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Differential interaction of Mycosphaerella brassicicola and brassica cultivars

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Abstract

Isolates of *Mycosphaerella brassicicola*, originating from various locations in Europe, differed in their virulence on a differential set of brassica cultivars, as measured by the number of lesions per leaf. Hypersensitivity and significant cultivar-isolate effects were observed, indicating a differential host–pathogen interaction. Although expression of resistance depends on plant development, the differential host–pathogen interaction was found in all plant stages tested. This is the first report on the existence of physiological specialization of *M. brassicicola*.

Additional keywords: *Brassica*, epidemiology, resistance, ringspot.

Introduction

The ringspot disease of brassica crops is restricted to areas with high humidity and moderate temperatures. The disease is not only found in coastal areas, as in north-west Europe and Australia (Nelson and Pound, 1959), but also in certain mountain areas in more tropical climates (Punithalingam and Holliday, 1975; Frinking and Geerds, 1987; Gonzalez and Montealegre, 1987).

Ringspot of brassica crops is caused by *Mycosphaerella brassicicola* (Duby) Lindau. For dispersal and infection the fungus depends on ascospores. No imperfect stage of *M. brassicicola* is known (Dring, 1961; Zornbach, 1990). The presence of ringspot in isolated geographical regions with specific climatic conditions for the development of the disease, and the dependence of the fungus on its sexual stage, may have resulted in the development of different populations of the fungus with specific genetic characteristics.

The host range of *M. brassicicola* is not restricted to varieties (in the botanical sense) of *Brassica oleracea* L. only. Varieties of *Brassica campestris* L. and *Brassica napus* L. are also susceptible to ringspot disease (Nelson and Pound, 1959). It is not known whether isolates of *M. brassicicola* differ in their adaptation to different varieties within one species. Previous studies showed that differences in levels of resistance between cultivars of one variety may occur (Zornbach, 1990; Van den Ende, 1992), but physiological specialization of the fungus has not yet been reported (Dixon, 1981).

Specificity implies that genetic variation in the host and the pathogen are correlated (Vanderplank, 1982). When pathogenic races can attack some but not all cultivars of the host, resistance is expressed in a qualitative way and distinct races can easily be identified.

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If all relevant races of the pathogen can attack all relevant cultivars of the host, physiological specialization may be based on quantitative differences in disease expression. Several factors influence disease expression, and therefore affect conclusions with respect to specificity. Disease development of ringspot on brassica plants depends on leaf age (Van den Ende, in preparation). If fully developed leaves or cotyledons are present on susceptible plants of brassica spp., plants can be infected by *M. brassicicola* (Hartill and Sutton, 1980). Testing of plants in different stages of development can reveal an age-cultivar-isolate interaction.

Differential adaptation of pathogen isolates to certain host genotypes can complicate screening strategies for the selection of disease-resistant host varieties. Knowledge of the virulence structure of the pathogen population will therefore be of value in developing effective strategies for breeding for resistance to this disease. To test the hypothesis that physiological specialization of *M. brassicicola* does exist, several isolates of the fungus obtained from different European regions were tested on cotyledons and on plants in the third and seventh leaf stage of brassica cultivars.

Materials and methods

Biological material. Isolates evaluated in these experiments were taken from different geographic regions and from different cultivars of *B. oleracea* (Table 1). Eight single spore isolates of *M. brassicicola* were obtained from infected leaves from the field. To collect the spores, lesions were cut out of infected leaves and soaked in demineralized water. After 30 min lesions were placed on wet filter paper in the lid of a petri dish. The bottom of the petri dish was filled with a thin layer of water agar. After sealing the petri dish with parafilm, it was placed upside down under light (8 000 lux) at 15 °C. Within a few days (1–2) the ascospores were shot into the water agar and could be transferred to growing media. Isolates were grown on V8 agar (Miller, 1955) at 17 °C under alternating light (12 h UV (380 nm) – 12 h dark) conditions.

Brassica cultivars were provided by Dutch breeding companies. Susceptibility levels for *M. brassicicola* under field conditions in the Netherlands are known for most cultivars (Table 2). Plants were grown in polyethylene pots in the greenhouse at 17–20 °C in daylight, supplemented with artificial light (Philips, HPIT, 400 W). A potting mixture was used consisting of a decomposed sphagnum peat to which some clay and marl were added (TRIO 17; pH 5.4; organic matter 74%). Beginning at the 2-leaves stage, fertilizer was added weekly (Kristalon blue: 19% N, 6% P, 20% K, 3% Mg).

Table 1. Identity of isolates used in the study of physiological specialization in *Mycosphaerella brassicicola*.

Isolate	Region	Host
NH	the Netherlands	*Brassica oleracea* var. *capitata*
OP	the Netherlands	*Brassica oleracea* var. *botrytis*
FR	France	*Brassica oleracea* var. *botrytis*
GE	Germany	*Brassica oleracea* var. *capitata*
DK	Denmark	*Brassica oleracea* var. *capitata*
UK	United Kingdom	*Brassica oleracea* var. *gemmifera*

Table 2. Cultivars of brassica species with known field resistance against the Dutch NH isolate, used in the study on physiological specialization in *Mycosphaerella brassicicola*.

