Growth Stage Specific Resistance of Winter Rye to *Microdochium nivale* and *Fusarium* spp. in the Field Assessed by Immunological Methods

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ABSTRACT

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Microdochium nivale and various *Fusarium* spp. cause severe diseases at all cereal growth stages. To investigate the successive colonization of the basal parts of winter rye in the field, 12 inbred lines were inoculated artificially with *M. nivale* and 11 with *F. culmorum* at one location across 2 yr for each pathogen. Arbitrarily sampled shoot or stem bases were assessed for fungal protein content in the host tissue by indirect enzyme-linked immunosorbent assay (ELISA) at seven growth stages from the beginning of tillering (EC 21) to full maturity (EC 91). Foot rot lesions were rated on a 1 to 9 scale at milk ripening. For *M. nivale*, the highest protein content and best genotypic differentiation were found in EC 21 shortly after snow melt. During further growth of the plants, *M. nivale* protein content decreased substantially and increased again between anthesis and full maturity. In contrast, *Fusarium* spp. showed a continuous increase in host tissue colonization from tillering to full maturity. In the early growth stages, fungal protein content was highly variable between years. Genotypic differentiation for resistance was significant at the earliest (EC 21) and a late (EC 75) host growth stage for *M. nivale* and at all growth stages tested for *Fusarium* spp. Heritability estimates reached the highest value at EC 21 for the *M. nivale* ELISA ($h^2 = 0.59$) and at milk ripening for the *Fusarium* spp. ELISA ($h^2 = 0.91$). *Microdochium nivale* and *Fusarium* spp. all caused foot rot symptoms at milk ripening leading to a mean foot rot rating of 3.3 and 3.8, respectively. No significant correlation existed between the resistance at early and adult-plant growth stages. For resistance to *Fusarium* spp., moderate to high genotypic correlations ($r = 0.64-0.86$) were found only among the adult-plant heading, anthesis, and milk ripening stages. Selection for adult plant resistance to Fusarium foot rot is not feasible during early host growth stages due to strong host genotype-growth stage interactions. Accordingly, resistance to *M. nivale* at early host growth stages (EC 21 and EC 25) cannot be used to predict resistance to *M. nivale* foot rot at milk ripening.

Additional keywords: *Secale cereale*, snow mold.

Microdochium nivale (F.) Samuels & I. C. Hallet (syn. *Gerlachia nivalis*, *Fusarium nivale*, teleomorph = *Monographella nivalis* (Schaffnit) E. Müller) and *Fusarium* spp. are widespread fungal pathogens of cereals. In early growth stages, *M. nivale* causes snow mold (3,6,23) and *Fusarium* spp. seedling blight (6,9). Later in the growing season, both pathogen groups may cause foot rot with necrosis of stem bases, and head blight in winter rye (*Secale cereale* L.) and winter wheat (*Triticum aestivum* L.) (3,6,7,13,18,23). The prevalent *Fusarium* spp. in temperate regions are *F. culmorum* (W. G. Sm.) Sacc., *F. graminearum* Schwabe (teleomorph = *Gibberella zeae* (Schwein.) Petch) and *F. avenaceum* (Fr.:Fr.) Sacc. (teleomorph = *Gibberella avenacea* R. J. Cook). In middle Europe, they are frequently associated with *Pseudocercosporella herpotrichoides* (Fron) Deighton causing foot rot in wheat and rye (7,18). Periodic epidemics of *Microdochium* or *Fusarium* diseases result in economically important yield losses caused by a thinned stand, pre-harvest lodging, or reduction of kernel number and size (6,13,23). Little is known about the epidemiological relationship between the *Microdochium*- and *Fusarium*-incited diseases at different host growth stages and the associations of host resistances with different host growth stages.

Appropriate fungicides for the control of *M. nivale* and *Fusarium* spp. are not currently available; thus, disease control must rely on breeding for resistance. Significant quantitative variation in resistance of winter rye to *F. culmorum*, *F. graminearum*, and *M. nivale* was reported (12,15,16). Under controlled environmental conditions, observed resistance reactions were highly specific to host growth stage at inoculation (12,15). To date, it has not been determined whether growth stage specific resistance is also expressed in the field.

Until recently, different foot rot pathogens infecting the same plant simultaneously could not be separated accurately by their disease symptoms in the field. By means of enzyme-linked immunosorbent assay (ELISA), it is possible to assess fungal protein content in host tissue in a highly specific, sensitive, and quantitative manner even when no disease symptoms are visible. Thus, application of ELISA is of special advantage for diseases involving a complex of fungi under field conditions in which infections remain latent for a substantial period of host growth. Visual disease ratings assess host plant reactions to fungal colonization while ELISA values measuring fungal protein content represent the pathogen growth within host tissue. In experiments for resistance selection, ELISA allows quantification of the extent to which genotypes are restricting pathogen growth. Resistance was shown to be associated with reduced pathogen colonization by recently developed ELISA systems in diseases caused by *M. nivale* and *F. culmorum* (1,11). The objectives of this study were to evaluate the colonization of winter rye by *M. nivale* and *Fusarium* spp. with ELISA during two growing seasons in the field, and to estimate the correlations of host resistance to fungal colonization at different growth stages.

