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Alternaria brassicicola and *Xanthomonas campestris* pv. *campestris* in organic seed production of *Brassicae*: Epidemiology and seed infection

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agriculture, nature
and food quality

Note 363

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Plant Research International B.V., Wageningen
September 2005 Note 363

This literature study has been carried out in the context of the Policy Support Research Programme 'Improvement of the quality of plant reproductive material for organic and sustainable agriculture' ('Verbetering van de kwaliteit van plantaardig uitgangsmateriaal voor biologische en duurzame landbouw (BO-04-003)'). This Programme is financed by the Netherlands Ministry of Agriculture, Nature and Food Quality.

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Epidemiology of *Alternaria brassicicola* in organic seed production of *Brassica*

Jürgen Köhl

1. Introduction

Alternaria brassicicola and *A. brassicae* are causing dark leaf spot of cultivated and wild crucifers (Smith *et al.*, 1988). Seeds, seedlings, leaves and pods can be damaged. Both pathogenic fungi can be seed-borne. Mycelium can be found growing superficially on the seeds but also internally. Saprophytically surviving mycelium on crop debris is another major inoculum source.

For the production of healthy seed, the prevention of dark leaf spot in seed crops is a pre-requisite. Foliar sprays of fungicides are common to prevent colonization of flowers and pods by the pathogens. For seed production in organic systems, the disease is a major threat. 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.

In this literature survey, knowledge on the epidemiology of dark leaf spot in seed production of *Brassica* is evaluated. This 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.

2. *Brassica* seed production: Pathogens and seed production systems

2.1 Role of fungal pathogens in *Brassica* seed production

In vegetable *Brassica* seeds, *A. brassicicola* is the dominating *Alternaria* spp. (Maude & Humpherson-Jones, 1980; Humpherson-Jones, 1985; Maude *et al.,* 1984), whereas in oilseed rape *A. brassicae* is more common (Humpherson-Jones, 1989). In *Brassica* vegetable production, both *Alternaria* spp. can occur and cause dark leaf spot. More *A. brassicae* is found on *Brassica* vegetables in areas with oilseed rape production. Corlett & MacLatchy (1996b) stress that *A. brassicicola* causes serious disease problems in vegetable production but not in oilseed rape. Babadoost & Gabrielson (1979) surveyed 85 seed production fields of cabbage or Brussels sprouts in Western Washington. They found *A. brassicicola* in 67 fields, but *A. brassicae* only in 6 fields. Humpherson-Jones (1983, 1985) assessed commercial *Brassica* seed lots in UK for infection of by *Alternaria* spp. and *Leptosphaeria maculans,* the causal organism of blackleg, another seedborne disease. Seed lots of oilseed rape were heavily infested by *L. maculans*, whereas seed lots of *B. oleracea* often were not infested. Since pathogenic as well as non-pathogenic *L. maculans* isolates can occur in seeds, pathogenicity tests were carried out. Virulent isolates were found on oilseed rape seed but not on seed of *B. oleracea*.

2.2 Seed production systems

Most research on pathogenic *Alternaria* spp. in seed production of *Brassica* is reported from England during a period between 1980 and 1990. Crops were established by direct drilling in June or July (Maude & Humpherson-Jones, 1980) and matured in June to August of the following year. Mature crops were cut, windrowed for drying during several weeks and threshed.

In organic production of cabbage and other *Brassica* species under Dutch conditions, diseases caused by *Alternaria* spp. and *Mycosphaerella brassicicola* are a major threat. Seed transmitted *Alternaria* can form a substantial risk in seed production (Lammerts van Buren, 1994). Pathogen-free seeds can successfully be produced in organic systems when disease-free areas are chosen for production.

Currently, *Brassica* seeds are produced in organic systems by a few Dutch seed companies. Various cropping systems are used which differ in production costs and the risks for disease problems envisaged in the crop during seed production and on the harvested seeds. Based on their experience and expectations, the representatives of the relevant seed companies advised to focus in the project on epidemiology of diseases in organic seed production on *A. brassicicola* in field grown *Brassica* seed crops. Special interest was expressed to obtain insight in critical periods during which the pathogen can potentially contaminate the developing seeds externally and, more important, internally. This knowledge can subsequently be used to develop preventive measures for use at such critical control points. Depending on the 'critical periods', preventive measures may aim at manipulation of microclimatic conditions within the crop, sanitation measures, such as removal of plant debris, and application of plant protection products registered in organic farming or other means.

