($P<0.01$) for plant height, 0.82 ($P<0.01$) for necrosis on leaf and 0.84 ($P<0.01$) for necrosis on ear. Efficiency of the lattice design (compared to complete block design) was 102.7 for SNLF, 108.7 for SNEA, 100.9 for plant height and 106.4 for days to ear emergence, indicating little effects of incomplete blocks. This allowed the calculation of ANOVA as randomised complete block design.

Significant differences ($P<0.01$) between the 16 populations ($\sigma^2_\nu$) as well as between the F2 families within a population ($\sigma^2_{F,P}$, Model I) were found for SNEA, SNLF, DEA and HCM. For SNEA, components of variance between populations compared to variance within populations had the same magnitude.
whereas for SNLF the component of variance between families was higher (0.88) than between populations (0.56). The variance components between the F4 headrows derived from different F3 plants but the same F2 plant ($\sigma^2_{RFPB}$, Model III) were two (SNLF) to four (SNEA) times lower than between the F2 families ($\sigma^2_{FP}$).

Values for broad sense heritability for SNEA and SNLF of each population are given in Table 7, together with the genetic variance components of SNEA and SNLF. Genetic variances and consequently heritabilities are very low in some cases (e.g. Arina x SN- for SNAE, SN+ x lena for SNLF) and very high in other cases (e.g. SN- x Greif for SNEA, SN- x SN+ for SNLF). Values for heritability differ strongly for SNEA and SNLF for SN+ Zenith, Arina x SN-, Forno x SN- and Boval x Zenith, which is in accordance with the different directions of heterosis for SNEA and SNLF in Table 5.

Table 7: Genetic components of variance and heritability for Septoria nodorum on ear and leaf (SNEA and SNLF)

<table>
<thead>
<tr>
<th></th>
<th>$\sigma^2_g$ SNEA</th>
<th>$h^2$ SNEA</th>
<th>$\sigma^2_g$ SNLF</th>
<th>$h^2$ SNLF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zenith x Greif</td>
<td>-0.002</td>
<td>x</td>
<td>0.570</td>
<td>0.50*</td>
</tr>
<tr>
<td>lena x Zenith</td>
<td>0.065</td>
<td>0.55*</td>
<td>-0.228</td>
<td>x</td>
</tr>
<tr>
<td>lena x SN-</td>
<td>0.047</td>
<td>0.62**</td>
<td>1.425</td>
<td>0.81**</td>
</tr>
<tr>
<td>lena x Forno</td>
<td>0.043</td>
<td>0.70**</td>
<td>0.509</td>
<td>0.62**</td>
</tr>
<tr>
<td>SN+ x Zenith</td>
<td>0.075</td>
<td>0.66**</td>
<td>0.893</td>
<td>0.75**</td>
</tr>
<tr>
<td>SN+ x lena</td>
<td>0.060</td>
<td>0.57*</td>
<td>0.071</td>
<td>0.15ns</td>
</tr>
<tr>
<td>SN+ x Boval</td>
<td>0.085</td>
<td>0.53*</td>
<td>0.336</td>
<td>0.52*</td>
</tr>
<tr>
<td>Arina x lena</td>
<td>0.081</td>
<td>0.75**</td>
<td>1.630</td>
<td>0.88**</td>
</tr>
<tr>
<td>Arina x SN-</td>
<td>0.006</td>
<td>0.16ns</td>
<td>0.591</td>
<td>0.74**</td>
</tr>
<tr>
<td>Arina x Forno</td>
<td>0.038</td>
<td>0.59*</td>
<td>0.095</td>
<td>0.22ns</td>
</tr>
<tr>
<td>SN- x Zenith</td>
<td>0.084</td>
<td>0.72**</td>
<td>0.455</td>
<td>0.64**</td>
</tr>
<tr>
<td>SN- x Greif</td>
<td>0.139</td>
<td>0.83**</td>
<td>1.293</td>
<td>0.74**</td>
</tr>
<tr>
<td>SN- x SN+</td>
<td>0.121</td>
<td>0.79**</td>
<td>2.813</td>
<td>0.95**</td>
</tr>
<tr>
<td>Forno x SN-</td>
<td>0.025</td>
<td>0.30ns</td>
<td>1.369</td>
<td>0.82**</td>
</tr>
<tr>
<td>Boval x Zenith</td>
<td>0.042</td>
<td>0.39ns</td>
<td>0.877</td>
<td>0.76**</td>
</tr>
<tr>
<td>Boval x lena</td>
<td>0.144</td>
<td>0.77**</td>
<td>0.541</td>
<td>0.56*</td>
</tr>
</tbody>
</table>

x not determined due to negative genetic components of variance

$\sigma^2_g$ genotypic variance component, $h^2$ broad sense heritability

** P<0.01, * P<0.01, n.s. P>0.05
Figure 1 shows the mean for SNEA of the parents, the F1 values, the F4 means and the range in F4 for all 16 populations in the field trial 1996. Figure 2 shows the same for SNLF. The F4 mean deviated in most crosses from the midparent value indicating non additive gene actions. For SNEA, the F4 generation of nine crosses showed higher level of resistance than expected from the parental mean, while the F4 of two crosses were more susceptible. The population mean was in some crosses even outside the range of the parents (e.g. Forno x SN-). For SNLF, the F4 mean of seven crosses showed higher level of resistance than expected from the parental mean, whereas six crosses were more susceptible. Deviations of F4 means from midparent values for SNEA were not associated with deviations observed for SNLF. Coefficients of correlation between the genetic variance components and the phenotypic differences between the parental lines of each cross were low for SNEA ($r = 0.27$, n.s.) and somewhat higher for SNLF ($r = 0.53$, $P< 0.05$). This discrepancy is illustrated in the Figures 3 to 5 showing three different segregation patterns for SNEA, with Arina x Lena (resistant x resistant) and Arina x SN- (resistant x susceptible) with similar distributions but different ranges of the parents, and Forno x SN- (susceptible x susceptible) with a narrow range of the parents but a larger distribution. Similar distribution patterns for SNLF are shown in Figures 6 to 8. This indicates that the amount of variability in segregating populations can not be predicted from the parental difference for severity indices SNEA or SNLF.
Figure 1: Mean values of parents, F1 value and F4 mean for *Septoria nodorum* on the ear (SNEA) for all 16 populations, including the range in the F4.

<table>
<thead>
<tr>
<th></th>
<th>Pm = mother</th>
<th>Pf = father</th>
<th>F1 = F1 value</th>
<th>F4 = F4 mean</th>
<th>Range in F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zenith x Greif</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
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<tr>
<td>Ilena x Zenith</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
</tr>
<tr>
<td>Ilena x SN-</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
</tr>
<tr>
<td>Ilena x Forno</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
</tr>
<tr>
<td>SN+ x Zenith</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
</tr>
<tr>
<td>SN+ x Ilena</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
</tr>
<tr>
<td>SN+ x Boval</td>
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<td>▲</td>
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<td>▲</td>
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<tr>
<td>Arina x Ilena</td>
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<tr>
<td>Arina x SN-</td>
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<tr>
<td>Arina x Forno</td>
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<tr>
<td>SN- x Zenith</td>
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<tr>
<td>SN- x Greif</td>
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<td>▲</td>
</tr>
<tr>
<td>SN- x SN+</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
</tr>
<tr>
<td>Forno x SN-</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
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<tr>
<td>Boval x Zenith</td>
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<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
</tr>
<tr>
<td>Boval x Ilena</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
</tr>
</tbody>
</table>
Figure 2: Mean values of parents, F1 value and F4 mean for Septoria nodorum on the leaf (SNLF) for all 16 populations, including the range in the F4

- ▲ Pm = mother
- ▲ Pf = father
- ▲ F1 = F1 value
- △ F4 = F4 mean
- → Range in F4
Figure 3: Frequency distribution of SNEA classes. The means of the parents, the F1 values and the F4 means for population Anna x lena are indicated by arrows.

Figure 4: Frequency distribution of SNEA classes. The means of the parents, the F1 values and the F4 means for population Anna x SN- are indicated by arrows.

Figure 5: Frequency distribution of SNEA classes. The means of the parents, the F1 values and the F4 means for population Foro x SN- are indicated by arrows.
Figure 6: Frequency distribution of SNLF classes. The means of the parents, the F1 values and the F4 means for population lena x Zenith are indicated by arrows.

Figure 7: Frequency distribution of SNLF classes. The means of the parents, the F1 values and the F4 means for population SN-x Zenith are indicated by arrows.

Figure 8: Frequency distribution of SNLF classes. The means of the parents, the F1 values and the F4 means for population SN+ x Boval are indicated by arrows.
3.5 Discussion

High correlations were found between the field trial in 1995 and 1996 for SNEA and SNLF of the parental lines ($r = 0.95$ and $0.88$, respectively, $P<0.01$) as well as to the disease indices calculated for the same cultivars by Keller et al. (1994). This indicates, that the resistance level can be determined in an accurate way on a 1-row and 5-row plot basis after artificial infection and multiple ratings. Multiple recordings are essential for assessment of disease development (Walther, 1990) and for reducing errors in estimating percentage of diseased area. Therefore, the resistance level determined for the F3 and for F4 generation of the 16 crosses provides a reliable basis to compare the resistance under field conditions to the susceptibility to Septoria nodorum metabolites in vitro (Part 2).

Heritability estimates for SNEA and SNLF were higher, lower or comparable to those reported by Brönnimann (1975) and Rosielle and Brown (1980), who screened only a limited number of genotypes. This points out the necessity to screen a broad genetic spectrum to study inheritance mechanisms. Heritability estimates differed in some cases strongly between SNEA and SNLF (SN+ x Zenith, Arina x SN-, Forno x SN- and Boval x Zenith, Table 7), which is in accordance with the different directions of heterosis for SNEA and SNLF (Table 5). The different magnitudes and the differences for SNEA and SNLF of the heritability estimates found in this study reflect the complex inheritance mechanisms of resistance to SNB.

Effects of heterosis in the F1 were in most cases negative for SNEA and SNLF, i.e. the F1 was more susceptible than the midparent value, indicating dominance for susceptibility as found by Brönnimann (1975) and Fried and Meister (1987). Heterosis was more important for SNEA than for SNLF. Production of wheat hybrid seeds would therefore not be a tool for improving septoria resistance. However, heterosis effects diminue in later generations due to the lower effect of variance of dominance. It is therefore possible, that
breeders discard populations in the F2 that seem to be susceptible. But as shown before, even in such populations (for example Forno x SN- for SNEA or Lena x Forno for SNLF, Figure 1 and 2) highly resistant genotypes occurred in later generations. However, large populations and strong selection pressure are necessary to identify such genotypes (Fried and Meister, 1987).

The results obtained by calculating general combining ability (GCA) indicate, that on the basis of the resistance reaction of a parent, its performance in hybrid combinations can not be predicted. Although Arina, the parent with the highest level of resistance on the ear (Table 3) had the highest negative GCA for SNEA (-0.24, reduction of necrosis), Lena, with almost the same level of resistance, showed only a small GCA. Specific combining ability (SCA) and GCA were of the same magnitude, indicating that in the F1 dominance and other nonadditive gene effects are of the same importance as additive effects. This was also reported by Wilkinson et al. (1990).