MATERIALS AND METHODS

Genotypes tested. Twelve self-fertile inbred lines (L5, L185, L201, L301, L305, L306, L307, L311, L312, L161, L286, L287) from the Hohenheim hybrid rye breeding program (10), mainly belonging to the two main heterotic groups Petkus and Carsten,

were used for this study. One line (L286) was not evaluated in the experiments with *F. culmorum* due to the low emergence rate in 1992. The lines represent modern breeding materials and were practically homozygous.

Field testing and artificial inoculation. The *M. nivale* experiments were conducted at the "Oberer Lindenhof" (OLI) experimental station in Southwest Germany (705 m above sea level, mean annual temperature 6.4 C, mean annual precipitation 912 mm) in 1990 and 1991, and the *F. culmorum* experiments at Stuttgart-Hohenheim (HOH, 400 m above sea level, 8.5 C mean annual temperature, 685 mm mean annual precipitation) in 1991 and 1992. At OLI, *M. nivale*-incited snow mold occurs frequently in normal stands indicating a high level of natural soil-borne inoculum. At HOH, the incidence of natural *Fusarium* diseases of winter rye is low. Field plots were integrated in a rotating system with no cereal forecrop (OLI, mustard seed; HOH, rape seed and grass fallow in 1991 and 1992, respectively). Inbred lines were drilled in six-row microplots. Rows were 1.2 m long with 0.21 m between rows (plot size 1.5 m^2) and arranged as a randomized complete block design with four replicates.

Preparation of inoculum and inoculation procedures followed those described for *M. nivale* by Miedaner et al (15) and for *Fusarium* spp. by Höxter et al (12), respectively. Genotypes were artificially inoculated by spreading air-dried wheat-grain material that had been colonized with *M. nivale* var. *nivale* or with *F. culmorum* and then ground in a mill (0.5–1 mm pore size) at a rate of 30 ml per row. For both pathogen species, equal volumes of five isolates of different geographic origins were mixed to simulate a complex pathogen population. Plants were inoculated in November after most plants had reached the three-leaf stage (EC 13 on the Zadoks scale) (25). In the *F. culmorum* experiments, inoculation was repeated in February at the four-leaf stage (EC 14). Directly after the second inoculation, relative humidity of 100% was maintained for 4 wk by covering all field plots with a transparent, punctured polyethylene film (500 punctures m^{-2}). In November, plants were left uncovered because natural relative humidity was high.

Preparation of samples and ELISA procedures. In both experiments, 20 shoots or stems per plot were harvested arbitrarily for ELISA determinations at the onset of tillering (EC 21), mid-tillering (EC 25), jointing (EC 31), heading (EC 51), anthesis (EC 65), milk ripening (EC 75), and full maturity (EC 91), respectively. After removing the leaf sheaths, the main shoot of each plant was washed, and a 2-cm piece (EC 21-25) or 10-cm piece (EC 31-91), respectively, was cut directly above the roots. All shoot or stem pieces per plot were sampled, freeze dried, and ground in a mill (pore size 0.5 mm, Cyclotec sample mill, Tecator, Höganäs, Sweden). The ground plant material was homogenized with 1 ml of phosphate-buffered saline per 0.1 g of dry matter with a hand homogenizer (Linaris, Basel, Switzerland) and stored at −20 C until ELISA determinations.

For measuring *M. nivale* protein content, a recently developed ELISA described in detail by Höxter et al (11) was used. Total protein fraction extracted from shake cultures of *M. nivale* var. *nivale* served as antigen for immunization of rabbits and the purified antibodies were used for an indirect ELISA. The antibodies reacted in a highly sensitive manner to a wide array of *M. nivale* isolates. The detection limit, defined as the concentration of *M. nivale* lyophilized mycelium producing absorbance

values ≥0.1 at 405 nm, was 0.02 μg mycelium ml^{-1} in buffer and 0.3 μg mycelium ml^{-1} in plant sap. No cross-reaction of antibodies with host plant protein or mycelium of six *Fusarium* spp. (*F. culmorum, F. graminearum, F. avenaceum, F. poae, F. tricinctum, F. sporotrichoides*) or *Pseudocercosporella herpotrichoides* was observed (11). In the first two growth stages (EC 21, EC 25), the amount of *M. nivale* protein within host tissue reached such high values that plant homogenates had to be more diluted with buffer to confine ELISA absorbance to a 0-1 scale. To compensate for the different dilution factors, an ELISA coefficient was calculated (ELISA absorbance at 405nm × dilution factor)/sample weight (mg).