Species and varieties	Cultivar	Level of resistance
Brassica oleracea var. *botrytis*	CF02	susceptible
	CF07	resistant
	CF08	susceptible
	CF09	susceptible
Brassica oleracea var. *gemmifera*	BS01	partially resistant
	BS04	susceptible
	BS05	resistant
	BS06	susceptible
Brassica oleracea var. *capitata*	CA01	susceptible
	CA02	resistant
	CA03	partially resistant
	CA04	partially resistant
	CA05	partially resistant
Brassica pekinensis	CC01	unknown
Brassica napus	OR01	unknown

Inoculum preparation. Inoculations were carried out with suspensions of mycelium according to Van den Ende (1992). Isolates were collected over a period of 6 months. Therefore, some reduction in virulence due to prolonged storage of some of the isolates could have occurred. To minimize variation caused by age differences of the cultures, fresh cultures were started prior to inoculation. After a growth period of 3-4 weeks, inoculum was produced by grinding mycelium of *M. brassicicola* in distilled water for 4 min in a micro blender. To ensure a high disease severity 3 g sucrose per 100 ml was added to the inoculum. A hemocytometer was used to estimate the density of infection units per ml.

Inoculation procedure. The inoculum was divided into parts, according to the number of replications in the experiment. Each part was sprayed separately onto a set of differential cultivars with a micro ulva (Micron Sprayers, LTD, Bromyard, England). One can argue about the validity of the replications because of the similarity of inoculum used in each replication. However, preparation of different inocula for each replication would create too much variability in the results, because standardization of mycelial suspensions is hardly possible. The experimental design was of a split-plot nature with individual isolates of *M. brassicicola* assigned to flats with plants as main plots, and cultivars randomly assigned to cells (one plant or pot per cell) within flats as subplots. Main plots were replicated.

After inoculation, plants were transferred to a growth chamber with constant temperature (15°C) and low light intensity (1 000 lux) (16 h light; 8 h dark). Pots with plants were covered with plastic bags to ensure a high humidity. After six days of high humidity plants were transferred to the greenhouse (17–20 °C). Light conditions were not standardized, as light does not influence symptom developement after penetration of the fungus (Van den Ende, 1992). The first symptoms could be read 18 to 24 days after inoculation. The optimal moment for disease asssessment is when the most susceptible cultivar approaches its maximum score (Parlevliet, 1989). At 28 or 29 days after inoculation disease severity was assessed as the number of lesions per leaf.

Experiments

Cotyledons (CO). For the inoculation of cotyledons, seedlings of cauliflower (CF02, CF07, CF08, CF09), cabbage (CA01, CA02, CA03, CA04, CA05), Brussels sprouts (BS04, BS05, BS06), Chinese cabbage (CC01) and oilseed rape (OR01) were grown in small polyethylene pots (7 × 7 × 6 cm). Each pot contained five seedlings. Ten days after sowing cotyledons were inoculated with mycelial suspensions of two isolates (NH and FR, Table 1). Per isolate mycelial suspensions were prepared by grinding eight colonies of 28 days old in 250 ml distilled water (NH: 1.4×10^5 infection units per ml, FR: 1.5×10^5 infection units per ml). Approximately 2 ml inoculum was applied per pot. Each cultivar was inoculated in six replications. As a control treatment one pot of each cultivar was treated with a 3% sucrose solution in distilled water. The average number of lesions per cotyledon per pot was assessed 29 days after inoculation.

Third leaf stage (TL). Plants of cauliflower (CF02, CF07, CF08, CF09), cabbage (CA01, CA02, CA03, CA04, CA05), Brussels sprouts (BS04, BS05, BS06) and Chinese cabbage (CC01) were grown in small polyethylene pots (7 × 7 × 6 cm). Each pot contained one plant. Plants in the third leaf stage (third leaf unfolded and the fourth leaf just visible) were inoculated with mycelial suspensions of the isolates NH and FR. Per isolate mycelial suspensions were prepared by grinding 10 colonies of 20 days old in 250 ml distilled water (NH and FR: 0.7×10^5 infection units per ml). Approximately 3 ml inoculum was applied per plant. Each cultivar was inoculated in five replications. As a control treatment one plant of each cultivar was treated with water. After 29 days lesions on the first two leaves of each plant were counted.

Seventh leaf stage (SL). Plants of cauliflower (CF07, CF08, CF09), cabbage (CA01, CA02, CA03) and Brussels sprouts (BS04, BS06, BS07) were grown in polyethylene pots (10 × 10 × 12 cm). Each pot contained one plant. Plants in the seventh leaf stage (seventh leaf unfolded and eighth leaf just visible) were inoculated with mycelial suspensions of six isolates, NH, DK, FR, GE, OP, UK (Table 1). Per isolate mycelial suspensions were made by grinding 8 colonies of 28 days old in 100 ml distilled water. No estimations of infection units per ml were made. After 28 days lesions were counted on each of the leaves 4 to 7.

Statistical analyses. Data were analyzed using the Genstat computer software package (Genstat 5, release 2.1). Analysis of the raw data indicated a heterogeneous error. Therefore, disease severity means of each isolate–cultivar combination were subjected to an analysis of variance after logarithmic transformation of the data. The Genstat procedure for a split-plot design was used.

