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# Seedbed infection of white cabbage by Mycosphaerella brassicicola

J.E. VAN DEN ENDE*

Department of Phytopathology, Wageningen Agricultural University, P.O. Box 8025, 6700 EE Wageningen, the Netherlands

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**Abstract**

Three cultivars of white cabbage with different levels of resistance to *Mycosphaerella brassicicola* were tested for seedbed infection. Seedlings grown in seedbeds, to which infected plant debris was added as an inoculum, showed typical ring spot lesions on the cotyledons and first two leaves before seedlings reached the transplanting stage, whereas non-inoculated controls had few lesions only. Differences in levels of resistance between cultivars were present in seedlings grown under field conditions. Disease severity of transplants at the end of the season reflected disease severity of seedlings before transplanting in each cultivar.

To lower the risk of a severe epidemic of ringspot at the end of the growing season, the seedbed should be protected from infection by *M. brassicicola*.

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

## Introduction

In the Netherlands, ring spot disease of *Brassica* spp., caused by *Mycosphaerella brassicicola* (Duby) Lindau, is a major disease in white cabbage (*Brassica oleracea* var. *capitata*). Under conditions of rainfall, high humidity (2 days of 18 h RLV > 90%) and moderate temperatures (5–20 °C), the disease can cause severe damage to the host (Hartill, 1977; Staunton and Ryan, 1978; Ryan and Staunton, 1983; Frinking and Geerds, 1987; Zornbach, 1990; Van den Ende, 1992a). During severe epidemics most of the exposed, older leaves become extensively spotted. Young leaves of white cabbage are usually without symptoms and they seem to become susceptible only shortly before they finish expansion (Hartill, 1977, 1978; Hartill and Sutton, 1980). Therefore, seedlings of white cabbage are usually thought to be uninfected, though circumstances in spring can be favourable for disease development. Several authors presumed the importance of seedbed sanitation in the control of ringspot (Weimer, 1926; Chupp and Sherf, 1960), but experimental data on seedbed infection are not available. Usually, infection of *M. brassicicola* is considered to occur after the transplanting stage, that is at the age of 8 to 10 weeks (Frinking and Geerds, 1987; Meier, 1985; Zornbach, 1990). By the time the plants reach this stage of susceptibility, conditions usually no longer favour disease development, as temperatures rise (> 20 °C) and long periods of high humidity become rare during the Dutch summer. In late summer and autumn plants are fully developed and climatic con-

* Present address: Experimental Orchard 'de Schuilenburg', Research Institute for Plant Protection, 4041 BK Kesteren, the Netherlands.

ditions can be optimal for disease development. Accordingly, ring spot is considered to be a problem in the Netherlands at the end of the growing season only, and little attention is paid to early infection in spring (Van den Ende et al., 1986).

In greenhouse studies, severe infection by *M. brassicicola* was found on inoculated cotyledons and on the first two leaves of inoculated 3-week-old seedlings of white cabbage, cauliflower and Brussels sprouts. Clear differences in resistance of seedlings to *M. brassicicola* were found between the brassica cultivars (Van den Ende, 1992b). This result indicated the possibility of seedbed infection of young plants by *M. brassicicola*. If under field conditions seedlings of white cabbage are susceptible to *M. brassicicola*, occasional infection in spring may result in a build-up of the disease to damaging amounts by harvest time at the end of the growing season.

As no quantative data were available about infection of young plants before the transplanting stage under field conditions, three field experiments were conducted (1989, 1990, 1991) to study seedbed infection and its effect on the severity of ringspot disease at the end of the growing season. As no information was available on the resistance of seedlings to *M. brassicicola* between different cultivars under field conditions, seedbed experiments were carried out with several white cabbage cultivars, with known and different responses to *M. brassicicola* under greenhouse conditions.

## Materials and methods

*Seedbed infection.* Three white cabbage cultivars with different levels of resistance to *M. brassicicola* (CA01: susceptible, CA02: resistant, CA03: partially resistant) were sown in two seedbeds on 24 April, 1989, in Wageningen. Seedbeds (1.5 × 1.5 m) were surrounded by 0.5 m high walls and located at 10 m distance to avoid cross infection between the seedbeds. Within each seedbed each of the three cultivars was sown in two rows of 150 seeds per row. Rows were 15 cm apart. In a second experiment seeds of the same three cabbage cultivars were sown on 3 May, 1990, in two larger seedbeds. The seedbeds were surrounded by 0.8 m high walls and seperated by 20 m distance to avoid cross infection between the seedbeds. Each seedbed (6 × 6 m) was divided in three blocks (1 × 1 m), at 1.5 m distance between the blocks. Per block each of the three cultivars was sown in one row of 150 seeds, at 25 cm row distance.