For detection of *Fusarium* spp. protein, a recently developed indirect ELISA described by Beyer et al (1) was used. Antibodies were raised by immunization of rabbits with total soluble protein of still cultures of *F. culmorum*. The purified antibodies showed no significant cross-reaction with host plant protein or mycelium of *M. nivale, Pseudocercosporella herpotrichoides, Cochliobolus sativus,* and *Ceratobasidium cereale*. The detection limit, defined as the concentration of *Fusarium* protein from lyophilized mycelium producing absorbance values ≥ 0.1 at 405 nm, was 0.2 μg protein ml^{-1} in buffer and 0.5 μg protein ml^{-1} in plant sap. In this ELISA, the same dilution factor could be used for all host growth stages. The original ELISA absorbance values are given in the Results. The *Fusarium* ELISA detected *F. culmorum, F. graminearum, F. avenaceum, F. crookwellense, F. equiseti, F. poae,* and *F. sporotrichoides* with the same sensitivity. In all experiments analyzed by ELISA, noninoculated, healthy inbred lines grown in a glasshouse and harvested in the respective growth stages served as a control and produced absorbance values ≤0.1 at 405 nm.

To determine the incidence of the major foot rot pathogens in the two experiments, 100 stems per replicate with foot rot lesions were arbitrarily sampled at milk ripening (EC 75). The lesions were cut out, surface disinfested, and incubated on a synthetic nutrient-poor agar as recently described (16). The growing colonies were identified according to their conidial morphology.

Foot rot rating. The stems sampled for ELISA at milk ripening (EC 75) were also scored for foot rot lesions using a 1–9 scale, where 1 = no lesion visible, 2 = pinpoint lesion, 3 = lesion covering up to 1/4 of the stem circumference, 4 = lesion covering about 1/4 to 1/2 of the stem circumference, 5 = lesion covering 1/2 to 3/4 of the stem circumference, 6 = lesion covering more than 3/4 of the stem circumference, 7 = lesion girdling the stem, no softening of the tissue, 8 = lesion girdling the stem, moderate softening of the tissue, 9 = stem fully necrotic and softened.

Statistical analyses. Analyses of variance were calculated from single-plot data at individual growth stages. For each environment, errors were homogeneous and no deviations from normality were found (20). For estimating host genotype–growth stage interaction, the various growth stages of the *Fusarium* experiments were analyzed as a series of experiments (5). Repeatability and broad-sense heritability estimates (h^2) (8) were based on entry means across replications (in individual environments) and across environments, respectively (8,24). Coefficients of genotypic correlation as defined by Falconer (8) were calculated among host growth stages; determination of their standard errors followed established procedures (17). Genotypic correlations and analyses of variances were computed with the program packages PLABCOV

TABLE 1. Incidence of *Fusarium culmorum*, other *Fusarium* spp., *Microdochium nivale*, and *Pseudocercosporella herpotrichoides* in plants inoculated with *M. nivale* or *F. culmorum*

Location[a]	Inoculated fungus	Year	Isolated fungi (%)			
			Fusarium culmorum	*Fusarium* spp.	*Microdochium nivale*	*Pseudocercosporella herpotrichoides*
OLI	*M. nivale*	1990	1.3	37.4	41.3	10.7
		1991		20.0	32.0	58.0
HOH	*F. culmorum*	1991	98.0	22.8	0.0	14.0
		1992	92.8	20.3	9.8

[a] OLI = Oberer Lindenhof, HOH = Hohenheim.

and PLABSTAT, respectively (21,22). The effects of replicates, genotypes, and years were assumed to be random.

RESULTS

Frequency of isolation of foot rot fungi. Among the *M. nivale*-inoculated plants, this fungus was isolated with an incidence of about 50% across both years (Table 1). *Fusarium* spp. occurred quite frequently in the same samples. *Fusarium culmorum* was found in high frequencies (>90%) in plants artificially inoculated with this pathogen at HOH in both years; other *Fusarium* spp. played a minor role. Thus, the results based on ELISA should be valid for *F. culmorum. Pseudocercosporella herpotrichoides* occurred in low frequencies in all experiments. Thirty to 36% of all necrotic stems examined were infected with more than a single pathogen.

Microdochium nivale. In both years, the highest amount of *M. nivale* protein was detected at the onset of tillering (EC 21) just after snow melt (Fig. 1). During rapid growth of the host plants from stem elongation to heading (EC 31–EC 51), the fungal protein content in the plant homogenate decreased substantially. At anthesis (EC 65), the fungal protein content started to increase again until full maturity (EC 91). In both years, the highest genotypic standard deviation was found at the earliest growth stage (EC 21); it then dropped and only at later growth stages did it increase again (Table 2). Combined over years, only EC 21 and EC 75 showed significant genotypic variation. Heritability estimates were medium ($h^2 \approx 0.6$) at those growth stages exhibiting significant genotypic variation over both years. Mean foot rot rating at milk ripening was 3.7 in 1990 and 2.8 in 1991 on a 1–9 scale. Significant genotypic variation for foot rot rating, however, was observed in 1991 only. ELISA absorbance values and foot rot ratings at EC 75 were not significantly correlated ($r = 0.54$). Genotype–growth stage interaction was significant when computed over those growth stages with significant genotypic variation in both years (EC 21 and EC 75, Table 3). Coefficients of phenotypic correlation among growth stages for ELISA coefficients were low, ranging from $r = -0.25$ to $r = 0.41$ for both years. Slightly higher correlations were observed in 1991 between the successive stages EC 21 and EC 25 ($r = 0.62$, $P = 0.05$) and between EC 75 and EC 91 ($r = 0.54$, $P = 0.1$). Between EC 21 and EC 75 a significantly negative correlation occurred ($r = -0.66$, $P = 0.05$).