To study a possible leaf age effect on the isolate–cultivar interaction the log transformed data of the SL experiment were subjected to an analysis of variance using the Genstat procedure for a split-split-plot experiment.

Results

Cotyledons. In Fig. 1 data are represented as the average number of lesions per cotyledon. Statistical analysis on the log transformed data showed a significant cultivar–isolate interaction effect (Table 3). Main effect (isolate) and subplot effect (cultivar) are significant too. Considerable differences in susceptibility to the Dutch isolate (NH) of *M. brassicicola* existed between cultivars. These differences were not always in agree-

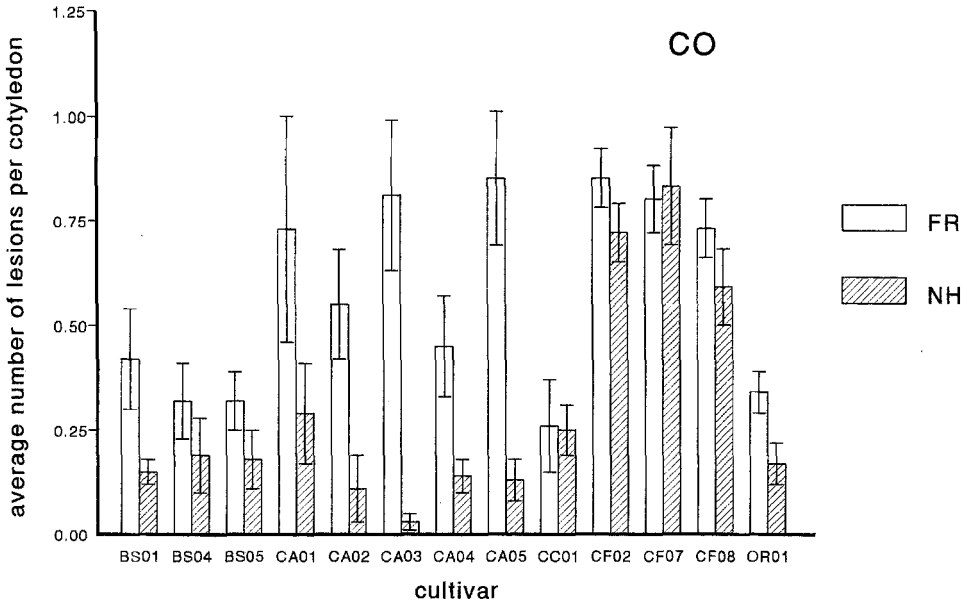


Fig. 1. Average number of lesions per cotyledon (CO) for two isolates of *Mycosphaerella brassicicola* (NH and FR) and 13 cultivars of Brussels sprouts (BS01, BS04, BS05), cabbage (CA01, CA02, CA03, CA04, CA05), chinese cabbage (CC01), cauliflower (CF02, CF07, CF08) and oilseed rape (OR01). Per bar standard errors are indicated.

Table 3. Analysis of variance of the log transformed average numbers of lesions per leaf on several brassica cultivars of three plant developmental stages caused by two isolates of *Mycosphaerella brassicicola* (NH and FR). Cotyledons (CO): 13 brassica cultivars. Third leaf stage (TL): 12 brassica cultivars. Seventh leaf stage (SL): 9 brassica cultivars.

Source of variation	CO		TL		SL	
	df[a]	MS	df[a]	MS	df[a]	MS
Replication	5	0.0060^{ns}	4	0.076^{ns}	3	0.18^{ns}
Isolate	1	0.29**	1	5.49**	1	0.60^{ns}
Main plot error	5	0.0036	4	0.21	3	0.071
Cultivar	12	0.45**	11	0.44**	8	1.49**
Cultivar • isolate	12	0.017**	11	0.28**	8	0.68**
Subplot error	109 (11)	0.0043	86 (2)	0.82	40 (8)	0.093
Total	145 (11)		117 (2)		63 (8)	

df = degrees of freedom.
MS = Mean Square.
[a] (. .) = number of missing values; ^{ns} = F-value is not significant; * = F-value significant for $P < 0.05$; ** = F-value significant for $P < 0.01$.

ment with the field data of the cultivars as provided by the breeding companies (Table 2). A striking difference in susceptibility to the Dutch (NH) and the French (FR) isolate existed between the cabbage cultivars (CA01 to CA05). Cultivars which are known to be resistant or partially resistant against the Dutch isolate of *M. brassicicola* (CA02, CA03, CA04 and CA05), showed high disease severity levels when tested with the French isolate. The cotyledons of Brussels sprouts (BS01, BS04, BS05) and cauliflower (CF07, CF08, CF09) did not show much difference in susceptibility for the two isolates. Cotyledons of chinese cabbage (CC01) and oilseed-rape (OR01) were susceptible to both the NH and FR isolates.