Coefficients of correlation over all populations between F3 lines, on 1-row-plot basis in 1995, and F4 lines, on 5-row-plot mean basis in 1996, were quite high ($r = 0.71$ for SNEA and $r = 0.80$ for SNLF). Scott et al. (1982) found much weaker correlations for disease ratings of the same genotypes in different environments, which he explained by genotype x environment interactions. These interactions were minimal in our trials, which is indicated by the low variance of the replicated entries with the parental lines in both years. This was probably due to the high infection pressure and conditions favourable for disease development in both years. It can therefore be concluded, that the efficiency of selection on 1-row-plot basis is quite high, if artificial infection is applied. In some populations, however, correlations between F3 and F4 lines were low. For example for SNLF and the cross SN+ x Boval ($r = 0.34$). In this case, the F1 shows heterosis for resistance (see Fig. 2), whereas the F4 mean had a lower level of resistance than both parents. One explanation could be the different level of heterozygosity (25% in F3 versus 12.5% in F4 for a single locus). Ecker et al. (1989) suggested, that at least 3 to 5 genes are involved in
the inheritance of resistance. The probability for homozygosity for 5 loci is 0.51 in F4 and 0.23 in F3. If dominance effects play a major role, as indicated by the considerable heterosis observed in some crosses, the level of heterozygosity may explain low correlations in some crosses. Moreover, the F3 and F4 generations were assessed in different years. It could be possible that the importance of genotype - environment interaction varies between the different populations. Another reason may be the assessment of 1-row versus 5-row plots. In a single row of F3 lines there may be short and tall plants due to remanent heterozygosity. Tall plants may show a higher level of resistance than the short ones. Assessing mainly tall plants that overgrow the short ones may cause bias in the estimation. In the 5-row plots of F4 lines, where each row is a rather homogenous head row, this effect can be eliminated.

The association from resistance to tall, late maturing plants found in this study for the progenies of the 16 crosses (significant negative correlations for both traits) was in coherence with earlier studies about septoria resistance in wheat (Scott et al, 1982). However, for the 8 parental lines, no association was found between SNB and plant height. This was due to the two short strawed cultivars lena and Greif (95 cm), which both showed a high level of partial resistance on the ears. This leads to the suggestion, that linkage rather than pleiotropy explains the association of resistance with plant height, because in these two cultivars there must be genes involved that have no effect on plant height (or ear emergence) but on effective partial resistance (Scott et al, 1982). On the other hand, pleiotropic effects of plant height may result from the fact that tall cultivars can escape the pathogen or have microclimatic conditions, that prevent the pathogen to develop (King et al., 1983).

The low coefficients of correlation between SNEA and SNLF found in this study lead to the conclusion that these traits are inherited independently. In the F1, the correlation is even negative. An explanation of this is shown in Figure 1 and 2. In the crosses SN+ x lena and SN+ x Boval, the F1 shows positive heterosis for SNLF, but negative heterosis for SNEA, thus representing a
contrary reaction. Therefore different genes and/or gene actions seem to be involved in the expression of glume blotch and leaf blotch resistance. This is in agreement with the findings of Fried and Meister (1987), that resistance on the heads is inherited independently from the resistance on the leaves and that both are inherited quantitatively.

The reduction of thousand kernel weight due to the artificial infection showed no significant correlation between SNEA and SNLF for the parental cultivars (Table 8). The reduction for Greif, who showed a high level of partial resistance in terms of necrosis on ear and leaf due to the infection with SNB was higher than for Boval, who showed a high susceptibility on the ear. Greif may have a great input of energy in the defence reaction, whereas Boval may be able to compensate losses of photosynthetic active tissue (Rooney and Hoad, 1989). The complex of partial resistance and tolerance has been described for many wheat cultivars (Brönnimann, 1975, Tvaruzek and Klem, 1994). On this point of view, it is obvious that breeders are confronted with a large number of problems to get an ideal cultivar that combines partial resistance on ear and leaf together with tolerance. Moreover the breeder selects for earliness and short straw and these traits are negatively correlated with resistance.

The variation observed in this study within and among the segregating populations suggest a quantitative inheritance pattern influencing the expression of the two traits. Continuous variations in SNB response have been reported in numerous studies (Loughman et al., 1994, Mullaney et al., 1982, Ecker et al., 1988, Bostwick et al., 1993). The components of variance between F2 families within a population were as high as (SNEA) or higher (SNLF) than those between populations. Therefore, a strong selection within a few populations may be as effective to find new resistant genotypes as selection in a large number of populations. The lower variation within F3 families (between F4 headrows) was due to the higher level of homozygosity in this generation. Assuming an additive-dominance model (Mather and Jinks, 1977), the components of variance within F4 populations tested in 1996 consist of the
variance between F2 families (=V_A + 1/16 V_D), (V_A: additive genetic variance of F2 generation, V_D: non-additive genetic (dominance) variance of F2 generation), the variance between F3 progenies within F2 families (= 1/2 V_A + 1/8 V_D) and the variance between individuals within F3 progenies (1/4 V_A + 1/4 V_D + V_E). (V_E: environmental component of within family variance), which is in total 7/4 V_A + 7/16 V_D + V_E. Therefore, dominance effects should turn out to be most important in the F1, and moving from F1 to F4, the dominance variance V_D diminishes from 1V_D (F1) to 7/16 V_D (F4). The additive-dominance model is adequate to explain variance in septoria resistance, although some variation in the form of interaction between nonallelic genes may occur. The graphical presentation of the parental lines, the F1 values and the F4 means in Figure 1 and 2 prove this theory. In most cases, the F1 is more susceptible than the F4 (mean) and is closer to the susceptible parent, indicating that dominance for susceptibility as found by Brönnimann (1975) and Fried and Meister (1987) is involved in the inheritance. Ecker et al. (1989) suggested that there are more allelic combinations which increase susceptibility than allelic combinations which increase resistance. Nevertheless, for SNLF (Figure 2) in three crosses (lena x Forno, SN+ x lena and SN+ x Boval) and for SNEA in one cross (Forno x SN-) dominance for resistance exists. This indicates, that dominant genes for resistance against leaf blotch as found by Frecha (1973) at the seedling stage might also be expressed at the adult stage. On the other hand, such genes may be modified by other genes or certain gene combinations as described by Kleijer et al. (1977) and Laubscher et al. (1966). Looking at the means for SNEA of Forno x SN- or SN+ x Zenith or for SNLF of SN- x Zenith or lena x Forno one can observe positive deviation (towards more resistant). But also negative deviation (towards more susceptible) occurs, for example for SNEA of lena x Zenith or for SNLF of Arina x Forno, indicating the importance of gene x gene interactions.

When breeders want to improve a trait, they usually cross two parents that already express this trait in a high level. On the basis of the results of this study, this strategy may also be promising for breeding for septoria resistance,
as it is shown in Figure 1 for Zenith x Greif and SNEA or in Figure 2 for SN+ x Boval and SNLF. But there are also some peculiarities which might be of interest to breeders. The correlation between the genetic component of variance in the F4 and the phenotypic difference between the parents is low. This leads to the conclusion, that on the basis of the genetic value of the parents, the genetic variability induced by a specific cross can not be predicted. This is demonstrated in Fig. 1. In the cross Forno x SN-, the parents have almost the same value, but the range in F4 is large, and even though the parents represent two susceptible genotypes, a selection for a high level of resistance in the F4 would be possible. This is also true for the cross SN- x SN+. In the cross SN- x Greif on the other hand, there is a large range too, but a selection for higher levels of resistance than the more resistant parent seems not promising for SNEA but for SNLF. In some crosses, for example Lena x SN- or Arina x Forno, the range of F4 lines correspond more or less to the phenotypic difference of the parents.

In almost all crosses some progenies were found that were more resistant than the better parent (range in F4, Figure 1 and 2). The progenies with the highest level on resistance for SNEA were found in the crosses Arina x Lena, Zenith x Greif and Boval x Lena and for SNLF in the crosses SN- x Greif and SN+ Lena. This indicates that Arina and Lena, both with a high level of partial resistance on the ear, carry different resistance genes and that also parental lines with low level of resistance (Zenith and Boval for SNEA and SN- and Lena for SNLF) contribute positive alleles increasing resistance. Thus transgression breeding can be a tool to breed for higher levels of resistance to SNB.
4 Part 2: *In vitro* screening for resistance against *Septoria nodorum* blotch in wheat

4.1 Abstract

This study was carried out with the aim to develop an *in vitro* test for the identification of genotypes resistant to septoria nodorum blotch. The basis for this project was a previous study in which a crude extract of *Septoria nodorum* was used as a selective agent (Keller et al., 1994). It was possible to distinguish resistant and susceptible cultivars in an *in vitro* test with zygotic embryos. In our project we wanted to test whether this *in vitro* test can also be used to detect resistant and susceptible genotypes in early segregating populations. Specific crosses between eight winter wheat lines showing contrasting resistance reaction for septoria nodorum blotch on leaves and ears were made. The resistance level of both leaf and ear was evaluated after artificial inoculation in the field for the parental lines, the F1 progenies as well as for segregating F3 and F4 populations. In addition, this plant material was tested *in vitro* using similar methods described by Keller et al (1994), i.e. culturing immature zygotic embryos and mature seeds on selective media. A good agreement between *in vitro* screening and field resistance on the ear was found for the parental lines, the F1 and F4 generation but not for the F3 generation. This leads to the conclusion that the *in vitro* screening might be integrated into wheat breeding programs. Populations showing a high susceptibility to the pathogen metabolites *in vitro* could be discarded. Another promising implementation for wheat breeding would be the screening of advanced breeding material or candidate partners in a crossing program for resistance on the ear. However, the *in vitro* screening is not precise enough to select single plants in early segregating populations.
4.2 Introduction

Septoria nodorum blotch (SNB) is a fungal disease of wheat (*Triticum aestivum* L.) causing leaf and glume blotch. It is a serious pathogen in many wheat growing areas throughout the world and may reduce yields up to 50% (Eyal et al., 1987). The causal agent is *Leptosphaeria nodorum* E. Müller (=*Phaeosphaeria nodorum* (E. Müller) Hedjaroude), anamorph = *Septoria nodorum* (Berk.) Berk. in Berk. & Broome (= *Stagonospora nodorum* (Berk.) Castellani & Germano).

The most ecological and economical approach to control this disease is the cultivation of varieties with a high level of resistance. In the available wheat gene pool, no complete resistance has been discovered until now, but genetic variation for partial resistance is present (Jeger et al., 1983, Tomerlin et al., 1984). Despite a considerable effort for selection towards septoria nodorum blotch resistance, the breeding progress using traditional methods is rather slow. This is mainly due to the complex inheritance of the resistance, which is controlled polygenically (Cunfer et al., 1988). Moreover, resistance on the ear is inherited independently from the resistance on the leaf (Fried and Meister, 1987, Bostwick et al., 1993). In addition to this, the assessment of disease development in the field, as it is usually done in the traditional wheat breeding programs, is often inaccurate due to environmental or physiological influences and it is very time consuming.