In both experiments inoculum was placed in one seedbed by strewing crushed dried cabbage leaves collected during the previous season between the rows. The dried cabbage leaves were for 50 to 60% of their surface covered by lesions of *M. brassicicola*. In the other seedbed seeds were sown in untreated soil on which no brassica crops had grown for years.

On 7 May, 1991, CA01, CA02 and CA03 were sown in four seedbeds. Three seedbeds were treated with inoculum similar to the previous experiments, one seedbed remained untreated. The seedbeds were surrounded by 0.8 m high walls. Distance between seedbeds treated with inoculum was 5 m, the control seedbed without inoculum was located 20 m away from the treated seedbeds to avoid cross infection. Each seedbed (6 × 6 m) was divided in three blocks (1 × 1 m), with 1.5 m distance between the blocks. Per block each cultivar was sown in one row of 150 seeds, at 25 cm row distance.

*Disease in transplants.* In the 1989 experiment, a sample of 200 seedlings per cultivar was taken from every seedbed 10 weeks after sowing. For every seedling, the number of leaves and of ringspot lesions per leaf were counted. In addition, 50 seedlings per cultivar were transplanted to two experimental plots, one for seedlings from the treated seedbed and the other for seedlings from the control seedbed. The experimental plots, measuring

9 by 5 m, were divided into three subplots of 2.5 by 5 m, with 0.5 m spacing between the subplots. Every subplot contained one cultivar of which the seedlings were planted in five rows of ten plants. Distance between plants and between rows was 50 cm. The experimental plots were separated from each other by maize over a distance of 60 m, to avoid cross infection. Within each sub-plot the resistant cultivar was planted between the susceptible and the partially resistant cultivar so that cross infection between the sub-plots was minimized. The rows were planted perpendicular to the direction of the prevailing wind. At the end of the growing season, on 19 October, 1989, plants were harvested. For each plant the number of leaves and of lesions per leaf caused by *M. brassicicola*, were recorded.

In the 1990 and 1991 experiments a sample of 100 seedlings per cultivar was taken from every seedbed 10 weeks after sowing. For every seedling the number of leaves and of ringspot lesions per leaf, were counted.

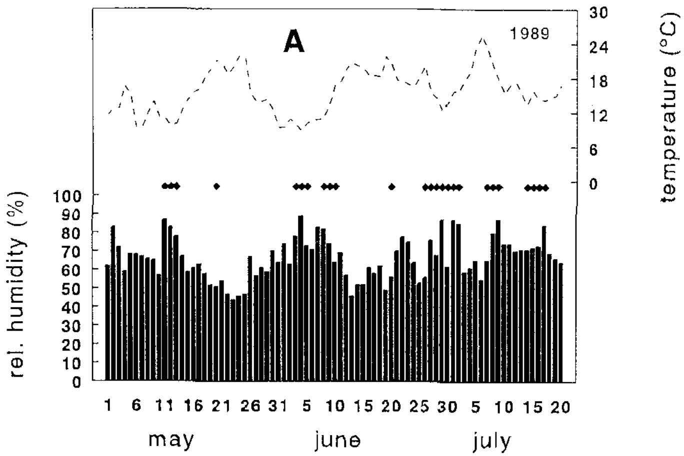
*Lesion type.* To gain more insight in differences in resistance between cultivars to ringspot under field circumstances, the lesion size of *M. brassicicola* on young seedlings was measured in the 1989 experiment. Lesions were either classified as a hypersensitive reaction, small (0–0.5 cm), medium (0.5–1.0 cm) or large (1.0–1.5 cm).