Third leaf stage. The number of lesions on the first two leaves per plant were averaged. In Fig. 2 the average number of lesions per leaf is shown. Statistical analysis on the log transformed data showed a significant cultivar–isolate interaction effect (Table 3). Main effect (isolate) and subplot effect (cultivar) were significant too. Differences in susceptibility between cultivars to the Dutch isolate (NH) were in agreement with the known field data (Table 2). Hypersensitive reactions as a result of infection with the NH isolate were found on the cabbage cultivars CA02 and CA03 and the Brussels sprouts cultivar BS05. Hypersensitive reactions were not found in the same cultivars when tested with the FR isolate, resulting in high numbers of lesions per leaf especially on the cabbage cultivars CA02 and CA03. Even in cauliflower, resistance to the NH isolate seemed to be ineffective against the FR isolate, as shown by the relatively high number of lesions on CF07 when tested with the FR isolate. Not all cultivars showed differences in disease severity

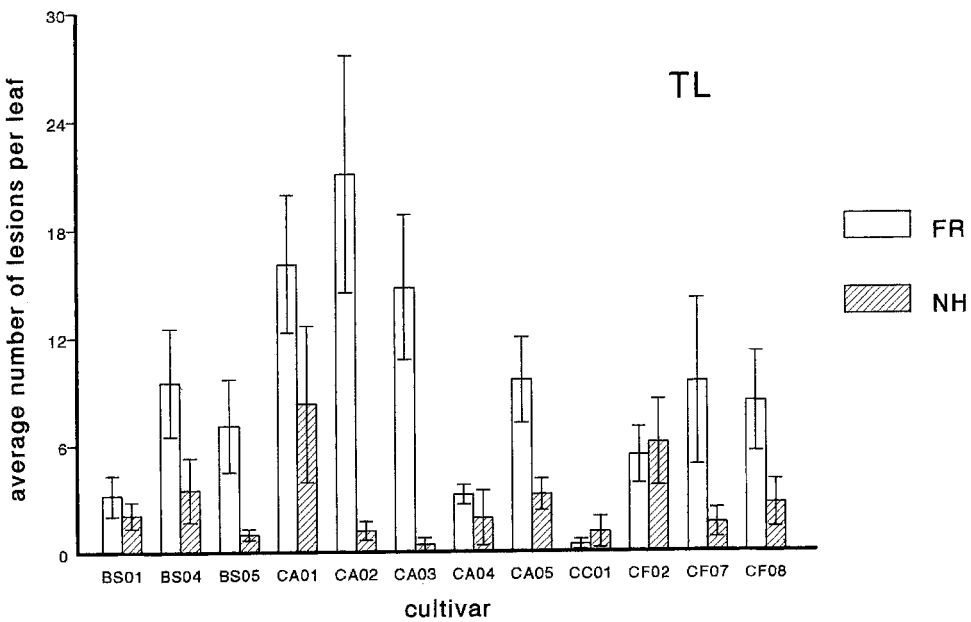


Fig. 2. Average number of lesions per leaf (TL) for two isolates of *Mycosphaerella brassicicola* (NH and FR) and 12 cultivars of Brussels sprouts (BS01, BS04, BS05), cabbage (CA01, CA02, CA03, CA04, CA05), chinese cabbage (CC01) and cauliflower (CF02, CF07, CF08). Per average number of lesions standard errors are indicated.

levels when tested with the NH and FR isolates. The partially resistant cultivars CA04 and BS01, which did not show hypersensitive reactions against the NH isolate but a relatively low number of lesions per plant, showed no significant differences between the NH and FR isolates.

Seventh leaf stage. Many of the lower leaves of the plants had withered or were dead at the time of assessment. Therefore, only the lesion numbers of leaves 4 to 6 were averaged. In Fig. 3 the average number of lesions per leaf is shown for the Dutch (NH) and French (FR) isolates. Statistical analysis of the log transformed data showed a significant cultivar–isolate interaction effect (Table 3). The main effect of isolates was not significant. Cultivars were significantly different. For the cabbage and Brussels sprouts cultivars differences in susceptibility between cultivars to the NH isolate were more pronounced than in the two previous experiments. These data were in agreement with the known field data (Table 2). Hypersensitive reactions were found in BS05, CA02 and CA03, but were absent when the cultivars were tested with the FR isolate. Results on the cauliflower cultivars showed a striking difference with the results found in the previous experiment (TL). The NH isolate showed a high number of lesions on the resistant CF07, whereas the inoculation with the FR isolate resulted in a low number of lesions per leaf. In younger plant stages no large differences could be found in disease severity of the cabbage cultivars when tested with the FR isolate (Fig. 1 and 2), but disease severity on the cabbage cultivars of older plant stages did differ (Fig. 3).