Phytotoxins produced by pathogenic fungi are the primary determinants in pathogenesis for many host pathogen interactions (Yoder, 1980). They induce typical disease symptoms in the absence of the pathogen. This is also true for the interaction between *Septoria nodorum* and wheat. The chemically characterised toxins belong to the mellein (Devys et al., 1994) and septorin (Barbier et al., 1994) class. Enzymes that can degrade cell walls are secreted, when *Septoria nodorum* is grown on media containing wheat cell walls as the
sole carbon source (Lehtinen, 1993). A chemically not characterised toxin was shown to cause necrotic symptoms (Kent and Strobel, 1976).

Alternative approaches to conventional breeding are based on marker assisted breeding or plant tissue and cell culture (Pauls, 1995). Using toxic metabolites produced by pathogens as selective agents in vitro, disease resistant plants have been obtained in various host-pathogen systems. (Daub, 1986, Van den Bulk, 1991, Ahmed and Sagi, 1993). In previous experiments, synthetic mellein, a metabolite produced by *Septoria nodorum*, showed no selective action to zygotic wheat embryos cultured in vitro (Keller et al., 1994). On the other hand, a crude extract of *Septoria nodorum* containing a mixture of toxins from the pathogen showed a clear selective action in vitro to wheat embryos originating from wheat varieties with different levels of partial resistance on the ear (Keller et al., 1994).

The aim of this study was to determine, whether this method can be applied to differentiate resistance levels of breeding material in early generations. Therefore, a large number of zygotic embryos in the F₁ and the segregating F₃ generations and F5 seeds from the segregating F4 generation originating from 16 crosses were screened in vitro. Crude extracts from *Septoria nodorum* containing metabolites of the pathogen were used as selective agents. The different sensitivity to these toxins observed in vitro was compared to the resistance reaction of the corresponding genotypes in the field after artificial infection (Part 1).

A good agreement between in vitro screening and field resistance on the ear was found for the parental lines, the F1 and F5 generation but not for the F3 generation. Possible explanations and implementations of the in vitro methods for wheat breeding are discussed.
4.3 Materials and methods

4.3.1 Plant material

The same eight winter wheat cultivars and lines that were used in the inheritance study of Part 1 were selected as parents. SN+ and Greif showed high level of resistance on the ear and the leaf, while Arina and Iena were resistant against SNB on the ear only and Boval against SNB on the leaf only. To test the in vitro selection in segregating populations, 16 crosses were made (Table 8) among the eight parental lines. The crosses were chosen in that way that all combinations of high and low level of resistance for SNB on the ear and the leaf occurred (Part 1). The F1 and F3 isolated immature zygotic embryos as well as F5 kernels of each population were used for in vitro screening.

Table 8: 16 specific crosses between eight parental cultivars and lines and number of randomly selected individual F2 plants per population

<table>
<thead>
<tr>
<th>Population No.</th>
<th>Female parent</th>
<th>Male parent</th>
<th>No. of F2 progenies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zenith</td>
<td>Greif</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>Iena</td>
<td>Zenith</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>Iena</td>
<td>SN-</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>Iena</td>
<td>Forno</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>SN+</td>
<td>Zenith</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>SN+</td>
<td>Iena</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>SN+</td>
<td>Boval</td>
<td>23</td>
</tr>
<tr>
<td>8</td>
<td>Arina</td>
<td>Iena</td>
<td>21</td>
</tr>
<tr>
<td>9</td>
<td>Arina</td>
<td>SN-</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>Arina</td>
<td>Forno</td>
<td>24</td>
</tr>
<tr>
<td>11</td>
<td>SN-</td>
<td>Zenith</td>
<td>24</td>
</tr>
<tr>
<td>12</td>
<td>SN-</td>
<td>Greif</td>
<td>23</td>
</tr>
<tr>
<td>13</td>
<td>SN-</td>
<td>SN+</td>
<td>28</td>
</tr>
<tr>
<td>14</td>
<td>Forno</td>
<td>SN-</td>
<td>22</td>
</tr>
<tr>
<td>15</td>
<td>Boval</td>
<td>Zenith</td>
<td>20</td>
</tr>
<tr>
<td>16</td>
<td>Boval</td>
<td>Iena</td>
<td>23</td>
</tr>
</tbody>
</table>
4.3.2 Preparation of *Septoria nodorum* extract

Autoclaved wheat kernels from the cultivar Arina were inoculated with a mixture of a broad spectrum of 140 isolates collected in Switzerland each year. Together with the same amount of autoclaved water in 250 ml Erlenmayer flasks, the mixture was multiplied first for one week at room temperature and then for four months at 4°C. The content was air dried and then milled, according to the description of Fried (1989). The resulting powder was well mixed. Crude extracts of *Septoria nodorum* were produced using a soxhlet apparatus (300 ml) and ethyl acetate as extracting agent (Sachse, 1992). After 15 h extraction, ethyl acetate was evaporated under vacuum at 50°C in a rotovapor and remaining traces of ethyl acetate were removed by a stream of nitrogen. 5 ml of water and 0.05% Tween 20 were added to the solution and put on a shaker overnight. Extracts were obtained by sterile-filtering the solution through a syringe. Extract solutions from ten extraction cycles were put together to one batch, giving about 50 ml of crude extract. One batch was prepared for the *in vitro* selection experiment of 1994 (batch 1). A second batch was prepared for the *in vitro* selection experiments in 1996 (batch 2). The second batch was produced according to the first batch with one exception: The soxhlet apparatus had a volume of 5000 ml, and the batch consisted therefore only on two extracts (150 ml).

4.3.3 *In vitro* test

4.3.3.1 Embryo culture

For the *in vitro* screening ears were harvested in the field or greenhouse at growth stage 83 to 85. Caryopses of the collected ears were surface sterilised for 3 min. in 95% ethanol. Immature zygotic embryos, about 2 mm long, were isolated under sterile conditions in a flow bench and transferred to *in vitro* culture. Embryo culture was induced on Murashige and Skooge (MS) medium (Murashige and Skooge, 1962) with 30 g/l sucrose, 2 mg/l 2,4-D, 8 g/l Difco
Agar and 2g/l Phytagel. Embryos in this stage show in culture direct shoot and root formation. Selective media were prepared by adding crude extract from *Septoria nodorum* (batch 1) to the MS medium (concentrations see below) at about 50°C and it will be called MSN medium. 7ml of medium was poured into 6 mm petri dishes at about 50°C. When the medium was solid, 12 embryos per petri dish were placed and grown at 26°C with a light intensity of 60µEm⁻²s⁻¹ for 16h per day. Assessment of germination was made after 7 to 10 days.

The concentration for the best selectivity of the extract in the MS medium was evaluated by testing a concentration range of 0, 1:100 to 1:1400 (v:v) of extract (batch 1) in medium. The variety Forno, with low level of resistance to SNB on the ear, and the variety Arina, with high level of resistance of the ear, were used as standards. For each concentration and each genotype, 100 zygotic embryos were cultured. In each experiment, 200 embryos (100 on MS medium, 100 MSN medium) of the 8 parental lines were cultured in the same way with two replications as reference. Relative germination rates on selective medium compared to the control were calculated and transformed with log (x + 1), giving the *in vitro* index (VI).

4.3.3.2 Culture of mature seeds

Whole kernels containing mature embryos were cultured *in vitro* in a germination dish (20cm x 13cm) containing 3 filter papers of the same size and 15 ml H₂O. This treatment was used as control medium. Adding crude extract (batch 2) to the water at concentrations 0 and 1:100 to 1:100, the optimal concentration for selectivity was determined screening again the variety Arina, with a high level of resistance on the ear, and Forno, with high susceptibility on the ear. No surface sterilisation was necessary prior to culture. Seeds were picked up with a vacuum cleaner connected to a metallic plate of the size of the germination dishes. This plate had 100 apertures in a regular arrangement. With this system regularly used for seed certification, 100 seeds were put onto the germination dishes in a short time. Germination was induced at 20°C and
normal day light. 100 seeds of each of the eight parents were placed on water containing dishes, and 100 seeds on dishes containing crude extract at the optimal concentration (This treatment will be called selective medium). Then, 200 seeds (100 on control medium, 100 on selective medium) were cultured. Seeds of each parental line were cultured in the same way with two replications as reference and the in vitro indices were calculated according to the embryo culture.

4.3.4 In vitro screening experiments

For a better comprehension for the experimental design, Figure 9 shows a scheme how the different generations were tested in vitro and in the field.

4.3.4.1 In vitro screening experiment 1, F1

200 F1 embryos from each of the 16 crosses were harvested from plants that were grown and crossed in the green house. 100 embryos were cultured on MS medium and 100 embryos on MSN medium and the in vitro index (VI) was calculated.

4.3.4.2 In vitro screening experiment 2, F3

In autumn 1993, F2 seeds of all 16 crosses were sown in 6-row-plots of 1.2m x 3.5m. In spring 1994, 18 to 28 individual plants out of each cross were selected randomly (Table 8) and digged up at growth stage 15 (Zadoks et al., 1974). They were planted into soil at 20 x 30cm space for each plant and labelled (352 in total). All ears except one of each individual plant were collected at growth stage 83 to 85. The remaining ear was left to produce seeds for field testing. 30 to 50 embryos of the 352 selected plants from the 16 populations were cultured on MS medium without extract as control as described above, and 30 to 50 on MSN medium containing the crude extract at the optimal concentration. In vitro indices were calculated as described above. All together,
in this *in vitro* selection experiment, about 30'000 F₃ embryos were cultured in the two treatments.

4.3.4.3 *In vitro* screening experiment, F₄ - F₅

In the meantime, each individual F₂ plant used for experiment 1 was propagated to five F₄ lines (5-row plot, Figure 9). From plots showing homogeneous resistance reactions between the five rows derived from different F₃ plants but the same F₂ plant, one line was harvested. From plots showing different resistance reactions between the 5 rows, the most susceptible and the most resistant row was harvested (550 in total). Then, 200 F₅ seeds from of the selected F₄ rows (100 on control medium, 100 on selective medium) were cultured *in vitro*. *In vitro* indices were calculated as described above.

4.3.5 Resistance screening in the field

In each of the field trials described below, the parental lines were included as reference. The trials were inoculated four times with a spore suspension (same isolates that were used for the production of crude extracts (Part 1)) of 1 million viable spores per ml (400 l/ha, 20 ml per row). Plant protection, artificial inoculation and scoring of resistance are described in Part 1.

Data of estimated percent diseased area on ear and leaf were transformed with the log (x+1), because they showed multiplicative characteristics. Scores of each recording were finally added up. This value was used to determine differences in the resistance reaction and will be used further on as SNEA for the resistance level on the ear and SNLF for the resistance level on the leaf.

4.3.5.1 Resistance screening in the field, F₁

Field trial 1 was carried out in order to compare the *in vitro* response on selective media of F₁ embryos to the resistance level of F₁ plants in the field
(Figure 9). This trial was sown as 3 row plots (16 plots) with the F1 plants in the middle row, and the female and male parental lines, in the left and right row, respectively.

4.3.5.2 Resistance screening in the field, field trial 2

Field trial 2 was carried out in order to compare the *in vitro* response on selective media of F3 embryos derived from the same F2 plant as the F3 plants screened for resistance in the field (F3 embryos vs. F3 plants, Figure 9). The parental lines were grown as 1-row plots with four replications.

