Epidemiology of dark leaf spot caused by *Alternaria brassicicola* in organic seed production of cauliflower

J. Köhl, C. van Tongeren, B.H. de Haas, and R.G. Driessen

***Abstract* – Dark leaf spot caused by *Alternaria brassicicola* is a seed-borne disease of *Brassicae*. Production of healthy seed is essential for the organic vegetable production. Literature on the epidemiology of the disease in organic seed production of *Brassica* was reviewed and an epidemiological field experiments was carried out. External and internal contamination of seeds with *A. brassicicola* increased steadily during their development. Colonisation of pod tissues as quantified by TaqMan-PCR increased exponentially. The developed knowledge can be used for optimizing cropping systems for organic seed production with lower risks for seed contamination by *Alternaria* spp. and to develop critical control points for disease management.[1]**

INTRODUCTION

Alternaria brassicicola can cause dark leaf spot of crucifers. Seeds, seedlings, leaves and pods can be damaged. The pathogen often is seed-borne but crop residues are another major inoculum source.

The use of organically produced seed not free from the disease may lead to an increased incidence of dark leaf spot in organic production of *Brassica* crops. For the production of healthy seed, the prevention of dark leaf spot in seed crops is a prerequisite.

The objective of our study was to review the literature on the epidemiology of dark leaf spot in organic production of *Brassica* and to follow the disease development in a field experiment. This knowledge can be used for optimizing cropping systems for organic seed production with lower risks for seed contamination and to develop critical control points for disease management.

LITERATURE REVIEW

Dark leaf spot disease is not restricted to leaves but can also damage fruit bearing branches and pods which turn black when colonised by *A. brassicicola.* Pre-mature ripening of the pods may lead to shedding of seeds (Maude and Humpherson-Jones, 1980; Köhl and van der Wolf, 2005). Seeds in infected pods tend to be shrunken and have low viability.

Superficial contamination of seed surfaces by necrotrophic pathogens is more common than internal colonisation of seeds after infection. External inoculum can be located in cracks of the seed coats so that conidia are protected from adverse environmental conditions but also from physical seed treatments. Internal infection of seeds by *A. brassicicola* was found in 89 out of 139 samples of basic seeds during an inventory carried out in 1976-1978 in the UK. There was a strong correlation between the number of external conidia per seed and the incidence of internally infected seeds.

Within internally infected seeds, *A. brassicicola* is mainly found in seed coats. In heavily infested seed lots, also the cotyledon tissue of the embryo can be infected. Interestingly, the incidence of internal infection was similar for small, shrivelled seeds and large, round seeds, so that a selection of healthy seeds on the basis of appearance and size was not possible. Both internal and external inoculum can survive for several years. The longevity of internal inoculum is higher than of external inoculum.

Germinating *Brassica* seeds are susceptible to infection by *A. brassicicola* by conidia contaminating the seed surface. After rupture of the testa, germination of conidia is stimulated and especially the hilum area and damaged parts of the testa can be infected by the pathogen. Immature seeds are more vulnerable than mature seeds.

Seedlings developed from infected seeds show typical symptoms of small discrete dark spots on the under-surface of the cotyledons or dark stripes on the hypocotyls. Seedlings from heavily infected seeds often die. Cotyledon infection under field conditions is often associated with seed coats sticking to cotyledons during emergence.

A significant correlation was found between internal seed infestation and symptom development on seedling under field conditions. However, surface sterilisation reduced the number of infected seedlings. The experimental data do not allow a clear conclusion on the importance of external versus internal inoculum (Maude and Humpherson-Jones, 1980).

Infection of seedlings by *A. brassicicola* after artificial inoculation of hypocotyls or cotyledons or naturally infested seeds depended on incubation temperature. Optimum temperature for development of typical wirestem symptoms was 25 °C. Little wirestem symptoms occurred at temperatures below 20 °C.

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FIELD EXPERIMENT

Material and Methods

Ten weeks old seedlings of open-pollinating cauliflower cv. Opaal RZ were planted at April 21st 2005 in two field plots in Groessen, central-east Netherlands. The distance between fields was 100 m. Each field was divided into four replicate sub-plots. Plants within replicate plots were grouped according to the date of beginning of flowering ('early plants'; 'late plants'). One field plot was artificially inoculated with *A. brassicicola* (+A.b.). Conidial suspensions (0.3 ml per leaf; 5 x 10^5 conidia/ml;) were sprayed on the eldest fully developed leaf with no symptoms of senescence of each plant, at May 31st, June 1st, June 8th, and June 14th.