In the same experiment four other isolates (DK, GE, OP, UK) of *M. brassicicola*

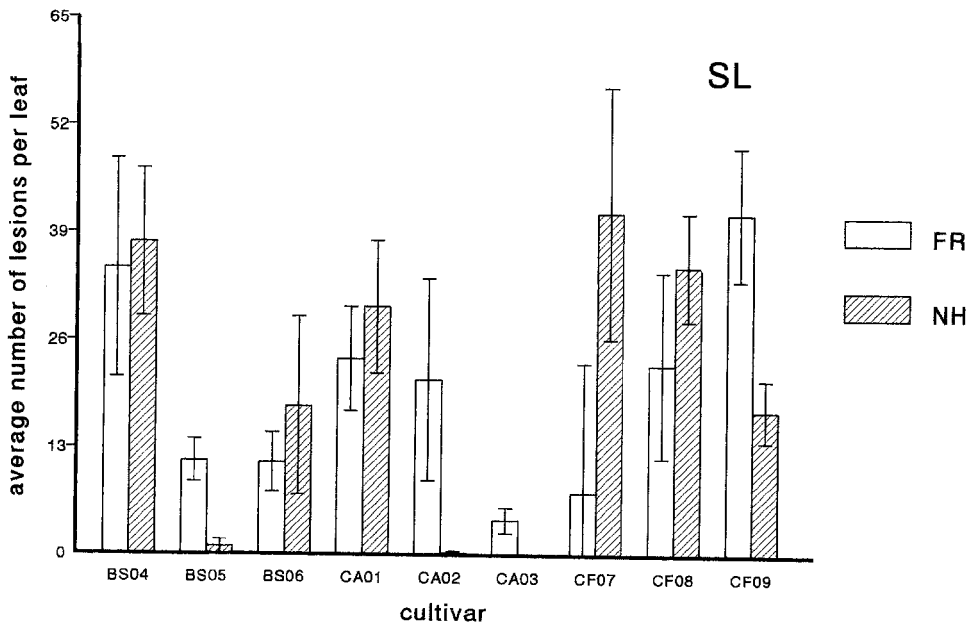


Fig. 3. Average number of lesions per leaf (SL) for two isolates of *Mycosphaerella brassicicola* (NH and FR) and 9 cultivars of Brussels sprouts (BS04, BS05, BS06), cabbage (CA01, CA02, CA03) and cauliflower (CF07, CF08, CF09). Per average number of lesions standard errors are indicated.

showed differential responses on the brassica cultivars too (Fig. 4). The analysis of all the transformed data of this experiment resulted in a significant cultivar–isolate interaction effect. Main effect (isolate) and subplot effect (cultivar) were also significant (Table 4). Except for the FR isolate, CA02, CA03 and BS05 showed hypersensitive reactions against all isolates. The cultivars in this experiment were all susceptible to the FR isolate, although differences in disease severity exists. The two isolates from the Netherlands (NH and OP) and the French isolate (FR) gave high disease severity levels on BS04, which was not the case for isolates from Denmark (DE), Germany (GE) and the United Kingdom (UK).

Influence of plant stage. The influence of leaf age on a possible isolate–cultivar interaction could be determined using the data from the experiment with plants in the seventh leaf stage at the time of inoculation. Transformed data of the average number of lesions per leaf from leaves 4 to 8 were subjected to a split-split-plot analysis of variance (Table 5). Main effects of isolate, cultivar and leaf age were present, and account for a high percentage of the total variance, as can be seen from the high magnitude of the respective mean sums of squares. Interaction effects of isolate–cultivar, isolate–age, age–cultivar and isolate–age–cultivar were significant.

To demonstrate the effect of leaf age, the average number of lesions per leaf for three leaf positions (4, 6, 8) are presented for two isolates (NH and FR) (Fig. 5). Resistance to the Dutch isolate (NH) in the cvs CA02, CA03 and BS05 was found in all three leaf levels, although some disease development took place at the oldest leaf level (leaf 4). In contrast,

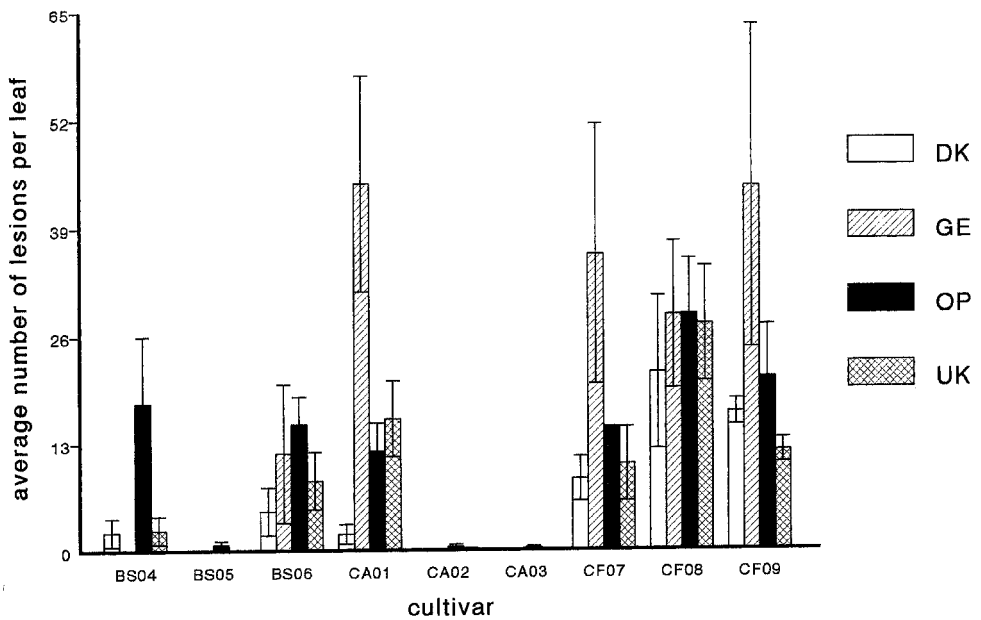


Fig. 4. Average number of lesions per leaf (SL) for four isolates of *Mycosphaerella brassicicola* (DK, GE, OP, UK) and 9 cultivars of Brussels sprouts (BS04, BS05, BS06), cabbage (CA01, CA02, CA03) and cauliflower (CF07, CF08, CF09). Per average number of lesions standard errors are indicated.