4.3.5.3 Resistance screening in the field, field trial 3

15 plants per F3 head row of field trial 2 were harvested. Each ear was threshed separately. The seeds (F4) from one F3 head row were sown as 5-row plots, where each row represented a single head row (progenie of a single F3 plant). The design was a rectangular lattice with three replications, including the 352 5-row plots of the 16 crosses and the eight parents as six replicated entries per replication (400 plots per replication).

4.3.6 Comparison of the *in vitro* methods with the resistance reaction in the field

*In vitro* indices (VI) of the embryo culture and the seed culture were correlated to the recorded resistance levels (SNEA and SNLF) in the field. *In vitro* indices of the parental lines were used to determine the effect of different *Septoria nodorum* extracts of batch 1 and batch 2 and to compare results of embryo culture with those of seed culture.
4.4 Results

4.4.1 Concentration range

By testing a concentration range of the crude extract of batch 1 in medium a clear selective action to wheat embryos was found for the two varieties Arina and Forno showing different resistance reactions on the ear in the field. The number of germinating embryos per 100 cultured embryos is shown in Figure 10 for each concentration of MSN medium and the MS medium. A selective action was observed between the concentrations 1:900 and 1:600. Higher concentrations were toxic for both genotypes, whereas lower concentrations did not reduce the germination rate of embryos. Within the selective range, the germination rate of the susceptible variety Forno was more reduced than that of the resistant variety Arina. For the further experiments, a concentration of 1:750 was chosen for batch 1. Similar results were obtained by testing a concentration range but cultivating mature seeds instead of embryos and using batch 2. The concentration for the best selectivity was in this case 1:350 (v:v).
Germination of embryos on media containing different concentrations of crude extract. Reaction of the two standard varieties Arina and Forno

Figure 10: Number of germinating embryos per 100 cultured embryos at different concentrations of crude extract of *Septoria nodorum* (batch 1) in medium. Reactions of the variety Arina (high level of resistance on the ear) and the variety Forno (low level of resistance on the ear)

4.4.2 *In vitro* screening of the parental lines

The *in vitro* screening of zygotic embryos using batch 1 differentiated the eight parents. Forno showed the most sensitive reaction to the extract *in vitro* and had an *in vitro* index (VI) of 0.03 whereas Arina showed the highest tolerance with a VI of 0.24. The number of germinating embryos per 100 cultivated embryos on MS medium and on MSN medium are given in Table 9. Almost the same results were obtained by cultivating mature seeds of the eight parental lines on control medium and on selective medium using batch 2. While on the control all varieties showed a germination rate of at least 96%, the range of VI on the selective medium was between 0.07 (19%, Forno) and 0.18 (51%, Arina). There was the same rank order for the VI compared to the embryo system, and the correlation between the two systems was very high ($r = 0.93$, $P<0.01$).
4.4.3 *In vitro* screening of F1 zygotic embryos

Germination rates of F1 embryos on MS control medium reached 100/100 over all crosses. VI on MSN medium of batch 1 ranged from 0.01 (3/100, Boval x Zenith) to 0.23 (72/100, Arina x lena).

4.4.4 *In vitro* screening of F3 zygotic embryos

The average germination rate of F3 embryos from all 16 populations on MS medium was 96%. VI ranged from 0.00 (0%) to 0.30 (100%) for F3 embryos derived from a single F2 plant. Averaged across the different F3 embryos of one population, VI ranged from 0.04 (lena x Zenith) to 0.23 (Forno x SN-).

4.4.5 *In vitro* screening of F5 mature seeds

The average germination rate of seeds from all 16 F4-populations on control medium was 97%. VI ranged from 0.05 to 0.30 for single progenies and the mean for each population ranged from 0.23 (69%, SN+ x Boval) to 0.28 (93%, Arina x lena). F4 plants derived from different F3 plants but the same F2 plant that showed different level of resistance against glume blotch differed in the *in vitro* screening for VI up to 0.18.
Table 9: Number of germinating embryos per 100 cultured embryos on control medium and on selective medium containing crude extract (batch 1) of Septoria nodorum together with the level of resistance SNEA and SNLF in the field in 1995 and 1996 (Part 1). Values of the eight parental lines.

<table>
<thead>
<tr>
<th>variety</th>
<th>control medium</th>
<th>selective medium</th>
<th>VI</th>
<th>SNEA 95</th>
<th>SNEA 96</th>
<th>SNLF 95</th>
<th>SNLF 96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arina</td>
<td>99</td>
<td>73</td>
<td>0.24</td>
<td>5.50</td>
<td>3.06</td>
<td>27.54</td>
<td>23.51</td>
</tr>
<tr>
<td>Greif</td>
<td>95</td>
<td>59</td>
<td>0.21</td>
<td>5.47</td>
<td>3.14</td>
<td>19.95</td>
<td>18.34</td>
</tr>
<tr>
<td>Iena</td>
<td>98</td>
<td>60</td>
<td>0.21</td>
<td>4.88</td>
<td>3.54</td>
<td>24.16</td>
<td>21.94</td>
</tr>
<tr>
<td>SN+</td>
<td>91</td>
<td>43</td>
<td>0.17</td>
<td>5.93</td>
<td>3.64</td>
<td>22.19</td>
<td>20.14</td>
</tr>
<tr>
<td>Zenith</td>
<td>95</td>
<td>29</td>
<td>0.12</td>
<td>6.58</td>
<td>3.38</td>
<td>29.52</td>
<td>22.30</td>
</tr>
<tr>
<td>SN-</td>
<td>94</td>
<td>24</td>
<td>0.10</td>
<td>8.08</td>
<td>4.36</td>
<td>30.54</td>
<td>25.23</td>
</tr>
<tr>
<td>Boval</td>
<td>98</td>
<td>19</td>
<td>0.08</td>
<td>8.82</td>
<td>5.59</td>
<td>24.79</td>
<td>20.53</td>
</tr>
<tr>
<td>Fomo</td>
<td>99</td>
<td>8</td>
<td>0.03</td>
<td>8.40</td>
<td>4.78</td>
<td>27.23</td>
<td>22.31</td>
</tr>
</tbody>
</table>

4.4.6 Parental lines: field - in vitro

The results from the different field trials are described in detail in Part 1. The aim of the present study was to estimate the value of in vitro selection by comparing the in vitro response of the different in vitro screenings to the resistance levels of the corresponding plant material in the different field trials.

Comparing the VI of the parental lines on selective media in vitro with the resistance reactions on the ears in the field (Table 9), significant (P<0.01) negative correlations were found between the two traits (Table 10). A genotype showing a high level of resistance in the field (low score) showed a high germination rate in vitro on selective media. This was true for both systems, the culture of zygotic embryos and the culture of mature seeds (Table 10). Between the resistance reaction on the leaves and the VI's, only slightly negative correlations between r= -0.25 (embryo, n.s.) and r =-0.38 (seeds, n.s.) were found. This is in accordance with the low coefficient of correlation between SNEA and SNLF (0.44 in 1995 and 0.34 in 1996, Part 1).
Table 10: Coefficients of correlation between two batches of extract, two culture systems and the resistance reaction on the ear of the parental lines in 1995 and 1996. SNEA index is the disease index ear described by Keller et al. (1994).

<table>
<thead>
<tr>
<th></th>
<th>SNEA 95</th>
<th>SNEA 96</th>
<th>SNEA index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1 embryos</td>
<td>-0.92**</td>
<td>-0.85**</td>
<td>-0.91**</td>
</tr>
<tr>
<td>Batch 2 embryos</td>
<td>-0.87**</td>
<td>-0.81**</td>
<td>-0.93**</td>
</tr>
<tr>
<td>Batch 1 seeds</td>
<td>-0.92**</td>
<td>-0.86**</td>
<td>-0.89**</td>
</tr>
<tr>
<td>Batch 2 seeds</td>
<td>-0.91**</td>
<td>-0.93**</td>
<td>-0.91**</td>
</tr>
</tbody>
</table>

** P<0.01

4.4.7 F1 - crosses field - *in vitro*

Correlation between the resistance level on the ears in the field (SNEA) and the *in vitro* screening (VI) -0.72 (P<0.01), whereas for SNLF and germination on MSN medium it was -0.17 (n.s.). Figure 11 shows the relation between VI and SNEA of the parental lines together with the VI of the F1 and the SNEA of the F1. In order to estimate the effect of heterozygosity, the slopes for both the parental lines and the F1 are shown.

![Graph showing correlation between resistance reaction on the ear in the field (SNEA) and sensitivity of zygotic embryos to a crude extract (batch 2) of *Septoria nodorum in vitro* (in vitro index)]

Figure 11: Relation between resistance reaction on the ear in the field (SNEA) and sensitivity of zygotic embryos to a crude extract (batch 2) of *Septoria nodorum in vitro* (in vitro index)
4.4.8 F3 - progenies: field - in vitro

In contrast to the parental lines and the F1 progenies, no significant correlation was found between the resistance reaction in the field and the sensitivity of zygotic embryos to a crude extract of Septoria nodorum in vitro in F3 progenies (data not shown). This is true for progenies from a single population, for all progenies over all populations and for the mean of the 16 populations. The highest negative correlation was found for the population lena x Forno (r = -0.29 n.s.).

4.4.9 F4 - progenies: field - in vitro

The correlation between the recorded SNEA of F4 headrows in the field and the VI of the in vitro screening over all populations and progenies was r = -0.47 (P<0.01, Fig. 12). Population mean for SNEA was highly correlated with the mean VI (r = -0.89 P<0.01, Fig. 13), indicating that the in vitro test allows to differentiate between populations for their resistance level on the ear. The correlation was negative for all populations with the lowest correlation for SN- x Greif (r = -0.11, P<0.05) and the highest correlation for SN+ x lena (r = -0.64, P<0.01). Figure 14 and 15 show the relation between the resistance level on the ear in the field (SNEA) and the VI for two of the 16 populations (population 13, SN- x SN+ and population 15, Boval x Zenith), that differ slightly in the slope of the regression. Table 11 shows a summary of the regressions for all populations with the corresponding R². Resistance level in the field for F4 head rows derived from different F3 plants but the same F2 plant an the in vitro screening of seeds of these headrows showed lower VI's for the higher SNEA score in 87% of the cases. These deviations observed in the field and the in vitro screening gave an indication for the segregation between F3 plants and the sampling effect if different F3 seeds were used for in vitro screening than for the field testing in F3 progeny test.
Figure 12: Regression of the relation between resistance level on the ear to *Septoria nodorum* in the field in the generation F4 and sensitivity of seeds to metabolites of the pathogen *in vitro*. Values of all individuals from the 16 populations are indicated. Possible selection limits for VI and SNEA are indicated as well.

\[ y = -8.06x + 5.86 \]
\[ R^2 = 0.21 \]

Figure 13: Regression of the relation between resistance level on the ear to *Septoria nodorum* in the field and sensitivity of seeds to metabolites of the pathogen *in vitro*. Values of the parental lines and the 16 population means in the generation F4 are indicated.

\[ y = -24.94x + 10.22 \]
\[ R^2 = 0.83, \text{ parental lines} \]

\[ y = -26.42x + 10.54 \]
\[ R^2 = 0.79 \]

\text{mean of the 16 populations}
population 13, SN- x SN+

![Graph](image)

\[
y = -7.74x + 5.80 \\
R^2 = 0.38
\]

Figure 14: Regression of the relation between resistance level on the ear to *Septoria nodorum* in the field in the F4 generation and sensitivity of seeds to metabolites of the pathogen *in vitro*. A possible selection limit for *in vitro* index VI and disease severity on the ear in the field SNEA are indicated.

population 15, Boval x Zenith

![Graph](image)

\[
y = -10.33x + 6.56 \\
R^2 = 0.35
\]

Figure 15: Regression of the relation between resistance level on the ear to *Septoria nodorum* in the field in the generation F4 and sensitivity of seeds to metabolites of the pathogen *in vitro*. A possible selection limit for *in vitro* index VI and disease severity on the ear in the field SNEA are indicated.
Table 11: Slopes of the regression between VI and SNEA of the 16 populations and the mean of all populations. Values of $a$, $b$ and $R^2$.