Fusarium spp. The fungal protein content of *Fusarium* spp. increased continuously during the growing season, reaching the highest values at full maturity (Fig. 2). Mean ELISA absorbance values during EC 21 to EC 65 were considerably higher in 1992 than in 1991. At the two l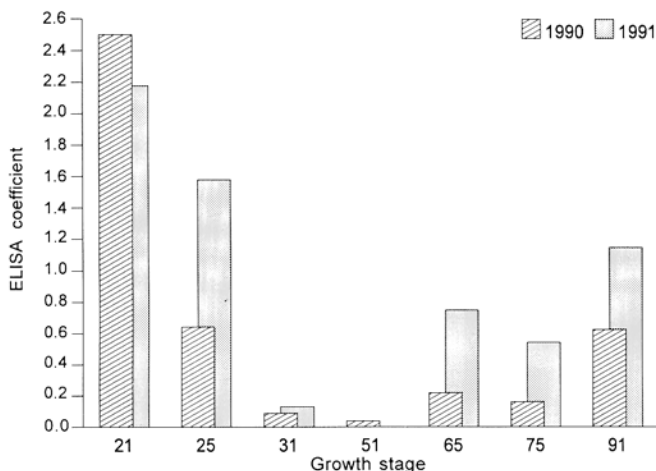atest growth stages, however, almost the same amount of *Fusarium* protein was detected in both years. Significant genotypic variation of ELISA absorbance values was observed at all growth stages except EC 31 in both years (Table 4). The genotypic variance tended to increase from EC 25 to EC 75. Heritability estimates were medium to high (0.6–0.9). Combined over both years, all growth stages showed significant genotypic variation. Foot rot rating at milk ripening averaged 4.0 in 1991 and 3.7 in 1992 on a 1–9 scale and detected significant genotypic variation in both years with a high heritability estimate. ELISA absorbance values and foot rot ratings were correlated at EC 75 with $r = 0.73$ ($P = 0.05$) averaged over both years. Coefficients of phenotypic and genotypic correlation between early- and adult-plant growth periods were generally low and not significant (Table 5). Moderate to high genotypic associations were found among the later growth stages (EC 51, EC 65, and EC 75). ELISA absorbance values at full maturity (EC 91) were correlated significantly with those at EC 75, but not with absorbance values at other growth stages.

Calculated over all tested growth stages, a highly significant genotypic variance occurred in both years (Table 6). Genotype–growth stage interaction variances were significant, too, and three to four times greater than genotypic variance. Combined over years, genotypic variance was no longer significant and genotype–growth stage interaction variance showed significance only at the 10% level. All interactions with year, however, were highly significant. Calculated over the adult-plant period from EC 51 to EC 75 only, genotypic variance was significant within and combined over years (Table 6). Genotype–growth stage interaction variance was not significant across adult-plant stages in the 2-yr analysis. Also, genotype–growth stage–year interaction variance

TABLE 2. Estimates of genotypic standard deviation (σ_g) within and combined over 2 yr[a]

Trait/plant growth stage[b]	1990	1991	1990 + 1991	h^2
		σ_g		
ELISA coefficient				
Tillering (EC 21)	1.743[+c]	0.760**	1.202[+]	0.59
Mid-tillering (EC 25)	—[d]	0.651**	0.349	0.33
Jointing (EC 31)	0.029	0.018	0.006	0.05
Heading (EC 51)	0.022	ND[e]	ND[e]	ND[e]
Anthesis (EC 65)	0.066	0.247**	0.108	0.44
Milk ripening (EC 75)	0.039[+]	0.107*	0.068[+]	0.61
Full maturity (EC 91)	0.070[+]	0.109[+]	—[d]	—[d]
Foot rot rating[f]				
Milk ripening (EC 75)	0.25	0.29**	0.24	0.50

[a] With broad-sense heritability (h^2) for enzyme-linked immunosorbent assay (ELISA) coefficient (ELISA absorbance at 405 nm × dilution factor/sample weight) and foot rot rating of 12 winter rye inbred lines inoculated with *Microdochium nivale*.
[b] On the Zadoks scale (25).
[c] +, *, and ** = significant genotypic variation (*F* test) at probability levels $P = 0.10$, 0.05 and 0.01, respectively.
[d] Negative estimate.
[e] No data in 1991.
[f] Based on a 1 (no lesion visible) to 9 (stem fully necrotic and softened) scale.