3. Damage caused by *Alternaria brassicicola* in *Brassica*

3.1 Seed production

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 & Humpherson-Jones, 1980). Seeds in infected pods tend to be shrunken and have low viability.
Chirco & Harman (1979) inoculated cauliflower and broccoli plants at petal fall (of oldest flowers of the plants, flowering started at apex), mid-pod (immature pods) or ripe pod stage (seeds fully formed in ripe pods) with *A. brassicicola.* Plants were inoculated at relatively high temperature (20 or 27 °C) and pods were enclosed in plastic bags for 7 days (plastic bags opened during 7 h per day). Inoculation at petal fall stage resulted in reduced seed weight. Germination under laboratory conditions as well as field conditions were significantly reduced for seeds obtained from pods treated with *A. brassicicola* at petal fall or mid-pod stages. Inoculation of ripe pods did not affect field emergence of seeds. Seed infection by *A. brassicicola* was increased after inoculation of plants with the pathogen in comparison to seed from plants only treated with water. Highest seed infections were found on plants inoculated early at petal fall. Surface sterilisation reduced seed infection in many, but not in all cases. The experiment of Chirco & Harman (1979) was carried out under conditions of artificially high humidity combined with high temperatures during and after inoculation with *A. brassicicola*. Furthermore, seeds were harvested and threshed after pod maturation. It cannot be concluded from the data at which stage seeds within pods were infested by *A. brassicicola* during maturation.
Superficial contamination of seed surfaces by necrotrophic pathogens is more common than internal colonisation of seeds after infection (Maude, 1996). 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. More external colonisation is found in the area of the seed hilum which can be explained by mycelial growth of the pathogen through the suture of silicas (pods) and the funicles (Maude, 1996).
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 (Maude & Humpherson-Jones, 1980). On average 4.9% of the seeds were internally infected. In the same study, 96% out of 112 assessed commercial seed lots were infested, on average 18% of the seeds were internally infected. Seed lots of cabbage with internal infection by *A. brassicicola* were also assessed for external inoculum. Up to 500 conidia per seed were found. 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 (Maude & Humpherson-Jones, 1980). 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, shriveled 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. Maude & Humpherson-Jones (1980) state that longevity of internal inoculum is much higher than of external inoculum.

3.2 Seedling production

Germinating *Brassica* seeds are susceptible to infection by *A. brassicicola* by conidia contaminating the seed surface (Knox-Davies, 1979). 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 when tested in the laboratory. Under greenhouse or field conditions, symptom development is generally less severe than under lab conditions and damping-off caused by *A. brassicicola* is less common, e.g. 19% of seedlings developed from

contaminated seeds showed disease symptoms when sown in the greenhouse and 6% showed symptoms under field conditions. Cotyledon infection under field conditions was often associated with seed coats sticking to cotyledons during emergence (Maude & Humpherson-Jones, 1980).
Maude & Humpherson-Jones (1980) found a significant correlation 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.
Infection of seedlings by *A. brassicicola* after artificial inoculation of hypocotyls or cotyledons or naturally infested seeds depended on incubation temperature (Bassey & Gabrielson, 1983a). Optimum temperature for development of wirestem symptoms was 25 °C. Little wirestem symptoms occurred at temperatures below 20 °C.

4. Epidemiology of *Alternaria brassicicola* in *Brassica* seed production

4.1 Infestation of vegetative crops

Crops for seed production can be infested by *A. brassicicola* from infected seeds, infested crop debris present in the field or transferred by wind from infested mature crops. Especially during cutting and trashing of windrowed crops huge amounts of conidia were released and captured at distances of up to 1800 m in spore traps consisting of Petri dishes containing a selective medium (Humpherson-Jones & Maude, 1982a). It was concluded that sufficient distances and optimum regional distribution of vegetative and maturing seed crops may prevent early infestation of vegetative seed crops. In modern production systems, especially in areas with low temperatures during winter, vegetative crops are not established by seeding in the field, but transplants are produced in greenhouses or tunnels during winter and transplanted in the field in spring. In such a system, inoculum produced on mature crops is no threat for the new crops since there are no overlapping growing seasons. Conidia of *A. brassicicola* formed on crop residues or alternative hosts such as weeds may play an important role as inoculum source in such cropping systems but no quantitative data are available on this aspect. The disease may also enter the vegetative crop phase during seedling production from infested basic seed. Transmission of *A. brassicicola* from infested seed to seedling was investigated by Maude & Humpherson-Jones (1980). Under glasshouse conditions, 12-19% of infested seeds produced seedlings with symptoms. However, the rate of disease transmission was lower under field conditions. Even from seed which was affected by the pathogen for 100%, only 6% of the developing seedlings showed symptoms of disease.