From each plant with early flowering, six arbitrarily chosen flowers were sampled at June 28th. Ten pods per plants were sampled in the same way at July 12th, July 26th, August 9th, and August 23rd. Sampling dates for plants with late development were July 12th for flowers and July 26th, August 9th, August 23rd, and September 1st. In the field inoculated with *A. brassicicola*, the mature plants with early development were cut at September 3rd and those with late development at September 10th. In the naturally infested field, plants with early development were cut at September 11th and those with late development at September 21st. Ten arbitrarily chosen pods per replicate were separated by hand from the plants for assessment of seeds and pod tissues. The remaining plant material was threshed and obtained seeds were cleaned.

Pods were cut with a sterile scalpel and young seeds were removed using sterile forceps to avoid contact of seeds with pathogen inoculum possible present on the outer pod surface. Two hundred seeds per sample were incubated for 10 days at 20 °C with 12 h blacklight per day on CW-medium (Wu and Chen, 1999). Another sub-sample of 200 seeds was surface sterilised in 0.5 % HCl solution for 10 minutes before incubation. Each seed was inspected for growth of colonies of *A. brassicicola.*

Flowers and pod tissues sampled at the various sampling dates were freeze dried and subsequently pulverized. DNA was extracted from 10 to 15 mg sub-samples using DNeasy plant mini kit (Qiagen, Westburg, Germany). Species-specific primers and a probe were developed and TaqMan reactions were carried out to quantify *A. brassicicola*, using DNA of the fungus for calibration and green fluorescent protein (GFP) coding sequence of *Aequorea victoria* as internal standard.

Preliminary results

Contamination of developing seeds within the pods increased steadily during time (Fig. 1A). This was also found for internal infections (Fig. 1B). Colonisation of pods by *A. brassicicola* increased exponentially from late June until harvest in September (Fig. 1C). No differences were found between early and late plants for same sampling dates indicating that disease progression may depend more on environmental factors than on development stages of the host. Epidemics in plots with artificial inoculation developed faster.

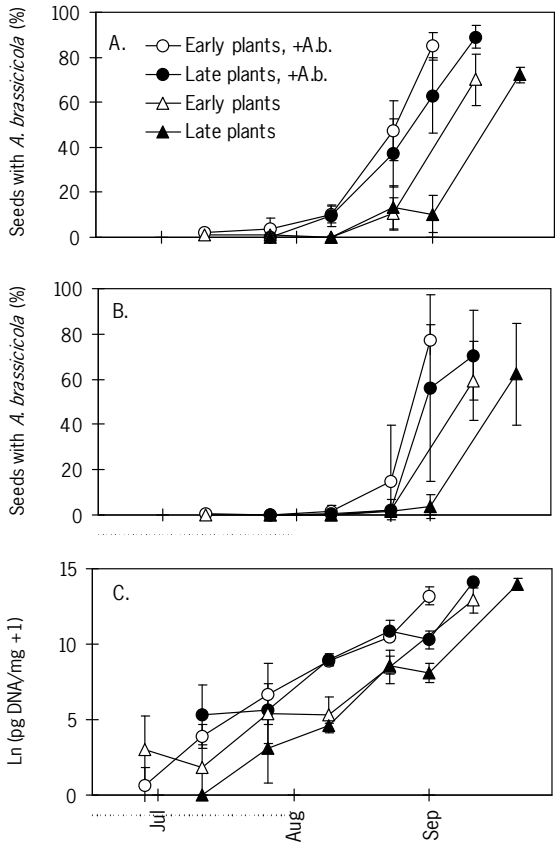

Figure 1. Contamination of seeds with A. brassicicola *assessed by plating on CW-medium without (A) and with surface sterilisation (B) and colonisation of flowers (first sampeling date) and pods assessed by TaqMan-PCR (C). Bars indicate standard deviation. +A.B.: artificial field inoculation with* A. brassicicola*.*

CONCLUSIONS

Preliminary results indicate that disease control should aim at slowing down disease progression in the crop rather than protect pods with developing seeds during certain critical stages. Further evaluation of the obtained seed samples is ongoing. Ripeness, germinability, vigour, disease transmission to seedlings and the effect of warm water treatments will be assessed. The field experiment will be repeated in 2006.

ACKNOWLEDGEMENT

This work is funded by the Dutch Ministry of Agriculture, Nature and Food quality.

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