TABLE 3. Estimates of variance components (σ^2) over 2 yr[a]

Source of variation	Degrees of freedom	σ^2
Genotype (G)	11	0.378[+b]
G × Growth stage (S)	11	0.683*
G × Year (Y)	11	0.185
S × Y	1	0.035
G × S × Y	11	0.009
Pooled Error	52	0.814

[a] For enzyme-linked immunosorbent assay (ELISA) coefficient (ELISA absorbance at 405 nm × dilution factor/sample weight) of young- (EC 21) vs. adult-plant (EC 75) growth stages for 12 winter rye inbred lines inoculated with *Microdochium nivale*.
[b] + and * = significant at probability levels $P = 0.1$ and 0.05, respectively.

Fig. 1. Enzyme-linked immunosorbent assay (ELISA) coefficients (ELISA absorbance at 405 nm × dilution factor/sample weight) of 12 winter rye inbred lines at successive host growth stages in field experiments inoculated with *Microdochium nivale* at Oberer Lindenhof in 1990 and 1991. Growth stage on the Zadoks (25) scale.

was no longer significant. Genotype-year and growth stage–year interaction variances were highly significant.

DISCUSSION

Sensitive and specific ELISA techniques allow analysis of fungal colonization in situ during the entire growing season. This is especially important for field experiments in which mixtures of foot rot pathogens, varying with location and year, are often observed (7,16,18,23). The isolation frequencies reported here confirm this. Among the *M. nivale*-inoculated plants, this fungus was isolated from about 50% of all necrotic stems at milk ripening. This might be attributable to a reduced competitiveness of *M. nivale* in adult host growth stages and does not necessarily indicate a low infection frequency during winter. In contrast, ELISA detected such high amounts of *M. nivale* protein in samples taken in spring (EC 21, EC 25) that plant homogenates had to be diluted 10 times more than in the other growth stages. The simultaneous occurrence of *Fusarium* spp. in the *M. nivale*-inoculated plants at milk ripening (Table 1) means that both pathogen groups contributed to foot rot ratings. This might explain the moderate correlation between foot rot ratings and ELISA values for *M. nivale*. However, ELISA values are not affected by this mixed fungal colonization because the *M. nivale* ELISA shows no cross-reactions with *Fusarium* spp. (11).
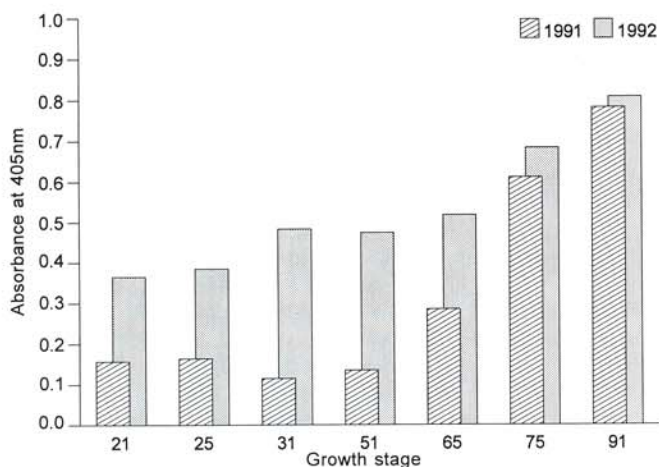

Fig. 2. Enzyme-linked immunosorbent assay absorbances (at 405 nm) of 11 winter rye inbred lines at successive host growth stages in field experiments inoculated with *Fusarium culmorum* at Hohenheim in 1991 and 1992. Growth stage on the Zadoks (25) scale.

The results of this study clearly demonstrated different behaviors of *M. nivale* and *F. culmorum*. Infection by *M. nivale* is favored by snow falling on lightly frozen or unfrozen soil (3). The snow cover maintains high humidity, prevents photosynthesis of the host, and protects fungal mycelium from sunlight (2). Accordingly, shortly after winter (EC 21) we found the highest ELISA coefficient, as well as significant genotypic variation. During intensive growth of the plants in spring, *M. nivale* protein content in the stem base of winter rye decreased to a minimum in reference to plant dry matter. This may be caused by a faster growth of the host relative to that of *M. nivale* during the period from tillering to anthesis. Additionally, the rapid growth of the host may activate mechanisms restricting pathogen growth. This should be especially valid when basic processes of host physiology play an important role in *M. nivale* resistance, as proposed by Bruehl and Cunfer (2). *Microdochium nivale* protein content increased again from anthesis to maturity as the stem base was aging. At milk ripening, *M. nivale* protein could be detected at the stem base by ELISA, illustrating that this pathogen also colonizes host tissue at adult-plant stages. It cannot be decided from our data whether the mycelium penetrating during the adult-plant growth period (EC 65–EC 91) had survived in the leaf sheaths infected during winter or whether it resulted from new soil-borne inoculum. Bruehl et al (3) previously observed that *M. nivale* occurs as a foot rot–inducing pathogen primarily when duration of snow cover is short. Indeed, in our experiments snow cover was about 4 wk in both years. Genotypes with high levels of colonization as seedlings may have had greater mortality and

TABLE 5. Coefficients of phenotypic (above diagonal) and genotypic (below diagonal) correlation among selected plant growth stages[a]