4.2 Spread of disease

In overwintering *Brassica* seed crops, *A. brassicicola* can survive on the litter. Humpherson-Jones & Maude (1982a) found several hundred up to a maximum of 40.000 conidia of *A. brassicicola* produced per g of overwintered litter when sampled in April. These conidia are considered the primary inoculum inciting epidemics in seed crops. Conidia were also sampled in the air above crops during two growing seasons. There was a slow increase of air load with *A. brassicicola* conidia during the season with significant peaks when the crops were cut and harvested with a maximum spore load of 12.000 conidia per m^3 air. During the growing season conidia were mainly found on dry days following periods of rain with long leaf wetness periods. Most spores were released during the day when relative humidity decreased in the crop, the minimum number of conidia was released during the moist periods at night. This diurnal periodicity of spore release was also found by Kennedy *et al.* (1999) and Chen *et al.* (2003). In a study in oilseed rape, spore release was observed mainly during 7.00 and 21.00 h, with a maximum spore release during 13.00 and 15.00 h (Kennedy *et al.*, 1999). Sporulation and spore release in the field could be predicted by using models based on temperature and humidity. Chen *et al.* (2003) found diurnal periodicity of spore flights with peaks at 10:00 h. Spore release and flights were mainly affected by rain and wind. Spores were spread over short distances, so that it can be assumed that they retain within the crop canopy (Chen *et al.*, 2003). Spread over longer distances of up to 1800 m was found only during trashing of infested crops (Humpherson-Jones & Maude, 1982a). Spore trapping at different heights showed that most conidia were transported over short distances of several centimeters resulting in a gradient in spore load within the canopy from bottom to top (Humpherson-Jones & Maude, 1982a; Chen *et al.,* 2003). Disease development started on pods close to the soil earlier in the season and progression was faster compared to pods at 1.25 or 2.5 m canopy height. This can be explained by the different inoculum level in combination with a more favourable micro-climate in the lower canopy levels. The effect of early or late infection of pods on the infection of seeds has not been followed in this study.
The spread of dark leaf spot caused by *A. brassicicola* in cabbage crops was studied by Fontem *et al.* (1991). They found steep gradients of disease severity in distance from infected plants used as inoculum source. This pattern of the spread of the disease within the crop indicated a short distance dispersal of conidia. Also Chen & Price (2002) found a short distance spread of the disease in Chinese cabbage.

A detailed study was carried out by Humpherson-Jones & Maude (1982a) to follow the disease development in the period between cutting and threshing after windrowing. In general, seeds threshed at cutting showed less infection compared to seeds of windrowed plants. Surface sterilisation of seeds was not included in this study. Thus, it cannot be concluded from the data whether external contamination increased on seeds from windrowed plants due to a higher inoculum load produced on the pods during windrowing and contaminating seeds externally during threshing. Seeds may also be infected internally during the period of windrowing.

4.3 Survival in crop debris

The capacity of leaf and stem debris of cabbage to serve as inoculum source of *A. brassicicola* was assessed by Humpherson-Jones (1989). Leaves were collected in seed production crops of cabbage infested with *A. brassicicola* and *A. brassicae*, placed on soil outdoors for several weeks and the potential of both *Alternaria* spp. to produce conidia on the leaves in moist chamber was measured regularly. Conidia were produced on leaves which had been exposed to field soil for up to 12 weeks. On average approximately 10^5 conidia of both *Alternaria* spp. were formed per leaf. On infested stem segments, conidia production was observed even after 23 weeks of exposure to field soil. Approximately 10^3 conidia per cm stem segment were produced. The viability of conidia produced on leaf or stem debris was higher than 75%. Since no observations on sporulation capacity on cabbage stems were made after field exposure for periods longer than 23 weeks, there is no information provided on the maximum period during which *Alternaria* spp. are able to produce conidia on stem debris. Survival of *Alternaria* spp. in stem debris may be different for stems on soil surface or ploughed in the soil. Stem debris ploughed up to the soil surface in subsequent years may still contain *Alternaria* inoculum. It is concluded by Humpherson-Jones (1989) that crop debris is the major inoculum source of *A. brassicicola* and *A. brassicae* in areas in which the disease is established. However, long-term survival of both pathogens in debris has not been investigated.