<table>
<thead>
<tr>
<th>Population</th>
<th>$a$</th>
<th>$b$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-6.72</td>
<td>5.17</td>
<td>0.20</td>
</tr>
<tr>
<td>2</td>
<td>-6.84</td>
<td>5.63</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>-7.59</td>
<td>5.77</td>
<td>0.14</td>
</tr>
<tr>
<td>4</td>
<td>-5.23</td>
<td>5.21</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>-7.49</td>
<td>5.34</td>
<td>0.14</td>
</tr>
<tr>
<td>6</td>
<td>-11.89</td>
<td>6.67</td>
<td>0.41</td>
</tr>
<tr>
<td>7</td>
<td>-3.20</td>
<td>5.15</td>
<td>0.05</td>
</tr>
<tr>
<td>8</td>
<td>-10.50</td>
<td>6.20</td>
<td>0.19</td>
</tr>
<tr>
<td>9</td>
<td>-8.76</td>
<td>5.83</td>
<td>0.19</td>
</tr>
<tr>
<td>10</td>
<td>-5.64</td>
<td>5.19</td>
<td>0.11</td>
</tr>
<tr>
<td>11</td>
<td>-4.06</td>
<td>4.65</td>
<td>0.12</td>
</tr>
<tr>
<td>12</td>
<td>-2.80</td>
<td>4.48</td>
<td>0.01</td>
</tr>
<tr>
<td>13</td>
<td>-7.74</td>
<td>5.80</td>
<td>0.38</td>
</tr>
<tr>
<td>14</td>
<td>-4.91</td>
<td>5.44</td>
<td>0.13</td>
</tr>
<tr>
<td>15</td>
<td>-10.33</td>
<td>6.56</td>
<td>0.35</td>
</tr>
<tr>
<td>16</td>
<td>-4.52</td>
<td>5.30</td>
<td>0.07</td>
</tr>
<tr>
<td>Mean</td>
<td>-8.06</td>
<td>5.86</td>
<td>0.21</td>
</tr>
</tbody>
</table>

4.4.10 Correlation between generations

In this study, a broad data set concerning resistance against septoria nodorum glume blotch in the field and the in vitro response was obtained over different generations. A question arises now, whether it would be possible to predict the level of resistance of the offspring by applying an in vitro screening of the parental lines. The correlation between the mean VI of the parental lines and the VI of the F1 zygotic embryos was $r=0.70$ ($P<0.01$). Between the VI of the parental lines and the SNEA of the F1 in the field the correlation was $r=-0.62$ ($P<0.05$). Correlations were somewhat lower between the mean VI of the parental lines and the mean VI of the F5 seeds ($r=0.59$, $P<0.05$) and between the mean VI of the parental lines and the mean SNEA of F4 ($r=-0.62$, $P<0.05$), indicating that a certain prediction of a population's resistance level is possible.
when an \textit{in vitro} screening of the crossing partners is carried out. The correlation between VI of F1 and VI of F4 was $r=0.60$ ($P<0.05$) and $-0.73$ ($P<0.01$) between VI of F1 and the mean SNEA of F4. These correlations show, that there is a clear interaction between the sensitivity to the toxins of the crude extract \textit{in vitro} and resistance level on the ear in the field in the material screened in the different generations.

4.5 Discussion

In this study, we found a high correlation between the different sensitivities of zygotic embryos or seeds of the parental lines to the toxic metabolites in the crude \textit{Septoria nodorum} extract and the resistance reaction on the ear. \textit{In vitro} selection techniques have widely been used aiming to improve crops with regard to different traits such as stress tolerance, salt or aluminium tolerance or selection of new amino acid pathways. However, most emphasis was put on \textit{in vitro} selection for disease resistance. \textit{In vitro} selection methods aim to select novel resistance at the cellular or plant tissue level and then to regenerate these selected cells or tissues to adult plants and to screen them for resistance (Van den Bulk, 1991). Pathogen metabolites in the form of crude extracts or culture filtrates of many pathogenic fungi showed toxic activity \textit{in vitro} to protoplasts, microspores, calli and embryos of a wide spectrum of crops studied (Daub, 1986). In these systems, plants that survive the selection pressure \textit{in vitro} are expected to show a higher level of resistance when transferred to the field. In our study, however, the \textit{in vitro} indices were used to determine the level of resistance of the donor plant and not of the individual embryos or seeds. A wheat line showing tolerance to the pathogenic metabolites \textit{in vitro} is expected to show a high level of resistance in the field. Therefore it should be possible to select resistant genotypes based on the \textit{in vitro} screening of a seed sample. If the \textit{in vitro} index is high, the remaining seed of this line will be put in the field for further selection, whereas lines with a high susceptibility \textit{in vitro} could be discarded.
We wanted to evaluate if such a detection of resistance at the *in vitro* level is possible not only between parental lines but also in early generations. Although a large variation in the sensitivity to the pathogen metabolites was observed in the F3 generation, the variation of the resistance reaction in the field was not in accordance with the *in vitro* response. Since we found a very high correlation for the parental lines, this was a very astonishing result. Possible explanations could be the different level of homozygosity in the F3 generation (75%) compared to the parental lines (100%) and that different F3 genotypes (although derived from the same F2 plant) were tested in the field and the *in vitro* screening. If only 3 resistance loci would segregate in our populations, this would result in 27 different genotypes in the F2 and depending on the degree of heterozygosity, seeds from each F2 will segregate in the F3. Since the resistance reaction to *Septoria nodorum* is a quantitative trait, segregation for several resistance loci is expected on our populations. Ecker et al. (1989) suggested, that at least 3 to 5 genes are involved in the inheritance of resistance to *Septoria nodorum* on the ear. The probability that a genotype is homozygous for 5 loci is 0.24 in the F3 generation, indicating that heterozygosity might still be very important in the *in vitro* as well as the field trial.

In the *in vitro* selection experiment, a number of embryos as big as possible had to be used. Therefore, only one ear (about 40 kernels) of a single F2 plant was left to produce a head row progeny. If this head row had a higher or lower level of partial resistance than the mean of the ears that were screened *in vitro*, this would be a sampling error and a high correlation between these two traits over the screened progenies could not be expected. In addition, for the *in vitro* screening of the parental lines, there were always enough embryos and enough plants available that were in the optimal growth stage for the embryo culture. This was not true for the F3 embryos used in this system. Due to the large number of entries, it was not possible to harvest all embryos in the optimal stage. Although in the control this had no visible effect, one can not exclude that older and bigger embryos showed a higher level of tolerance to
the extract *in vitro* than younger and smaller ones. The total number of cultured embryos per genotype was smaller than it was for the parental lines or the F1. Therefore we have three possible explanation for the lacking correlation between *in vitro* and field for the F3 embryos: The degree of heterozygosity, a sampling effect resulting from segregation and the reliability of the embryo culture system. Here it has to be noted, that many studies dealing with *in vitro* selection and segregating populations were abandoned at this early stage due to the missing correlation between *in vitro* and field characteristics in this early generation (for review see Daub, 1986).

To study the effect of heterozygosity, F1 plants were produced and compared with parental lines. Although the F1 were 100% heterozygous, VI and SNEA showed similar correlations ($r=-0.72$) as the parental lines ($r=-0.82$). This indicates that the sampling effect in the segregating F3 is more important than the degree of heterozygosity. However, comparing the slopes of the regression between VI and SNEA for the F1 and the parental lines (Figure 11), it is obvious that there is an influence of the degree of heterozygosity, because the slope of the parental lines is steeper. So, if in early generations the genotypes differ in their degree of heterozygosity for the resistance loci, the overall correlations might be reduced.

In order to evaluate the hypothesis of sampling effect in F3 due to segregation, ten progenies from the F3 head rows were derived from a single F2 plant were screened for resistance to *Septoria nodorum* in the field. And in fact, also in the F4 generation, a considerable variation in the resistance reactions between the 10 progenies all derived from the same F2 plant was detected. On the other hand, no more obvious segregation within a head row was detected. Since each row was assessed for the resistance reaction to *Septoria nodorum*, the sensitivity to the pathogen metabolites *in vitro* should then be better correlated to the resistance reaction of a single head row in the field. In order to prove this, an improved method of *in vitro* selection had to be found, because it was not possible to harvest such a large number of ears in the correct growth stage.
for the embryo culture. Moreover, it would not have been possible to culture such a large number of embryos. We tried therefore to use mature seeds for the *in vitro* test. If the pathogen metabolites would diffuse through the caryopsis to the embryo, this would have the same effect as the direct contact of an embryo to these metabolites in the growth medium. Actually this was the case as we found a high correlation firstly between disease indices of the parental lines and the sensitivity to the metabolites *in vitro* and secondly between the sensitivity of embryos and the sensitivity of seeds of the parental lines. Therefore mature F5 seeds harvested from each F4 head row were used for the *in vitro* screening for all 550 progenies across the 16 populations. This system is very easy to handle and allows to screen a large number of genotypes in a short time, which is a prerequisite for a successful *in vitro* selection method. The correlation of -0.47 between the sensitivity to the pathogen metabolites *in vitro* and the resistance reaction on the ear of all progenies over all populations (Fig. 12) partly proved the hypothesis, that the segregation in the F3 was too high and therefore the sampling error too important to find a significant correlation between *in vitro* screening and resistance in the field. The comparison between the deviation for SNEA between F4 headrows derived from the same F2 plant and the VI's of these headrows showed lower VI's for the higher SNEA score in 87% of the cases. However, in some cases a contrary reaction was observed, i.e. a line with a higher SNEA was less susceptible *in vitro* than the line with the lower SNEA, which reduced the overall correlation. Although the correlation for the F4 is significant due to the high number of entries and nevertheless is high for a quantitative trait, it wouldn't be sufficient for breeders to select for resistance or to detect susceptibility in segregating populations. On the other hand, taking the mean sensitivity to the pathogen metabolites *in vitro* and the mean resistance level on the ear of each population, a high correlation between these two traits was found. This leads to the conclusion, that this method could be integrated into wheat breeding programs. Populations showing a high susceptibility to the pathogen metabolites *in vitro* could be discarded. The slopes of the regression between VI and SNEA are similar for all populations.
(Table 11), indicating that the sensitivity to toxic compounds *in vitro* of the
different populations is genotype independent which again is a prerequisite for a
successful application of *in vitro* selection. Would it then be possible to select
*in vitro* extremely good genotypes within the promising populations or would the
*in vitro* test just allow to discard extremely bad genotypes? In Figure 14 and 15
we can see the relation between VI and SNEA for single individuums of two
different populations. It is obvious, that it is not possible to select on the single
progeny level. It is true that most progenies showing tolerance *in vitro* have a
low SNEA in the field. The same is true for the high susceptibility *in vitro* (high
SNEA in the field). However, there are also genotypes with high VI but high
susceptibility in the field (Fig 15). By defining a selection limit (Fig. 14 and 15),
for VI (>0.275) we will select some susceptible genotypes and also discard
some highly resistant (SNEA<3.5) progenies, but most of the progenies with
week resistance could be eliminated. Therefore, a selection among F4 based
on *in vitro* screening might be possible within a population if the correlation is
high enough ($R^2>0.25$). However, if we use such selection limits for F4
progenies across all populations (Fig. 12), selection based on *in vitro* screening
seems not promising.