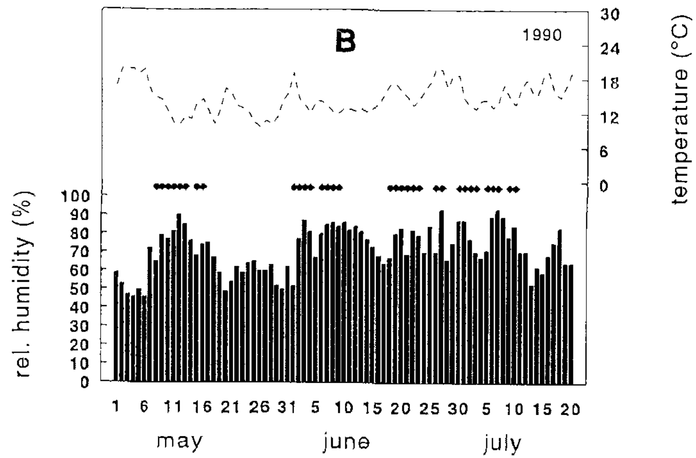
*Weather data.* In all seedbed experiments temperature and relative humidity were recorded by use of termohygrographs (Thies, Göttingen). Data on daily precipitation were obtained from a weather station at a distance of 500 m from the experimental plots.

*Statistical analysis.* Data were analyzed using the Statgraphics computer software package (Statgraphics, release 4.0). Analysis of the raw data indicated a heterogeneous error. Therefore, data of 3 succeeding years were subjected to an analysis of variance after logarithmic transformation of the number of lesions per cultivar. Because the data set involved small values, $\log_{10}(X + 1)$ was used instead of $\log_{10} X$, where X is the number of lesions per plant (Gomez and Gomez, 1984). Differences between treatments and between cultivars were tested for significance at 95% probability with LSD.

## Results

In all 3 years rainfall together with long periods ($\geqslant$ 2 days) of high humidity (daily average > 80%) and moderate temperatures ($5 < T < 20$ °C) were recorded during the period that plants were growing in seedbeds (Fig. 1). In 1989 and 1990, the first lesions of *M. brassicicola* appeared on the susceptible cultivar (CA01) in the treated seedbed 5 and 6 weeks after sowing, respectively. In the 1991 experiment, lesion development began 9 weeks after sowing. Results of lesion counts on seedlings 10 weeks after sowing are presented in Fig. 2. Data are expressed as the logarithm of the average number of lesions per leaf plus 1. High numbers of lesions were found on the susceptible cultivar in the treated seedbeds of 1989 and 1990. Seedlings of CA01 from the treated seedbeds showed typical ring spot lesions, predominantly on the cotyledons and the first two leaves (Fig. 3). The resistant cultivar (CA02) showed a clear hypersensitive reaction on the green leaves of the young plants (Fig. 4), whereas on the older yellowed leaves some lesion development took place. In the control seedbeds only very few lesions were found on the susceptible cultivar and no lesions were found on the partially resistant and resistant cultivars. In the 1991 experiment, disease severity on seedlings of the susceptible cultivar was very low. The analysis of variance on the $\log_{10}$ transformed data of all three experiments showed significant effects for seedbed treatment and cultivar ($P < 0.01$). No significant



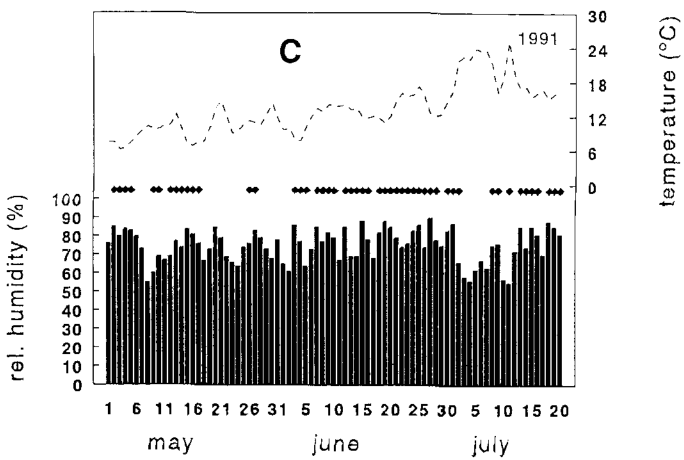




Fig. 1. Weather conditions during seedbed experiments in 1989 (A), 1990 (B) and 1991 (C). Bars represent daily average relative humidity (%), dotted lines represent daily average temperature (°C) and diamonds represent rain days.