Table 4. Analysis of variance of the log transformed average numbers of lesions per leaf on nine brassica cultivars in the seventh leaf stage caused by six isolates (NH, FR, DK, GE, OP, UK) of *Mycosphaerella brassicicola*.

Source of variation	df[a]	MS
Replication	3	0.080[ns]
Isolate	5	1.62**
Residual	15	0.094
Cultivar	8	5.98**
Isolate • cultivar	40	0.40**
Residual	122 (22)	0.070
Total	193 (22)	

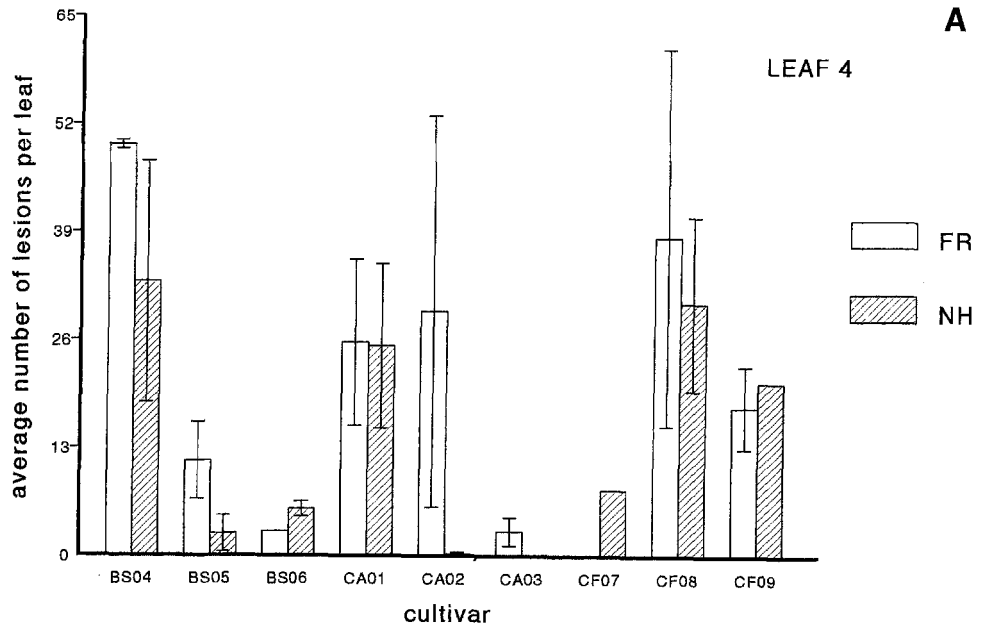
df = degrees of freedom.
MS = Mean Square.
[a] (. .) = number of missing values; [ns] = F-value is not significant; * = F-value significant for $P < 0.05$; ** = F-value significant for $P < 0.01$.

inoculation with the French isolate (FR) resulted in disease development in all three leaf levels of the same cultivars, although disease severity levels decreased with decreasing leaf age. On the oldest leaf level (leaf 4) CA03-FR showed a lower disease severity than CA02–FR, whereas in the younger leaf levels CA03–FR tended to a higher disease severity than CA02–FR.

Table 5. Analysis of variance for the log transformed numbers of lesions per leaf (5 different leaf ages per cultivar) on nine brassica cultivars caused by six isolates (NH, FR, DK, GE, OP, UK) of *Mycosphaerella brassicicola*.

Source of variation	df[a]	MS
Replication	3	0.83[ns]
Isolate	5	3.56**
Main plot error	15	0.23
Cultivar	8	14.19**
Isolate • cultivar	40	1.013**
Subplot error	143 (1)	0.30
Leaf age	4	18.47**
Isolate • leaf age	20	0.22**
Cultivar • leaf age	32	1.57**
Isolate • cultivar • leaf age	156 (4)	0.17**
Sub-sub plot error	527 (121)	0.085
Totaal	953 (126)	

df = degrees of freedom.
MS = Mean Square.
[a] (. .) = number of missing values; [ns] = F-value is not significant; * = F-value significant for $P < 0.05$; ** = F-value significant for $P < 0.01$.