Plant growth stage[b]	Plant growth stage[b]					
	EC 21	EC 25	EC 51	EC 65	EC 75	EC 91
Tillering (EC 21)		−0.26	0.19	0.25	0.06	0.04
Mid-tillering (EC 25)	−0.32		0.06	0.09	0.20	0.29
Heading (EC 51)	0.25	−0.01		0.50	0.69*[c]	0.19
Anthesis (EC 65)	0.37	0.14	0.64[++]		0.62*	0.07
Milk ripening (EC 75)	0.10	0.26	0.86[++]	0.73[++]		0.42
Full maturity (EC 91)	0.08	0.35	0.23	0.09	0.45[+]	

[a] For enzyme-linked immunosorbent assay (ELISA) absorbance of 11 inbred lines inoculated with *Fusarium culmorum* averaged over 2 yr (correlations with EC 31 were not computed since no significant genotypic variation occurred between genotypes).
[b] On the Zadoks scale (25).
[c] * = significant genotypic variation (*F* test) at probability level *P* = 0.05; [+], [++] = estimate higher than once or twice its standard error, respectively.

TABLE 6. Estimates of variance components (σ^2, multiplied by 10) within and combined over two yr[a]

Source of variation	EC 21–EC 91		EC 51–EC 75	
	Degrees of freedom	σ^2	Degrees of freedom	σ^2
1991				
Genotype (G)	10	0.017**[b]	10	0.058**
G × Growth stage (S)	60	0.045**	20	0.061**
Error	198	0.032	66	0.043
1992				
Genotype (G)	10	0.013**	10	0.031**
G × S	60	0.048**	20	0.010
Error	198	0.069	66	0.083
1991 + 1992				
Genotype (G)	10	0.006	10	0.022[+]
G × S	60	0.012[+]	20	0.007
G × Year (Y)	10	0.009**	10	0.022**
S × Y	6	0.069**	2	0.071**
G × S × Y	60	0.035**	20	0.028
Error	394	0.050	131	0.063

[a] For enzyme-linked immunosorbent assay (ELISA) absorbance of all host growth stages (EC 21–EC 91) vs. adult-plant growth period (EC 51–EC 75) of 11 inbred lines inoculated with *Fusarium culmorum*.
[b] [+] and ** = significant at probability levels *P* = 0.10 and 0.01, respectively.

TABLE 4. Estimates of genotypic standard deviation (σ_g) within and combined over 2 yr[a]

Trait/plant growth stage[b]	σ_g			h^2
	1991	1992	1991 + 1992	
ELISA absorbance (405 nm)				
Tillering (EC 21)	0.018*[c]	0.031*	0.015*	0.62
Mid-tillering (EC 25)	0.016*	0.035*	0.015*	0.61
Jointing (EC 31)	0.004	0.042	0.031*	0.58
Heading (EC 51)	0.013[+]	0.057**	0.029**	0.76
Anthesis (EC 65)	0.097**	0.044[+]	0.057**	0.77
Milk ripening (EC 75)	0.128**	0.084**	0.101**	0.91
Full maturity (EC 91)	0.088**	0.143**	0.095**	0.90
Foot rot rating[d]				
Milk ripening (EC 75)	0.31**	0.98**	0.56**	0.88

[a] With broad-sense heritability (h^2) for enzyme-linked immunosorbent assay (ELISA) absorbance and foot rot rating of 11 winter rye inbred lines inoculated with *Fusarium culmorum*.
[b] On the Zadoks scale (25).
[c] [+], *, and ** = significant genotypic variation (*F* test) at probability levels *P* = 0.10, 0.05, and 0.01, respectively.
[d] Based on a 1 (no lesion visible) to 9 (stem fully necrotic and softened) scale.

could not be sampled later. Resulting survivors might have had lower infection as seedlings, but exhibited a greater colonization in later growth stages. This would explain the significant negative correlation between EC 21 and EC 75 in 1991 ($r = -0.66$, $P = 0.05$). Accordingly, Cook (6) reported important *M. nivale* foot rot in wheat from areas with little or no snow cover during winter, but long, cool, and wet periods during stem elongation. In our study, significant genotypic variation for resistance appeared again in the adult-plant growth period with highest heritability at EC 75. However, the genotypic standard deviation at EC 75 did not nearly approach the high value observed at EC 21 over both years. Summarizing *M. nivale* results, greatest genotypic variation among both years was found in the earliest host growth stage (EC 21) when plants just had survived snow cover. Phenotypic correlation between EC 21 and EC 25 was one of the highest found in our study ($r = 0.62$, $P = 0.05$). The nonsignificant correlation between young- (EC 21) and adult-plant (EC 75) growth stages was most probably caused by a high genotype–growth stage interaction. This illustrates that resistance to snow mold and *M. nivale* foot rot are likely attibutable to different mechanisms in the host.