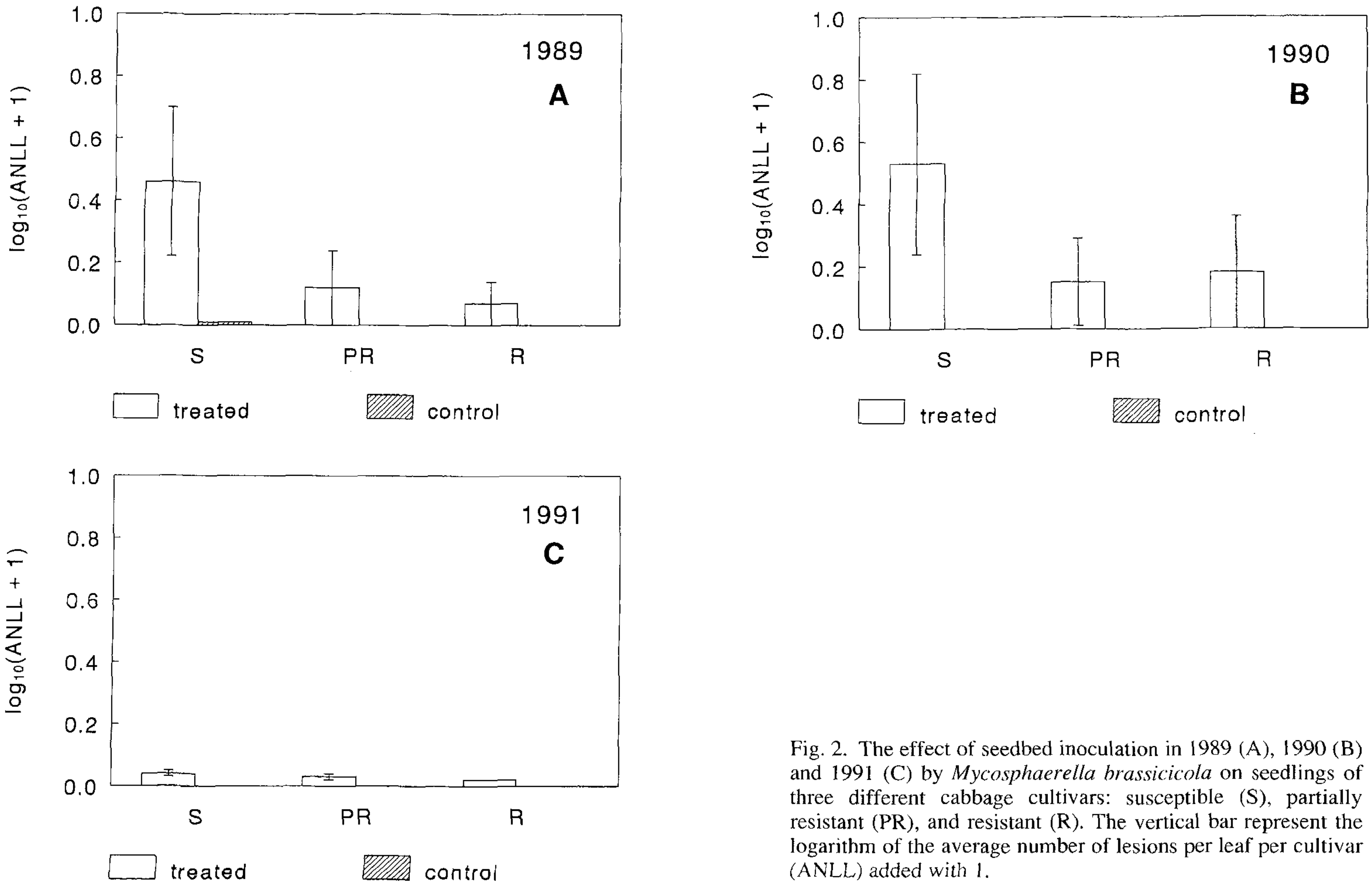



Fig. 2. The effect of seedbed inoculation in 1989 (A), 1990 (B) and 1991 (C) by *Mycosphaerella brassicicola* on seedlings of three different cabbage cultivars: susceptible (S), partially resistant (PR), and resistant (R). The vertical bar represent the logarithm of the average number of lesions per leaf per cultivar (ANLL) added with 1.

Fig. 3. Lesions of *Mycosphaerella brassicicola* on a susceptible cultivar seedling.

Fig. 4. Hypersensitive reaction of a cultivar seedling resistant to *Mycosphaerella brassicicola*.

interaction effects were present. Disease severity in the treated seedbed was significantly higher than in the control seedbed (LSD, $P = 0.05$). The number of lesions of the susceptible cultivar was significantly higher than that of the partially resistant and resistant cultivar (LSD, $P = 0.05$).