An application of the *in vitro* method together with the septoria screening within
the wheat breeding program at the Swiss Federal Research Station for
Agroecology and Agriculture (FAL-Reckenholz) over several years would
provide more reliable data about the usefulness of this method.

Another promising implementation for wheat breeding would be the screening
of advanced breeding material or candidate partners in a crossing program for
resistance on the ear. Since they show a high level of homozygosity and we
found high correlations for the parental lines between VI and SNEA, detection
of highly resistant or highly susceptible germplasm *in vitro* should be possible.
Comparing the VI of the parental lines with the SNEA of the F1 and F4, we
found exactly the same correlations for both (-0.62). This indicates, that it is
possible to predict in a a certain amount the resistance level of F1 progenies or
population in the F4 in the field when we screen the corresponding parental lines in vitro. These correlations show, that there is a clear interaction between the sensitivity to the toxins of the crude extract in vitro and resistance level on the ear in the field in the material screened in the different generations. On the other hand, in the resistance study (part 1), crosses with two parental lines susceptible to Septoria nodorum resulted in some progenies in the F4 showing a high level of resistance. This is an explanation why the correlation between these two traits is not higher. In the same study, dominance for susceptibility of the F1 was found which again explains the lack of a high correlation between the VI of the parental lines and the SNEA of the F1. Depending of the future development of wheat hybrids, the in vitro screening could also be applied to this material, as we found a relative high correlation in the F1 of the 16 crosses. However as found in part one, the production of hybrids is not the best tool to improve septoria resistance, as we found in most crosses dominance for susceptibility in the F1. Depending on the success of an implementation of the in vitro selection method, it would also be of interest, if this system works also for other species, for example triticale, where the production of hybrids shows very promising results. The in vitro screening could be applied either to breeding material or to screen the female and male crossing partners or the F1 hybrids itself.

Many studies have been conducted to screen for resistance on the seedling stage. Reactions observed in the greenhouse on seedlings or in the laboratory on detached leaves were often in accordance with reactions under natural conditions in the field (Wilkinson et al., 1990). Therefore, a combination of seedling screening for leaf resistance and an in vitro screening for ear resistance would be a powerful tool to select germplasm with more complete resistance on both organs. Such a combination would not be destructive, since the seedlings can later grow to maturity and provide seeds for the in vitro screening as well as for further propagation of promising breeding material. However, resistance reactions against Septoria nodorum observed at the seedling stage or at detached leaves were not always expressed at the adult

A complex of problems concerns the pathogen metabolites in the crude extract. The content of these selective acting metabolites varies from batch to batch (Keller et. al., 1994), and the concentration for the best selectivity has to be adjusted for each batch. Moreover, there is no knowledge about the nature of these metabolites. A purification of the extract would lead to a more defined solution. A fractionation of the extract could result in different selective fractions, which could then further be chemically analysed and characterised. This could lead to informations about the mechanisms responsible for the different sensitivities in vitro and under field conditions. In the frame of this study, we fractionated an extract by high performance liquid chromatography (HPLC) and this resulted in 12 fractions. Among these fractions there were five not belonging to the mellein or septorin families (Prof. Dr. R. Tabacchi, University of Neuchâtel, personal communication). One of the five fractions tended to show a selective action to wheat embryos originating of the two standard varieties Arina and Forno. But the amounts of these fractions were to small to confirm this. Nevertheless, this approach would be essential to further develop the in vitro selection method and studies on this field have to be continued.

5 General discussion

5.1 Background

The increasing disapproval of chemical agents in agriculture in recent years has resulted in a change from a conventional agriculture towards more integrated or biological ways of the production of agricultural goods. Besides adequate cultural techniques, the cultivation of more resistant cultivars is an approach to ecologically produce with minimal or no use of agrochemicals. The basic strategy of varietal development is therefore to combine resistance to all
diseases of economical importance with yield, quality and further agronomical
traits. This strategy is easier to describe than to introduce, since a great effort
is needed to improve resistance against each single disease. This is also true
for Septoria nodorum.

5.2 Reliability of the assessment of Septoria nodorum resistance

Resistance to septoria leaf and glume blotch has been reported to be governed
quantitatively and by several components of partial resistance. These
components have been analysed by several researchers (for review King et al.,
latent period is highly correlated to the field resistance. However, this final
resistance level at the adult plant is a result of the interaction between all
components and therefore is suitable to distinguish cultivars with different
levels of partial resistance. Moreover, it is possible to detect ear resistance
which is not possible at the detached leaf level or at seedling stage, because
foliar and ear resistance are controlled by separate genetic mechanisms (Fried
and Meister, 1987). In this study, high correlations were found between the
field trial in 1995 and 1996 for both traits, SNEA and SNLF of the parental lines
\( r = 0.95 \) and \( 0.88 \), respectively, \( P<0.01 \) as well as to the disease indices
calculated for the same cultivars by Keller et al. (1994). This indicates, that the
resistance level can be determined in an accurate way on 1-row and 5-row plot
basis after artificial infection and multiple ratings. Multiple recordings are
essential for assessment of disease development (Walther, 1990) and for
reducing errors in estimating percentage of diseased area. Therefore, the
resistance level determined for the F3 and for the F4 generation of the 16
crosses provides a reliable basis to compare the resistance under field
conditions to the susceptibility to Septoria nodorum metabolites in vitro (part 2).
The low variance of the replicated parents indicates, that environmental
influences played a minimal role in trials using artificial inoculation and that the
different resistance reactions are mainly due to the different genetic
mechanisms responsible for this trait. Spraying the trial with fungicides in order
to exclude interactions with other diseases was very effective, because the parental lines that were grown in the third non inoculated replication in the same field (used to determine the reduction of thousand kernel weight) showed very few symptoms from other pathogens as well as from Septoria nodorum.

5.3 Inheritance of resistance

Heritability estimates for SNEA and SNLF were higher, lower or comparable to those reported by Brönnimann (1975) and Rosielle and Brown (1980), who screened only a limited number of genotypes. This points out the necessity to screen a broad genetic spectrum to study inheritance mechanisms. Heritability estimates differed in some cases strongly between SNEA and SNLF (SN+ Zenith, Arina x SN-, Forno x SN- and Boval x Zenith, Table 7), which is in accordance with the different directions of heterosis for SNEA and SNLF (Table 5). The different magnitudes and the differences for SNEA and SNLF of the heritability estimates found in this study reflect the complex inheritance mechanisms of resistance to SNB.

Effects of heterosis in the F1 were in most cases positive for SNEA and SNLF, i.e. the F1 was more susceptible than the midparent value, indicating dominance for susceptibility as found by Brönnimann (1975) and Fried and Meister (1987). Heterosis was more important for SNEA than for SNLF. Production of wheat hybrid seeds would therefore not be a tool for improving septoria resistance. However, heterosis effects diminish in later generations due to the lower effect of variance of dominance. It is therefore possible, that breeders discard whole populations in the F2 that seem to be susceptible due to the high degree of heterozygosity. But as shown before, even in such populations (for example Forno x SN- for SNEA or Lena x Forno for SNLF, Figure 1 and 2) highly resistant genotypes occur in later generations. However, large populations and strong selection pressure in later generations are necessary to identify such genotypes (Fried and Meister, 1987).
The results obtained by calculating general combining ability (GCA) indicate, that on the basis of the resistance reaction of a parent, its performance in hybrid combinations can not be predicted. Although Arina, the parent with the highest level of resistance on the ear (Table 3) had the highest negative GCA for SNEA (-0.24, reduction of necrosis), Lena, with almost the same level of resistance, showed only a small GCA. Specific combining ability (SCA) and GCA were of the same magnitude, indicating that in the F1 dominance and other nonadditive gene effects are of the same importance as additive effects. This was also reported by Wilkinson et al. (1990).

Coefficients of correlation over all populations between F3 lines, on 1-row-plot basis in 1995, and F4 lines, on 5-row-plot mean basis in 1996, were quite high (r = 0.71 for SNEA and r = 0.80 for SNLF). Scott et al. (1982) found much weaker correlations for disease ratings of the same genotypes in different environments, which he explained by genotype x environment interactions. These interactions were minimal in our trial, which is indicated by the low variance of the replicated entries of the parental lines in both years. This was probably due to the high infection pressure and conditions favourable for disease development in both years. It can therefore be concluded, that the efficiency of selection on 1-row-plot basis is quite high, if artificial infection is applied. In some populations, however, correlations between F3 and F4 lines were low. For example for SNLF and the cross SN+ x Boval (r = 0.34). In this case, the F1 shows heterosis for resistance (see Fig. 2), whereas the F4 mean had a lower level of resistance than both parents. One explanation could be the different level of heterozygosity (25% in F3 versus 12.5% in F4 for a single locus). Ecker et al. (1989) suggested, that at least 3 to 5 genes are involved in the inheritance of resistance. The probability for homozygosity for 5 loci is 0.51 in F4 and 0.23 in F3. If dominance effects play a major role, as indicated by the considerable heterosis observed in some crosses, the level of heterozygosity may explain low correlations in some crosses. Moreover, the F3 and F4 generations were assessed in different years. It could be possible that the importance of genotype - environment interaction varies between the different
populations. Another reason may be the assessment of 1-row versus 5-row plots. In a single row of the F3 trial there may be short and tall plants because F3 lines are not homogeneous. Tall plants may show a higher level of resistance than the short ones. Assessing mainly tall plants that overgrow the short ones may cause a bias in the estimation. In the 5-row plots of F4 lines, where each row is a rather homogenous head row, this effect can be eliminated.