For *Fusarium* spp., mycelial colonization started at the earliest host growth stages and increased continuously till full maturity. In 1992, high ELISA absorbance was found as early as EC 21 owing to unusually mild winter and spring temperatures. Differences of ELISA values between both years decreased considerably in the two latest host growth stages, probably because the higher temperatures during early summer favor rapid *Fusarium* growth even if colonization in early growth stages is low. In contrast to *M. nivale, Fusarium* protein content did not decrease during jointing. Obviously, growth of this pathogen increases even during periods of intensive host growth. Despite the differing ELISA absorbance values at the early stages between the two years, at milk ripening almost the same protein content was found. The ELISA results indicate that *Fusarium* spp. are potent colonizers of intact, growing host tissue and can penetrate successively new leaf sheaths. This is in contrast to earlier findings (4) reporting that colonization of stem bases by *F. culmorum* was just a consequence of an early *P. herpotrichoides* attack or of host weakness attributable to unfavorable environmental conditions. When data over years and all growth stages were pooled, genotype–growth stage interaction was important. However, when calculated over the adult-plant period (EC 51-EC 75) only, this source of variation was less marked, as also illustrated by moderately strong genotypic correlations among the respective growth stages. Resistance selection for Fusarium foot rot, therefore, could have started with the onset of heading (EC 51) using ELISA. However, genotypic variance increased considerably at later stages, reaching a maximum at EC 75 and EC 91. In this late growth period, the highest heritabilities were also observed. The considerably weaker phenotypic and genotypic correlations of EC 91 to the other adult-plant growth stages EC 51, EC 65, and EC 75 for *Fusarium* protein content in our experiments might partly be caused by stimulated saprophytic growth of the pathogen in the ripening stem occurring independently from host resistance. Thus, the optimum growth stage for resistance selection seems to be EC 75. Analogous results were found for the colonization of winter wheat by *P. herpotrichoides* in the greenhouse (14).

Significant genotypic differentiation existed at most growth stages for ELISA values for *F. culmorum*, but only at EC 21 and EC 75 for *M. nivale* calculated over both years. This finding demonstrates that the genotypes differed in their ability to restrict pathogen growth and should lead to a high gain in resistance selection at the respective growth stages. However, for both pathogens, ELISA absorbances in the early growth period (EC 21–EC 25) were not consistently correlated with those of the adult-plant period (EC 51–EC 91). Resistances assessed in the field were specific to the growth stage of the plants. In the greenhouse, similiar growth stage specific resistances were found recently for infection of winter wheat with *P. herpotrichoides* (14). Artificial inoculations of winter rye inbred lines at young-plant growth stages under controlled environmental conditions also revealed

that resistance to *M. nivale* (15), and to *Fusarium culmorum* and *F. graminearum* (12), strongly depended on the host growth stage at inoculation. Because foot rot pathogens need almost the whole growing season before the first symptoms become visible at or just after heading (9), resistance at the different growth stages might be caused by physiological changes in the host tissue during plant development. Bruehl and Cunfer (2) previously proposed that snow mold resistance in wheat involves fundamental processes of host physiology such as carbohydrate storage and utilization, or the maintenance of a minimum level of metabolism under snow cover. Accordingly, Miedaner et al (15) reported that winter rye inbred lines without hardening prior to artificial inoculations with *M. nivale* were considerably less resistant than those pre-cultivated at cool temperatures. For *P. herpotrichoides* infections in the adult-plant growth period of winter wheat, Murray and Bruehl (19) found significant correlations among host resistance and various anatomical traits in the noninoculated plants, such as thickness of hypodermis and lignification of cell walls. These findings imply that preformed resistance mechanisms might play an important role in resistance to snow mold and fungi that induce foot rot. Expression of those resistances depends highly on host growth stage; this could, therefore, be the cause for the important genotype–growth stage interaction found for resistance to *M. nivale* and *F. culmorum* in our study.

In conclusion, *M. nivale* was shown to most effectively colonize host tissue during winter in the earliest growth periods. However, a considerable amount of protein of this pathogen was also found in adult plants causing foot rot symptoms. For *F. culmorum*, mean ELISA absorbance values increased steadily from the onset of tillering to full maturity. Although the tested inbred lines showed significant differences in ELISA absorbance values at most growth stages, strong genotype–growth stage interactions were found for resistances to both *M. nivale* and *F. culmorum* when analyses were calculated over the whole growing season. This indicates that resistance is caused by different mechanisms during host growth. The complex relationships between pathogen growth and host metabolism prevent a prediction of adult-plant resistance at early growth stages or vice versa. However, both pathogens could be found by ELISA at all host growth stages tested. To select cultivars with a reliable level of *M. nivale* or *Fusarium* resistance at the basal parts of winter rye during their whole life span, it is necessary either to conduct several resistance tests at different host growth stages or to monitor fungal protein content quantitatively in host genotypes by ELISA during the whole growing season, as reported here, and select only those genotypes that show low absorbance values at all economically relevant host growth stages.