A
LEAF 4
average number of lesions per leaf
65
52
39
26
13
0
FR
NH
BS04
BS05
BS06
CA01
CA02
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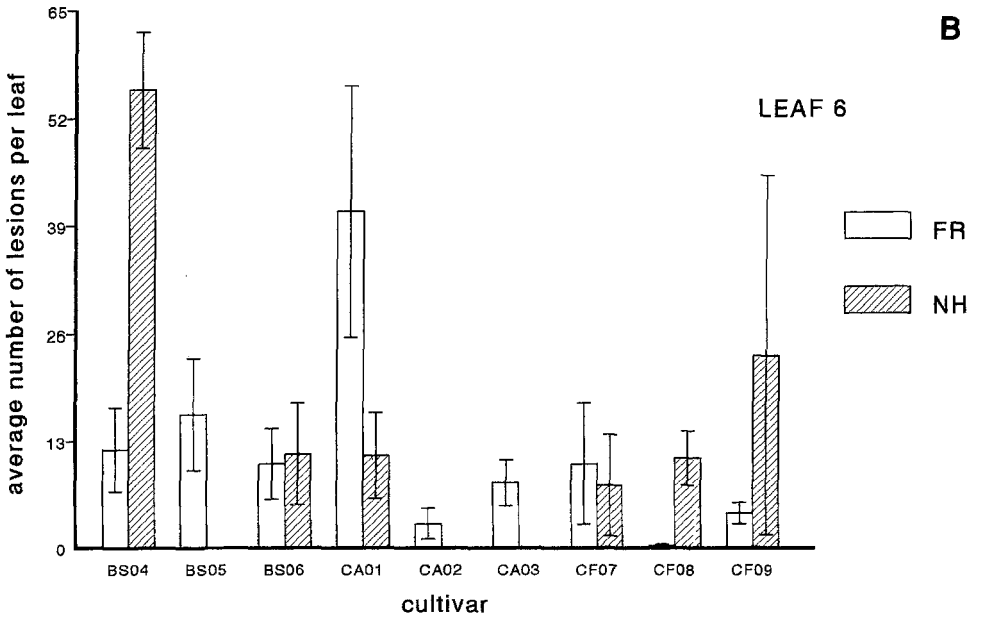
CF07
CF08
CF09
cultivar


B
LEAF 6
average number of lesions per leaf
65
52
39
26
13
0
FR
NH
BS04
BS05
BS06
CA01
CA02
CA03
CF07
CF08
CF09
cultivar

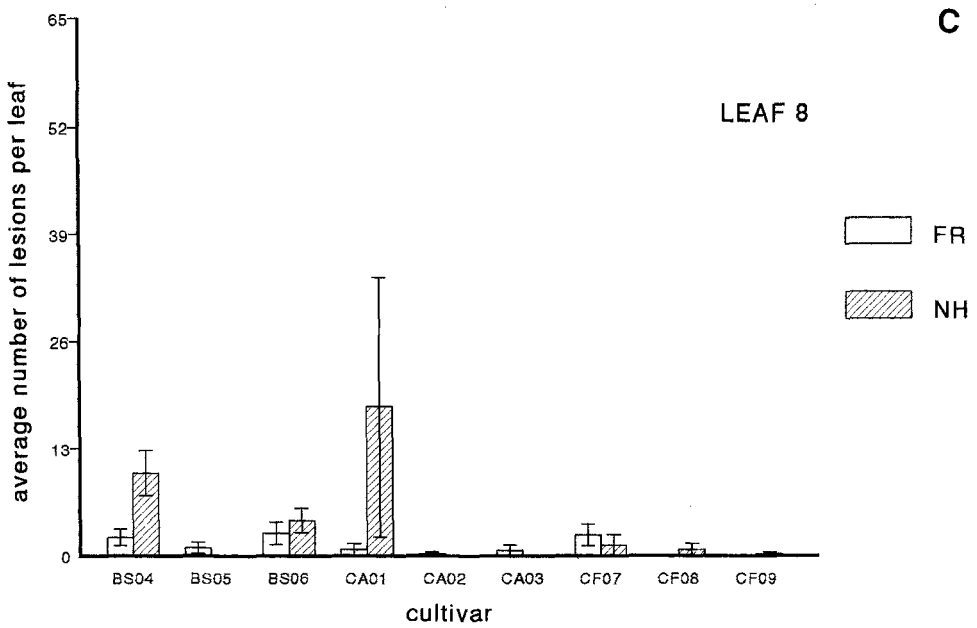


Fig. 5. Average number of lesions per leaf (SL) for two isolates of *Mycosphaerella brassicicola* (NH and FR) and 9 cultivars of Brussels sprouts (BS04, BS05, BS06), cabbage (CA01, CA02, CA03) and cauliflower (CF07, CF08, CF09) in relation to leaf number. Leaf age decreases with increasing leaf number. A = leaf number 4, B = leaf number 6, C = leaf number 8. Per average number of lesions standard errors are indicated.

Discussion

In brassica plants resistance against the NH isolate of *M. brassicicola* can either be a result of a hypersensitive reaction in the mesophyll of leaves (resistance type I), or a consequence of a difference between the number of succesful penetrations of leaves of different cultivars (resistance type II) (Van den Ende, 1992). Results in the present study show that differences in resistance levels exists between cultivars of brassica to other isolates of *M. brassicicola.*

Specificity in pathosystems is often indicated by significant isolate–cultivar interactions in the analysis of variance of an experiment where a number of pathogen isolates are tested on a set of host genotypes in all possible combinations (Kulkarni and Chopra, 1982; Vanderplank, 1982, 1984). Many authors have indicated that an interpretation of the analysis of variance can be misleading, when no information about the genetic background is available (Parlevliet and Zadoks, 1977; Winer, 1984; Jenns and Leonard, 1985; Carson, 1987). For instance, interaction effects can be due to higher order interactions such as the cultivar–isolate–environment (Kulkarni and Chopra, 1982; Zadoks and Van Leur, 1983). Therefore, the presence of an isolate–cultivar interaction in the analysis of variance is only an indication for the presence of physiological races of *M. brassicicola.* Hypersensitive responses on certain cultivars inoculated with the Dutch isolate (NH), and

the absence of such responses on the same cultivars when inoculated with the French isolate (FR) provide additional evidence that differential adaptation within the pathosystem *M. brassicicola–Brassica* spp. can occur.