The objective of breeding for resistance against *Septoria nodorum* is a cultivar which will yield well and produce good quality grain in environments that are otherwise favourable for disease development (Rosielle and Brown, 1980). Three resistance mechanisms working singly or in combination can assist in the achievement of this objective, namely escape resistance, true resistance and tolerance (Parlevliet, 1977). The association of resistance to tall, late maturing plants (Scott et al., 1982) was found in this study for the progenies of the 16 crosses (significant negative correlations for both traits). These are both possible escape mechanisms, since they result in a reduced probability of contact between host and parasite, or in a reduced rate or ease of penetration and colonisation. In tall crops, the pathogen may be prevented from reaching the upper parts of the leaves or the ear by the need to complete several cycles of splash dispersal (Scharen, 1964). However, in this trial this escape mechanism was not the main cause, because all plants were inoculated at least once on the ears. *Septoria nodorum* requires moisture for spore production and infection (Shipton et al., 1971) and the microclimatic conditions may limit further development. This limitation may also explain the association of resistance to late maturing, because later in the growing season, wetness duration is usually decreased. Moreover, *Septoria nodorum* infects older tissue more readily than younger and at a given time, the tissues of more advanced lines are therefore likely to be more susceptible than the tissues of later lines (Scott et al., 1982). However, inoculating the trial four times, all plants have been hit at the most sensitive stage and the influence of plant age was minimised. For the 8 parental lines, the association from tall, late maturing
plants was only true for days to ear emergence but not for plant height. This is due to the two short strawed cultivars Lena and Greif (95 cm), which both show a high level of partial resistance on the ears, which reflects true resistance. This leads to the suggestion, that pleiotropy rather than linkage explains the association of resistance with plant height (Scott et al., 1985), because in these two cultivars there must be genes involved that have no effect on plant height (or ear emergence) but on effective partial resistance, whereas tall cultivars may escape the pathogen or have a microclimatic conditions, that prevent the pathogen to develop (King et al., 1983). Height independent genetic variation in resistance to *Septoria nodorum* is therefore substantial for breeders.

The reduction of thousand kernel weight due to the artificial infection showed no significant correlation between SNEA and SNLF for the parental cultivars (Table 8). The reduction for Greif, who shows a high level of partial resistance in terms of necrosis on ear and leaf due to the infection with SNB is higher than for Boval, who shows a high susceptibility on the ear. This proves the existence of the third mechanism, tolerance. Greif may need a great input of energy in the defence reaction, whereas Boval may be able to compensate losses of photosynthetic active tissue (Rooney and Hoad, 1989).

The low coefficients of correlation between SNEA and SNLF found in this study lead to the conclusion that these traits are inherited independently. In the F1, the correlation is even negative. An explanation of this is shown in Figure 1 and 2. In the crosses SN+ x Lena and SN+ x Boval, the F1 shows positive heterosis for SNLF, but negative heterosis for SNEA, thus representing a contrary reaction. Therefore different genes and/or gene actions seem to be involved in the expression of glume blotch and leaf blotch resistance. This is in agreement with the findings of Fried and Meister (1987), that resistance on the heads is inherited independently from the resistance on the leaves and that both are inherited quantitatively.
The complex of partial resistance, escape mechanisms and tolerance has been described for many wheat cultivars (Brönnimann, 1975, Tvaruzek and Klem, 1994). On this point of view, it is obvious that breeders are confronted with a large number of problems to get an ideal cultivar that combines partial resistance on ear and leaf together with tolerance. Moreover, earliness and short straw should not negatively influence these traits.

The variation observed in this study within and among the segregating populations suggests a quantitative inheritance pattern influencing the expression of the two traits. Continuous variations in SNB response have been reported in numerous studies (Loughman et al., 1994, Mullaney et al., 1982, Ecker et al., 1988, Bostwick et al., 1993). The components of variance between F2 families within population are as high as (SNEA) or higher (SNLF) than those between populations. Therefore, a strong selection within a few populations may be as effective to find new resistant genotypes as selection in a large number of populations. The lower variation within F3 families (between F4 headrows) is due to the higher level of homozygosity in this generation.

Assuming an additive-dominance model (Mather and Jinks, 1977), the components of variance within F4 populations tested in 1996 consist of the variance between F2 families = $V_A + 1/16 \ V_D$, ($V_A$: additive genetic variance of F2 generation, $V_D$: non-additive genetic (dominance) variance of F2 generation), the variance between F3 progenies within F2 families = $1/2 \ V_A + 1/8 \ V_D$ and the variance between individuals within F3 progenies $1/4 \ V_A + 1/4 \ V_D + V_E$, ($V_E$: environmental component of within family variance), which is in total $7/4 \ V_A + 7/16 \ V_D + V_E$. Therefore, dominance effects should turn out to be most important in the F1, because moving from F1 to F4, the dominance variance $V_D$ diminishes from $1V_D$ (F1) to $7/16 \ V_D$ (F4). The additive-dominance model is adequate to explain variance in septoria resistance, although some variation in the form of interaction between nonallelic genes may occur. The graphical presentation of the parental lines, the F1 values and the F4 means in Figure 1 and 2 confirm this theory. In most cases, the F1 was more susceptible than the F4 (mean) and was closer to the susceptible parent, indicating that
dominance for susceptibility as found by Brönnimann (1975) and Fried and Meister (1987) is involved in the inheritance. Ecker et al. (1989) suggested that there are more allelic combinations which increase susceptibility than allelic combinations which increase resistance. Nevertheless, for SNLF (Figure 2) in three crosses (lena x Forno, SN+ x lena and SN+ x Boval) and for SNEA in one cross (Forno x SN-) dominance for resistance exists. This indicates, that dominant genes for resistance against leaf blotch as found by Frecha (1973) at the seedling stage might also be expressed at the adult stage. On the other hand, such genes may be modified by other genes or certain gene combinations as described by Kleijer et al. (1977) and Laubscher et al. (1966). Looking at the means of F4 populations for SNEA of Forno x SN- or SN+ x Zenith or for SNLF of SN- x Zenith or lena x Forno one can observe positive deviation (towards more resistant). But also negative deviation (towards more susceptible) occurs, for example for SNEA of lena x Zenith or for SNLF of Arina x Forno, indicating the importance of gene x gene interactions.

When breeders want to improve a trait, they usually cross two parents that already express this trait in a high level. On the basis of the results of this study, this strategy may also be promising for breeding for septoria resistance, as it is shown in Figure 1 for Zenith x Greif and SNEA or in Figure 2 for SN+ x Boval and SNLF. But there are also some peculiarities which might be of interest to breeders. For example the cross between highly resistant cultivars lena x Zenith and Arina x lena resulted in F4 mean with lower level of resistance than each parent. However, some of the progenies were more resistant (Fig. 1). The correlation between the genetic component of variance in the F4 and the phenotypic difference between the parents is low. This leads to the conclusion, that on the basis of the genetic value of the parents, the genetic variability induced by a specific cross can not be predicted. This is demonstrated in Fig. 1. In the cross Forno x SN-, the parents have almost the same value, but the range in F4 is large, and even though the parents represent two susceptible genotypes, a selection for a high level of resistance in the F4 would be possible. This is also true for the cross SN- x SN+. In the
cross SN- x Greif on the other hand, there is a large range too, but a selection for higher levels of resistance than the more resistant parent seems not promising for SNEA but for SNLF. In some crosses, for example lena x SN- or Arina x Forno, the range of F4 lines correspond more or less to the phenotypic difference of the parents.

In almost all crosses some progenies were found that were more resistant than the better parent (range in F4, Figure 1 and 2). The progenies with the highest level on resistance for SNEA were found in the crosses Arina x lena, Zenith x Greif and Boval x lena and for SNLF in the crosses SN- x Greif and SN+ lena. This indicates that Arina and lena, both with a high level of partial resistance on the ear, carry different resistance genes that can be combined and that also parental lines with low level of resistance (Zenith and Boval for SNEA and SN- and lena for SNLF) contribute positive alleles increasing resistance. Thus transgression breeding can be a tool to breed for higher levels of resistance to SNB.

5.4 In vitro screening

The analysis of the results of the field assessment of Septoria nodorum proved once more, that with the complicated mechanisms of inheritance, resistance breeding is still a big challenge. In order to optimise the selection for resistance, different methods such as screening of seedlings or detached leaves have been investigated (Bruno and Nelson, 1990, Karjalainen, 1984). It was suggested that the use of more than one screening method in a breeding program would be a more effective method for detecting and determining resistance to Septoria nodorum (Bostwick et al., 1993). Looking at the different biotechnological methods that are already applied in breeding programs (marker assisted selection), the use of such methods to screen for resistance seems to be a promising approach to improve the efficiency of the traditional breeding methods.
In vitro selection techniques have widely been used aiming to improve crops with regard to different traits. Together with selection of new amino acid pathways (Lange et al., 1995) and salt or aluminium tolerance, most emphasis was put on in vitro selection for disease resistance (Ahmed and Sagi, 1993). Pathogen metabolites in the form of crude extracts or culture filtrates of many pathogenic fungi showed toxic activity in vitro to protoplasts, microspores, calli and embryos of a wide spectrum of crops studied (Daub, 1986). However, in a considerable number of studies selection for resistance using toxic compounds was not successful. Knowledge about the role of toxic components in pathogenesis, the mode of action and properties of toxins and/or their interactions with host cells is still lacking for many host pathogen systems. Such information is needed to assess whether a selection procedure can be successful or not (Van den Bulk, 1991). Concerning the crude extract of Septoria nodorum used in this study, no detailed information about the toxins are available. Nevertheless it proved to be useful for in vitro selection due to its toxicity to wheat zygotic embryos or seeds caused by metabolites of the pathogen. Extracts that have been prepared of non infected wheat kernels showed a 10 fold lower toxicity to wheat embryos compared to extracts from infected kernels (Keller et al., 1994). These authors investigated in their study also mellein, a chemically characterised toxin produced by Septoria nodorum, and found that this compound showed toxic but no selective action to wheat embryos. However, one can not exclude, that mellein is acting selectively. Septona nodorum produces in culture at least six forms of mellein (Devys et al., 1994), from which only one was tested in the study of Keller et al. (1994), and it is therefore not known if one or more of the other forms are responsible for the selective action of the crude extract. The large number of isolates used to produce the crude extract makes sure that in vitro toxins closely resemble the natural toxins (Fadel and Wenzel, 1993), because it is known that in many pathogens there are big differences in quality and quantity between isolates with regard to the toxin production (Manka et al., 1985). A disadvantage of the crude extract is its content of toxic metabolites, which varies between different
extracts by as much as a factor of 4 (Keller et al., 1994), and the concentration for the best selectivity has to be adjusted for each new batch.

5.5 Correlation between field assessment and in vitro screening

Usually in vitro selection methods aim to select or to create novel resistance at the cellular or plant tissue level and then to regenerate these selected cells or tissues to adult plants and to screen them for resistance (Van den Bulk, 1991). Like any other method of indirect selection, the value of in vitro selection is dependent on the extent to which in vitro and field traits are correlated, on heritabilities of both in vitro and field assessment and on ease of measurement of both (Haines, 1993). Many important characters operate at the plant level, and these won't be expressed in cultured cells. Other traits may be expressed at the cellular level, but not in cultured tissues or at the plant level. Therefore progeny tests of in vitro selected regenerants are essential. Wenzel and Foroughi-Wehr (1990) found that progenies of several regenerants of barley, wheat and potato that were selected for improved resistance to toxic fractions of Helminthosporium, Fusarium or Phytophthora did not differ significantly in their level of resistance from the starting material.