4.4 Transmission by insects and other invertebrates

Various pest insects, such as pollen beetle and seed pod weevil, are feeding on *Brassica* spp. or may lay eggs in flowers and young pods which later serve as feed for larvae. Flowers of *Brassica* spp. are also visited by various beneficial insects playing an important role in pollination. In seed production, bees, bumble bees, but also flies are used to assure optimum pollination in production systems in the open field, tunnels or greenhouses.
Such beneficial or pest insects may act as vectors of fungal spores. Quak (1956) investigated under field conditions the possible role of pollen beetles (*Ceuthorrhynchus assimilis*) and seed pod weevils (*Meligethes aeneus*) as vectors of *Alternaria* spp. in oilseed rape. Presence of the pests in cages placed in the crop did not increase disease development on pods in comparison to pods from plants in cages without pests. Similar results were obtained at two locations.
Dillard *et al*. (1998) observed flea beetles (*Phsyllotreta cruciferae*) in cabbage fields feeding on plants infected by *A. brassicicola*. *A brassicicola* could be isolated from 20 to 30% of beetles caught in such fields. Viable pathogen conidia were found externally on the insect surface, mainly in the cavities of the exoskeleton, but also in the digestive tracts and beetle feces. The percentage of beetles with *A. brassicicola* increased during the growing season. Beetles collected in diseased fields were kept in cages with healthy cabbage plants. Disease was transmitted to plants grown under greenhouse. Development of leaf spots was as severe as on plants artificially inoculated with conidial suspensions of *A. brassicicola*, whereas untreated plants were almost free of disease symptoms.
Transmission of *A. brassicicola* by slugs has also been reported (Hasan & Vago, 1966) who observed feeding of slugs on infected cabbage leaves. Viable conidia of *A. brassicicola* were found in the excrements of slugs during a period of one week after being fed with infected leaves.

5. Prevention and control in organic seed production

Transmission of the disease from mature crops to vegetative crops can be prevented if crops are not established by drilling in the field but by planting transplants in spring. If crops are established by seeding, the distance between mature crops and newly seeded crops should be more than 1800 m (Humpherson-Jones & Maude, 1982a).
In integrated seed production, seeds are treated by fungicides (Maude *et al.*, 1984) and repeated foliar applications of fungicides, e.g. iprodione, are common to protect pods from infection. Seeds obtained from treated plants are significantly less infected by *A. brassicicola* if the disease was present in the field (Humpherson-Jones & Maude, 1982b). Since synthetic fungicides are not available in organic production, basic seed should be produced in disease-free areas (Lammerts van Buren, 1994) and tested for absence of the pathogen. However, if only pathogen-infested basic seed is available, seed treatments, e.g. with hot water, have to be developed.
Given the high temperature optimum for seedling infection (Bassey & Gabrielson, 1983a), climate control in production of seedlings is important. Temperatures above 20 °C should not occur in combination with humid conditions.
Humpherson-Jones & Phelps (1989) found that the sporulation process of *A. brassicicola* is inhibited by dry periods of at least 3 h. Experiments carried out under controlled conditions showed that *A. brassicicola* can sporulate in a wide temperature range between 5 and 30 °C, if the relative humidity is above 90% (Humpherson-Jones & Phelps, 1989). A moist period of at least 12 h is needed to complete sporulation. If a moist period was interrupted by a dry period at 70 or 80% r.h. for a few hours, the sporulation process was disturbed and was not continued in a following moist period. Several generations of spores can be produced on tissues given sufficiently long wetness periods of >12 h. Dry periods between such long moist periods did not affect the spore yield in subsequent sporulation periods. Climate control in the seed crop can thus be an important means to prevent sporulation of the pathogen on infected tissue by achieving short durations of wetness periods within the crop.
In field experiments with artificially inoculated plants serving as inoculum source in a crop of Chinese cabbage, Chen & Price (2002) found that the disease spread was driven by wind. More disease was found downwind of the inoculum source. The disease spread faster within rows than across rows. Orientation of rows not parallel but in right angles to the main wind direction was suggested to reduce the risks of disease development.
Protection of *Brassica* leaves from infection by *A. brassicicola* using antagonistic fungi has been investigated by Pace & Campbell (1974). Applications of *Aureobasidium pullulans* and *Epicoccum nigrum* on leaves reduced infection under controlled conditions. However, biological control of *A. brassicicola* under field conditions has not been reported.