Tests of plants in the third leaf stage revealed resistance type II. Some cultivars showed a lower number of lesions without the presence of a hypersensitivity reaction (BS01 and CA04, Fig. 2). It is remarkable that resistance type II against the NH isolate seems to be effective against the FR isolate too (BS01 and CA04), whereas resistance type I (CA02, CA03, BS05) does not seem to be effective against the FR isolate (Fig. 2).

The interaction effects age–cultivar–isolate and age–cultivar in the analyses of variance show that leaf age plays an important role in the expression of resistance in brassica cultivars, and consequently can influence conclusions about physiological specialization in *M. brassicicola*. Both types of resistance are influenced by leaf age. Discoloration of the mesophyll as a result of the hypersensitive reaction (resistance type I) seems to occur only in leaves and not in cotyledons. When leaves start yellowing with increasing age, the hypersensitive response is partially lost and lesions start to grow on leaves of previously resistant cultivars. Especially in Brussels sprouts and cauliflower cultivars which show early senesence, inoculation of resistant cultivars in the seventh leaf stage with the isolate NH resulted in a number of lesions on the oldest leaf. Cotyledons of Brussels sprouts and cauliflower cultivars started to wither much faster than cotyledons of cabbage cultivars which can explain the development of lesions on cotyledons of cultivars with high levels of resistance to the NH isolate (BS05 and CF07). The effect of leaf age on resistance type II seems to be present too. In some cases, resistance to some isolates increases with plant development though no hypersensitive response is present. As previous studies showed, in CA02 both types of resistance are present (Van den Ende, unpublished). The French isolate (FR) showed a higher number of lesions on cotyledons of CA02 then on CA03 (Fig. 1). As plants developed disease severity on CA03 decreased, while that on CA02 remained at the level of the most susceptible cultivar (CA01) (Fig. 2 and 3). The same is true with increasing leaf age (Fig. 5).

Resistance type II can be related to compounds in the wax layer of brassica leaves which restrain fungi from leaf penetration (Rawlinson et al., 1978; Hartill and Sutton, 1980; Conn and Tewari, 1989; Bansal et al., 1990). It is possible that the production of these specific compounds depends on leaf age. Further research should give more information about the resistance mechanism involved.

Main effects of isolates in the analysis of variance are difficult to interpret. Zornbach (1990) tested many isolates of *M. brassicicola* from different geographical regions on a single cabbage cultivar and stated that there were differences in aggressiveness between isolates from different parts of the world. As in the present study, he treated his plants with inoculum consisting of mycelial fragments. Standardization of this kind of inoculum is hard to accomplish, which can lead to high variability in the results. Differences in disease severity of plants after inoculation can therefore be a result of differences in inoculum concentration. However, in the present study, the difference between the isolates FR and NH is consistent for all the plant stages tested, although cotyledons did not show hypersensitive reactions when tested with the NH isolate. Because the other isolates were only tested in one experiment, reliable conclusions based on differences in disease severity of the cultivars tested cannot be drawn. The four other isolates (OP, GE, UK and DK) all resulted in hypersensitive reactions on CA02, CA03 and BS05, and were therefore easy to distinguish from the FR isolate. A remarkable difference exists between the number of lesions on the Brussels sprouts cultivar BS04 when tested with the FR-, OP- or NH isolate compared to testing with the GE-, UK- or DK isolate. More insight into specialization of these isolates might have been revealed in some of these isolates, if additional cultivars

had been added to the differential host set.

M. brassicicola isolated from certain host varieties in the field are also pathogenic on other host varieties. Adaptation of an isolate to specific host varieties does not seem to occur. The isolate OP from cauliflower showed a similar disease spectrum on the differential set of host genotypes as the isolate NH from cabbage. *M. brassicicola* isolated from *B. oleracea* was also infective on different species of brassica, as can be concluded from the symptoms on *B. campestris* and *B. napus*. Results of the present study confirm the findings of Zornbach (1990) in Germany. He demonstrated that isolates of *M. brassicicola* from oilseed rape were also pathogenic on cabbage, which explained the severe epidemics of ringspot in areas were both crops are grown next to each other.

The present study shows that physiological specialization of *M. brassicicola* is present in the isolates under study. Consequently, rapid adaptation of the pathogen to a particular host cultivar and loss of effective resistance is possible. Stability of resistance is assumed to be highest when many resistance genes and so pathogenicity genes are involved, and when recombination in the pathogen is strongly restricted (Parlevliet and Zadoks, 1977). Inbreeding of brassica cultivars tends to eliminate much of the genetic variability, as only major genes for resistance are being selected. Although the fungus is homothallic, cross fertilization between isolates of different origin can occur (Snyder, 1946), resulting in recombination in the pathogen. It is therefore to be expected that the incorporation of only major genes for resistance in cultivars will result in a rapid loss of effective resistance, due to a strong selection pressure for virulent races of the fungus.

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