LITERATURE CITED

1. Beyer, W., Höxter, H., Miedaner, T., Sander, E., and Geiger, H. H. 1993. Indirect ELISA for quantitative assessment of *Fusarium* spp. in rye. Z. Pflanzenkrankh. Pflanzenschutz 100:278-284.
2. Bruehl, G. W., and Cunfer, B. 1971. Physiologic and environmental factors that affect the severity of snow mold of wheat. Phytopathology 61:792-799.
3. Bruehl, G. W., Sprague, R., Fischer, W. R., Nagamitsu, M., Nelson, W. L., and Vogel, O. A. 1966. Snow molds of winter wheat in Washington. Wash. Agric. Exp. Stn. Bull. 677:1-21.
4. Butler, F. C. 1953. Saprophytic behaviour of some cereal root-rot fungi. I. Saprophytic colonization of wheat straw. Ann. Appl. Biol. 40:284-297.
5. Cochran, W. G., and Cox, G. M. 1957. Experimental Designs. 2nd ed. John Wiley & Sons, NY.
6. Cook, R. J. 1981. *Fusarium* diseases of wheat and other small grains in North America. Pages 39-52 in: *Fusarium* - Diseases, Biology, and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. The Pennsylvania State University Press, University Park.
7. Duben, J., and Fehrmann, H. 1979. Vorkommen und Pathogenität von *Fusarium*-Arten an Winterweizen in der Bundesrepublik Deutschland. I. Artenspektrum und jahreszeitliche Sukzession an der Halmbasis. Z. Pflanzenkrankh. Pflanzenschutz 86:638-652.
8. Falconer, D. S. 1989. Introduction to Quantitative Genetics. 3rd ed. Longman, NY.

9. Fehrmann, H. 1988. *Fusarium culmorum*. Pages 287-289 in: European Handbook of Plant Diseases. I. M. Smith, J. Dunez, D. H. Phillips, R. A. Lelliot, and S.A. Archer, eds. Blackwell Scientific Publ., Oxford.

10. Geiger, H. H. 1985. Hybrid breeding in rye (*Secale cereale* L.). Pages 237-265 in: Proc. Eucarpia Meeting of the Cereal Section on Rye. Part I. Svalöf, Sweden. 11-13 June 1985. Svalöf AB Publishing, Svalöf, Sweden.

11. Höxter, H., Miedaner, T., Sander, E., and Geiger, H. H. 1991. Quantitative assessment of *Microdochium nivale* in rye with ELISA. Z. Pflanzenkrankh. Pflanzenschutz 98:13-17.

12. Höxter, H., Sieber, G., Miedaner, T., and Geiger, H. H. 1992. Test systems for evaluating quantitative resistance to Fusarium foot rot in inbred lines of winter rye. Plant Breeding 108:274-282.

13. Jenkins, J. E. E., Clark, W. S. and Buckle, A. E. 1988. *Fusarium* diseases of cereals. HGCA Research Review No. 4, Home-Grown Cereals Authority, London.

14. Lind, V. 1992. Measuring the severity of eyespot disease induced by *Pseudocercosporella herpotrichoides* (Fron) Deighton in wheat cultivars at different growth stages. Plant Breeding 108:202-209.

15. Miedaner, T., Höxter, H., and Geiger, H. H. 1993. Development of a resistance test for winter rye to snow mold (*Microdochium nivale*) under controlled environment conditions in regard to field inoculations. Can. J. Bot. 71:136-144.

16. Miedaner, T., Ludwig, W. F., and Geiger, H. H. 1995. Inheritance of foot rot resistance in winter rye. Crop Sci. (In press.)

17. Mode, C. J., and Robinson, H. F. 1959. Pleiotropism and the genetic variance and covariance. Biometrics 15:518-537.

18. Moser, H., Ludwig, W. F., Miedaner, T., and Geiger, H. H. 1989. Basic studies towards breeding rye resistant to foot rot fungi in: Proc. Eucarpia Congr., 12th. Göttingen, Germany. 27 Feb. - 4 Mar. 1989. Vorträge für Pflanzenzüchtung 15:6-4. Th. Mann Publishers, Gelsenkirchen-Buer, Germany.

19. Murray, T. D., and Bruehl, G. W. 1983. Role of the hypodermis and secondary cell wall thickening in basal stem internodes in resistance to strawbreaker foot rot in winter wheat. Phytopathology 73:261-268.

20. Snedecor, G. W., and Cochran, W.G . 1967. Statistical Methods. Iowa State University Press, Ames.

21. Utz, H. F. 1991. PLABCOV: A computer program for the computation of variances and covariances. Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany.

22. Utz, H. F. 1991. PLABSTAT: A computer program for the statistical analysis of plant breeding experiments. Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany.

23. Wiese, M. V. 1987. Compendium of Wheat Diseases, 2nd ed. American Phytopathological Society, St. Paul, MN.

24. Wricke, G., and Weber, W. E. 1986. Quantitative Genetics and Selection in Plant Breeding. De Gruyter, Berlin.

25. Zadoks, J. C., Chang, T. T., and Kunzak, C. F. 1974. Decimal code for growth stages of cereals. Weed Res. 14:415-421.