6. Detection of *A. brassicicola*

A. brassicicola can be identified and distinguished from *A. brassicae* and *A. alternata* based on the appearance of the conidia (Corlett & MacLatchy, 1996a, b; Mathur & Kongsdal, 2003; Simmons, 1995). Maude & Humpherson-Jones (1982a) used prune lactose agar on which *A. brassicicola* can be distinguished from *A. brassicae* based on colony characteristics.
Prune lactose yeast agar supplemented with benomyl and DuPont exp fungicide DPX 3217 (Humpherson-Jones & Maude, 1982a) has been used as semi-selective medium, e.g. for spore trapping in the field. For seed testing, Wu & Chen (1999) developed a semi-selective medium. On this CW-medium, growth of various fungi is suppressed. Colonies of *A. brassicicola* have a powdery appearance and can easily be distinguished macroscopically from other *Alternaria* spp., including *A. alternata* (M. Asma, pers. communication) which is not possible on ARSA (Pryor *et al.*, 1994), another medium semi-selective for *Alternaria* spp. (C. van Tongeren, pers communication).
Seeds can be assessed using various tests, e.g. blotter method, deep freezing blotter method, agar plate method, tests on filter paper based on the use of 2,4-D to prevent seed germination, or germination tests (Anon. 1966; Bassey & Gabrielson, 1983b; Vannacci, 1981). Optimum temperature to detect *A. brassicicola* on infested seeds was 20 to 25 °C.
PCR-based detection of *A. brassicicola* on cruciferous seeds was developed by Iacomi-Vasilescu *et al.* (2002). The designed primer pairs were highly specific for the pathogen and could be used for detection of presence of *A. brassicicola* in seeds with infection levels of 10% or higher. The quantitative detection of *A. brassicicola* in host tissue of *Arabidopsis* by real-time PCR has been demonstrated by Brouwer *et al.* (2003) and Gachon & Saindrenan (2004). However, in these studies other *Alternaria* spp. were not present in the plant tissues and no information is given on the specificity of the used primers. Blast analysis of sequences of primers used by Brouwer *et al.* (2003) showed that these were not specific for *A. brassicicola* but were also found in strains of various *Alternaria* spp., *Ulocladium* spp. and other fungi (P. Bonants, pers. communication). Primers used by Gachon & Saindrenan (2004) are based on sequences of Cutinase A (ABU03393) gene of *A. brassicicola*. A blast analysis does not allow an estimation of the specificity of these primers because of the limited data in the data base. It is likely that other *Alternaria* spp. may contain cutinase genes too. For the application of real-time PCR for quantification of *A. brassicicola* in plant tissues sampled in the field or in seeds, primers with a high specificity are needed because various fungi, including those closely related to *A. brassicicola*, may be present. The primers published by Iacomi-Vasilescu *et al.* (2002) for *A. brassicicola* are not suitable for real-time TaqMan-PCR because the amplicon is too large and the forward primer partly overlaps with an ITS area most specific and thus ideal for probe development (R. van Hoof, pers. communication). For *A. brassicae*, such specific primers for specific and quantitative detection using real-time PCR have been developed by Guillemette *et al.* (2004).

7. Conclusions

Little information is available on organic production systems for seed production of *Brassica*, especially on specific problems caused by diseases and their prevention. From the information collected from literature in this review, the following measures for prevention of seed contamination by *A. brassicicaola* can be advised:

- Production of pathogen-free basic seed in disease-free areas;
- Thorough seed testing to ensure pathogen-free basic seeds;
- Climate control during seedling production avoiding temperatures above 20 °C in combination with humid conditions;
- Seed production fields at distance of at least 2 km from other *Brassica* crops or crop residues including oilseed rape;
- Establishment of crops with open canopy to reduce risks of long wetness periods;
- Control of pollen beetle, flea beetle and slugs as potential vectors;
- Control of weeds within the crop and wild plants in neighbourhood of crop which may serve as host;
- Monitoring of diseases in crop and removal of infected plants or plant parts;
- Removal of crop residues.