If the lesion type is taken into account the differences in susceptibility to *M. brassicicola* between the three cultivars are emphasized. Fig. 5 shows data from the 1989 seedbed experiment. Most lesions on the susceptible cultivar tend to be large, whereas on the resistant cultivar most infections result in a hypersensitive response or in small lesions.

At the end of the 1989 growing season the effect of the seedbed-inoculation was clear in all three cultivars (Fig. 6). Transplants of the susceptible cultivar from the treated seedbed showed severe infection by *M. brassicicola* in contrast to the transplants of the same cultivar from the control seedbed. Though relatively few lesions were counted on seedlings of the partially resistant and resistant cultivars, the seedbed inoculation nevertheless had effects on disease severity of both cultivars at the end of the growing season. Differences in disease severity between cultivars of the treated plot correspond to the difference in resistance to *M. brassicicola* of these cultivars.

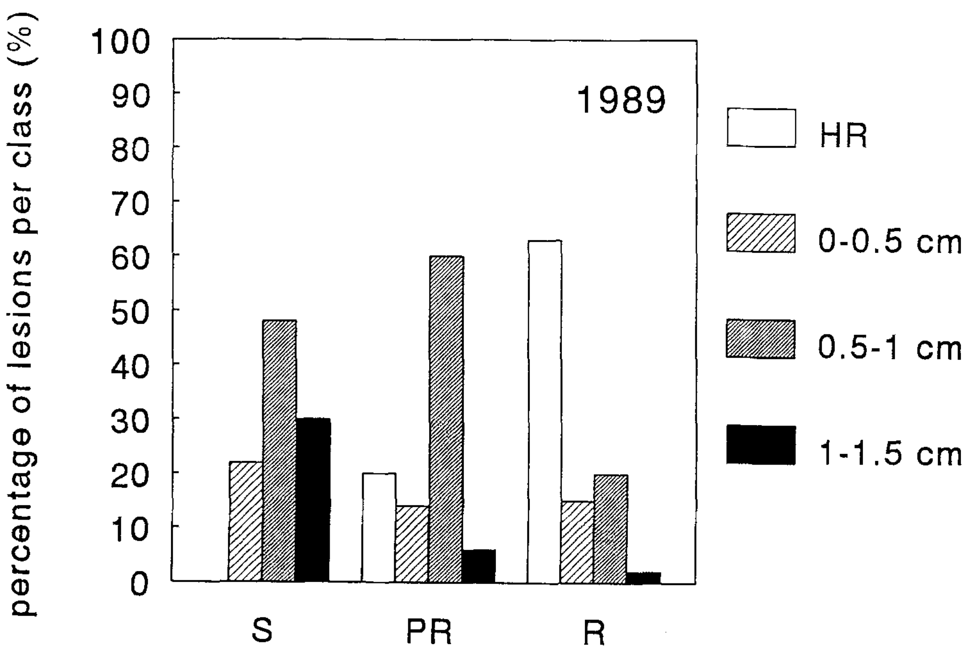



Fig. 5. Frequency distribution of lesion types of *Mycosphaerella brassicicola* on a susceptible (S), partially resistant (PR) and resistant (R) cultivar. Lesions are classified as not present (hypersensitive reaction, HR), small (0–0.5 cm), medium (0.5–1) or large (1–1.5).