One of the best ways to test whether resistance can be expressed at the in vitro level is to compare the in vitro response of resistant and susceptible varieties (Daub, 1986). In this study, we found a high correlation between the different sensitivities of zygotic embryos or seeds of the parental lines to the toxic metabolites in the crude Septoria nodorum extract and the resistance reaction on the ear. The in vitro indices were used to determine the level of resistance of the donor plant and not of the individual embryos or seeds. A wheat line showing tolerance to the pathogenic metabolites in vitro will be further developed in the field using part of the seeds that have been stored. On the other hand, lines with a high susceptibility will be discarded.
We wanted to evaluate if such a detection of resistance at the \textit{in vitro} level is possible for \textit{Septoria nodorum} resistance for homozygous breeding lines but also for early generations. Although a large variation in the \textit{in vitro} sensitivity to the pathogen metabolites was observed in the F3 generation, the variation of the resistance reaction in the field was not in accordance with the \textit{in vitro} response. Since we found a very high correlation for the parental lines, this was a very astonishing result. Possible explanations could be the different level of homozygosity in the F3 generation (75\%) compared to the parental lines (100\%) and that different F3 genotypes (although derived from the same plant) were tested in the field and the \textit{in vitro} screening. If only 3 resistance loci would segregate in our populations, this would result in 27 different genotypes in the F2 and depending on the degree of heterozygosity, seeds from each F2 will segregate in the F3. Since the resistance reaction to \textit{Septoria nodorum} is a quantitative trait, segregation for several resistance loci is expected in our populations. Ecker et al. (1989) suggested, that at least 3 to 5 genes are involved in the inheritance of resistance to \textit{Septoria nodorum} on the ear. The probability that a genotype is homozygous for 5 loci is 0.24 in the F3 generation, indicating that heterozygosity might still be very important in the \textit{in vitro} as well as the field trial.

In the \textit{in vitro} selection experiment, a number of embryos as big as possible had to be used. For this reason, only one ear (about 40 kernels) of a single F2 plant was left to produce a head row progeny. Therefore it is possible that this head row had a higher or lower level of partial resistance than the F2 plant which was evaluated \textit{in vitro} using embryos of several ears. This would be a sampling error and a high correlation between these two traits over the screened progenies could not be expected. When the parental lines were screened \textit{in vitro}, there were always enough embryos available, and there were also enough plants available that were in the optimal growth stage for the embryo culture. This is not true for the F3 embryos used in this system. Due to the large number of entries, it was not possible to harvest all embryos in the optimal stage. Although in the control this had no visible effect, one can not
exclude that older and bigger embryos showed a higher level of tolerance to the extract in vitro than younger and smaller ones. The number of cultured embryos was smaller than it was for the parental lines or the F1. Therefore we have three possible explanation for the lacking correlation between in vitro and field for the F3 embryos: The degree of heterozygosity, a sampling effect resulting from segregation and the reliability of the embryo culture system. Concerning the heterozygosity, one have to point out, that with the F1 we found a high correlation field - in vitro. Although 100% heterozygous, they were completely homogeneous and they showed all the same reaction to the toxins of the crude extract. It has to be noted, that many studies dealing with in vitro selection and segregating populations were abandoned at this early stage due to the missing correlation between in vitro and field characteristics (for review see Daub, 1986).

To study the effect of heterozygosity, F1 plants were produced and compared with parental lines. Although the F1 are 100% heterozygous, VI and SNEA showed similar correlations (r=-0.72) as for the parental lines (r=-0.82). This indicates that the sampling effect in the segregating F3 is more important than the degree of heterozygosity. However, comparing the slopes of the regression between VI and SNEA for the F1 and the parental lines (Figure 11), it is obvious that there is an influence of the degree of heterozygosity, because the slope of the parental lines is steeper. So if in early generations the genotypes differ in their degree of heterozygosity for the resistance loci, the overall correlations might be reduced.

In order to evaluate the hypothesis of sampling effect in F3 due to segregation, ten progenies from the F3 head rows all derived from a single F2 plant were screened for resistance to Septoria nodorum in the field. And in fact, also in the F4 generation, a considerable variation in the resistance reactions between the 10 progenies was detected. On the other hand, no more obvious segregation within a head row was detected. Since each row was assessed for the resistance reaction to Septoria nodorum, the sensitivity to the pathogen
metabolites *in vitro* should then be better correlated to the resistance reaction of a single head row in the field. In order to prove this, a new method for *in vitro* screening had to be found, because it was not possible to harvest such a large number of ears in the correct growth stage for the embryo culture. Moreover, it would not have been possible to culture such a large number of embryos. We tried therefore to use mature seeds for the *in vitro* test. If the pathogen metabolites would diffuse through the caryopsis to the embryo, this would have the same effect as the direct contact of an embryo to these metabolites in the growth medium. Actually this was the case as we found a high correlation firstly between disease indices of the parental lines and the sensitivity to the metabolites *in vitro* and secondly between the sensitivity of embryos and the sensitivity of seeds of the parental lines. Therefore mature F5 seeds harvested from each F4 head row were used for the *in vitro* screening for all 16 populations. This system is very easy to handle and allows to screen a large number of genotypes in a short time, which is a prerequisite for a successful *in vitro* selection method. The correlation of -0.47 between the sensitivity to the pathogen metabolites *in vitro* and the resistance reaction on the ear of all progenies over all populations (Figure 12) partly proved the hypothesis, that the segregation in the F3 was too high and therefore the sampling error too important to find a significant correlation between *in vitro* screening and resistance in the field. The comparison between the deviation for SNEA between F4 headrows derived from the same F2 plant and the VI's of these headrows showed lower VI's for the higher SNEA score in 87% of the cases. However in some cases a contrary reaction was observed, i.e. a line with a higher SNEA was less susceptible *in vitro* than than the line with the lower SNEA, which reduced the overall correlation. Although the correlation for the F4 was significant due to the high number of entries and nevertheless high for a quantitative trait, only 22% of the variation in the field can be predicted by *in vitro* screening. Therefore, *in vitro* screening is useful to select for resistant genotypes in the F4 generation but it is not sufficient to substitute all field test. On the other hand, taking the mean sensitivity to the pathogen metabolites *in vitro* and the mean resistance level on the ear of the populations, a high
correlation between these two traits was found (Figure 13). This leads to the conclusion, that this method is extremely useful to select between populations. Although in most populations some highly resistant genotypes were found (part 1), their frequency differed considerably. Populations showing a high susceptibility to the pathogen metabolites in vitro could be discarded, because chances to find highly resistant genotypes will be low. The slopes of the regression between VI and SNEA were similar for all populations (Table 11), indicating that the sensitivity to toxic compounds in vitro of the different populations is genotype independent which again is a prerequisite for a successful application of in vitro selection. Would it then be possible to select in vitro extremely good genotypes within the promising populations or would the in vitro test just allow to discard extremely bad genotypes? Based on our results (Table 11) this depends strongly on the cross. Only in six of 16 populations, we could predict a considerable amount (20-41%) of the resistance against septoria nodorum glume blotch based on the in vitro screening. Figure 14 and 15 show the relation between VI and SNEA for single progenies of such populations. Although most progenies showing tolerance in vitro had a low SNEA in the field and vice versa, some progenies did not show the expected resistance level in the field. Therefore we would lose resistant genotypes or select susceptible ones by defining a selection limit (Figure 14 and 15).

Another promising implementation for wheat breeding would be the screening of advanced breeding material or candidate partners in a crossing program for resistance on the ear. Since they show a high level of homozygosity and we found high correlations for the parental lines between VI and SNEA, detection of highly resistant or highly susceptible germplasm in vitro should be possible. Comparing the VI of the parental lines with the SNEA of the F1 and F4, we found exactly the same correlations for both (-0.62). This indicates, that it is possible to predict the average resistance level of F1 progenies or population in the F4 when we screen the corresponding parental lines in vitro. These correlations show, that there is a clear interaction between the sensitivity to the
toxins of the crude extract in vitro and resistance level on the ear in the field in the material screened in the different generations. On the other hand the resistance study of part 1 showed, that the variance within the populations could not be predicted. Even crosses with two parental lines susceptible to Septoria nodorum resulted in some progenies in the F4 showing a high level of resistance. This is an explanation why the correlation between these two traits is not higher. Depending on the future development of wheat hybrids, the in vitro screening could also be applied to this material, as we found a relatively high correlation in the F1 of the 16 crosses. Depending on the success of an implementation of the in vitro selection method, it would also be of interest, if this system works also for other species, for example triticale, where the production of hybrids shows very promising results. The in vitro screening could be applied either to screen the female and male crossing partners or the F1 hybrids itself.

In this study we found the in vitro screening to be useful to detect resistance to Septoria nodorum on the ear but not on the leaf. Nothing is known about the mechanisms responsible for this. But it is not astonishing. If high correlation exists between resistance on the ear and the in vitro indices, this correlation will not be the same for resistance on the leaf and the in vitro indices, since resistance on the ear and resistance on the leaf are at least partly governed by a different set of genes (Fried and Meister, 1987). On the other hand it is surprising, that we found such a high correlation between the in vitro response and the resistance on the ear in the field. As the propagation of the pathogen to produce extracts was initiated on kernels, it could be possible, that specific metabolites are produced on kernels, that would not be produced on leaves and that later show a specific interaction to embryos or kernels. A preliminary application to leaves of intact plants that were grown in the greenhouse showed no necrotic reactions to the crude extract. However the concentration of the metabolites was probably too low. It would be of interest to produce an extract based on wheat leaves.
Many studies have been carried out to screen for resistance on the seedling stage. Reactions observed in the greenhouse on seedlings or in the laboratory on detached leaves were often compared with reactions under natural conditions in the field. A combination of seedling screening for leaf resistance and an in vitro screening for ear resistance would be a powerful tool to select germplasm with more complete resistance on both organs. Such a combination would not be destructive, since the seedlings can later grow to maturity and provide seeds for the in vitro screening as well as for further propagation of promising breeding material. However, resistance reactions against Septoria nodorum observed at the seedling stage or at detached leaves were not always expressed at the adult plant stage under field conditions (Arseniuk et al., 1991, Trottet and Benacef, 1989, Nelson and Marshall, 1990).

5.6 Outlook

Taking into account the progress in microspore cultures in wheat, this technique could turn out to be a powerful tool in the field of in vitro selection. In combination with a selection pressure through toxic pathogen metabolites, such a microspore selection system would allow to screen millions of genotypes in a short time, to identify the expression of dominant as well as recessive traits, to apply a controlled and homogenous selection pressure and to rapidly develop new cultivars from the selected regenerants (Lashermes, 1991). This approach is different to the one presented in the present work, where a germplasm screening rather than selection was carried out. Toxin tolerant microspores could be selected, regenerated and then screened for resistance in the field. The haplodiploidisation procedures offer the possibility of developing completely homozygous lines from heterozgeous parents in a single generation. In addition to this, recessive genes contributing to resistance can be detected and expressed. Moreover, somaclonal and gametoclonal variation has the potential to broaden the genetic variability for resistance.
It would certainly be of interest to chemically analyse the crude extract. In the frame of this study, we fractionated the extract by HPLC and this resulted in several purified compounds. Among these compounds there were some fractions not belonging to the mellein or septorin families (Prof. Dr. R. Tabacci, personal communication). One fraction tended to show a selective action to wheat embryos. The amount of that fraction was too small to confirm this. Nevertheless, this approach would be essential to further develop the in vitro selection method. A defined selective acting fraction or a combination of them could lead to a more reproducible system for the detection of resistance at the in vitro level. As a possible future approach, such selective fractions might be useful to find genes which code for toxin degrading enzymes. Such genes, transferred to wheat, could lead to a more complete resistance against Septoria nodorum. However, in the current stage where detailed informations are lacking it is preferable to use the crude extract.
6 Literature


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