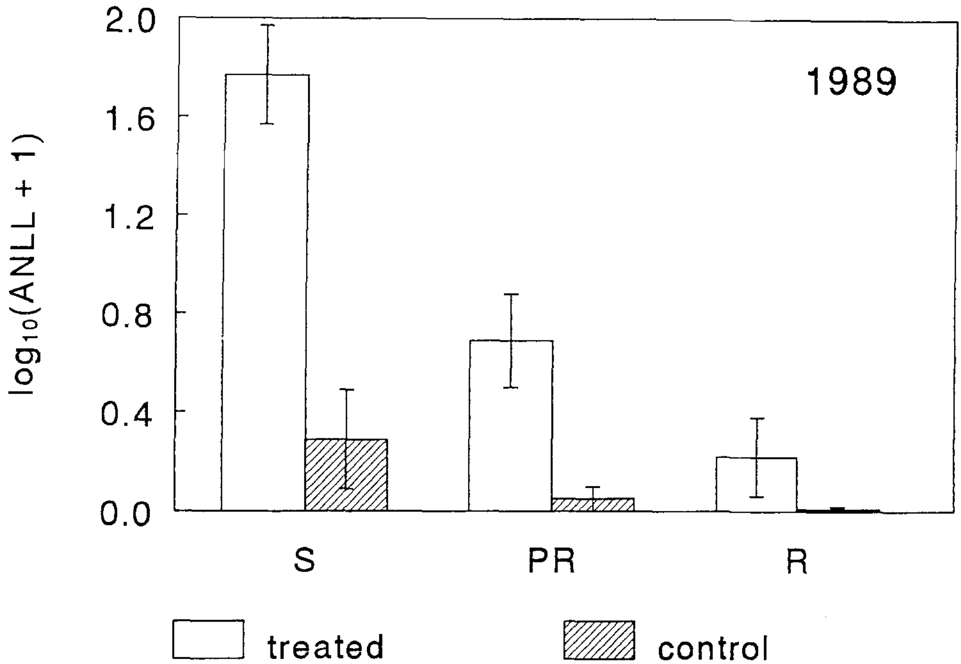



Fig. 6. The effect of seedbed inoculation of *Mycosphaerella brassicicola* on transplanted, fully grown plants of three different cabbage cultivars: susceptible (S), partially resistant (PR) and resistant (R). The vertical bar represent the logarithm of the average number of lesions per leaf per cultivar (ANLL) added with 1.

## Discussion

The seedbed experiments of 1989 and 1990 show that infection of young seedlings is likely to occur before transplanting if a source of inoculum is available and if weather conditions are suitable for infection. If an incubation period of approximately 20 days (Van den Ende, 1992b) is taken into account, the results show that in the field seedling infection can already occur at a plant age of 2–3 weeks. Although conditions for infection seemed to be optimal in all 3 years, disease severity levels in the 1991 experiment were very low. This was probably caused by a low quality of the inoculum used in this experiment. Sporulation of the particular dried leaf material was very poor, when tested in vitro. Infection by *M. brassicicola* is not restricted to the fully grown plants as suggested by Osmun and Anderson (1915) and Butler and Jones (1955). Cotyledons and the first two leaves of white cabbage seedlings are also susceptible to *M. brassicicola*. This result does not contradict the conclusion of Hartill (1977, 1980) that symptoms rarely develop until leaf expansion is complete, and never develop on rapidly expanding leaves. Two or three weeks after sowing, cotyledons and the first two leaves of cabbage are likely to be fully expanded. Therefore, infection of *M. brassicicola* is possible well before transplantation, which is at an earlier stage than suggested by Frinking and Geerds (1987) and Zornbach (1990).

Although statistically reliable conclusions cannot be drawn, the results of the 1989 experiment after transplantation indicated that infected seedlings can have a strong impact

on the severity of infection at the end of the growing season. As shown in studies with transplants older than 8 weeks, infection of plants early in the growing season will especially occur in regions where large scale cultivation of *Brassica* is practiced and host plants are grown year round (Jouan, 1972; Zornbach, 1990). In the spring, ascospores from infested winter crops can cause a high inoculum pressure for transplants, but also for plants in seedbeds. The possibility for infection of young plants in seedbeds in spring will certainly lead to a change in the current view on the control of ringspot during the growing season of cabbage in the Netherlands. The experimental results of this study support the recommendations by Weimer (1926) and Sherf and MacNab (1986) to locate seedbeds far from infected fields, to keep the seedbeds free from infected plant debris, and to have the seedbeds protected from the prevailing wind which might carry ascospores over a considerable distance.
Under field conditions differences in resistance of cultivars are present even when plants are in a very young stage of development, which confirms results found in greenhouse studies (Van den Ende, 1992b). Resistance is expressed as a lower number of lesions, a reduced lesion growth or as a hypersensitive response. The results of seedbed infection in the partially resistant and resistant cultivars may have been influenced by cross contamination from the severely diseased plants of the susceptible cultivar. Further research should exclude this possibility through better experimental design.

The present study suggests the possibility to select for resistance in cabbage seedlings in the field. Under field conditions, seedling resistance seemed to be correlated to adult plant resistance against *M. brassicicola*. This information may be useful to the plant breeders, who presently select for resistance in late fall when cabbage plants are fully grown.

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