Epidemiological research on *A. brassicicola* in organic seed production of *Brassica* should be directed at contamination of seed during their development in the field by locally produced inoculum. For such studies, the quantitative specific detection of *A. brassicicola* in the crop will be an important tool. Based on the results of such studies, measures can be developed for disease prevention or control in organic seed production of *Brassica*, such as climate control in the crop, disease control with fungicides available in organic farming during critical periods or vector control.

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Infection of Brassica seed with *Xanthomonas campestris* pv. *campestris*

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1. Introduction

Black rot of crucifers is characterized by blackened vascular tissues and foliar marginal V-shaped chlorotic or necrotic lesions (Cook *et al.*, 1952a). As the disease progresses, parenchyma cells surrounding vessels in the main stem turn black, and the plant becomes wilted, stunted and finally rots. The disease is found throughout the world and is one of the most destructive diseases of crucifers which cause considerable economic damage.

The disease is caused by the Gram-negative bacterium *Xanthomonas campestris* pv. *campestris*, (Xcc), a pathogen that can infect a wide range of plants within the crucifer family Brassiceae, including cabbage, cauliflower, kale, rape, radish, and black mustard, but also *Arabidopsis*, a plant used in molecular model studies. Epidemics caused by Xcc are polycyclic. The pathogen, which once established, repeatedly multiplies and spreads when conditions are favorable (Figure 1).

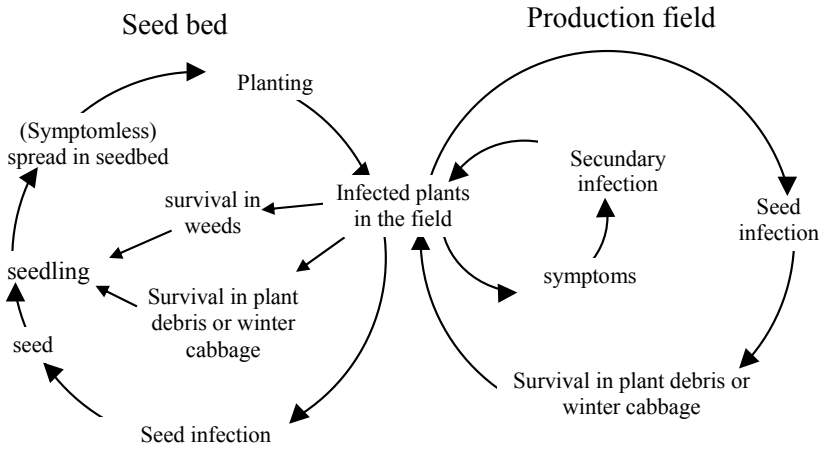

Figure 1. Disease cycle of black rot caused by Xanthomonas campestris pv. campestris in cabbage. For initial infections, debris of cabbage plants, weeds and introduction via irrigation water are most important. Dispersal in nurseries and in the fields are mainly due to splash dispersal.

The pathogen has a high degree of variability with respect to the virulence on host plants, serology, genetics, and with respect to physiological and biochemical properties (Alvarez *et al.*, 1994). A leaf spot disease of crucifers is caused by a closely related seedborne pathogen, *X. c.* pv. *armoraciae* (McCulloch 1929) Dye 1980, which is equivalent to *X. campestris* pv. *raphani* (Machmud, 1982).

In this survey, literature is extracted concerning infection of *Brassica* seeds with Xcc. First, important epidemiological features of Xcc are outlined briefly. Thereafter, routes for seed infections and the localization of Xcc within seeds in relation to disease incidence are summarized. The survey finalizes with some remarks concerning the risks for seed infections with Xcc in organic agriculture.

2. Epidemiological features

2.1 Initial infections

Diseases often start with infected seed as the initial inoculum, even though infected seeds often appear healthy (Cook *et al.*, 1952b). Other primary sources of infection can be soil, splashes and aerosols dispersed from adjacent infected fields, infected (perennial) weeds, infected machineries and materials and possibly insects.

Soil is a well-known source of inoculum. Xcc can only survive for 20-50 days free in soil, but for ca. two year in cabbage residues in soil (Schaad & White, 1974; Dzhalilov & Tiwari, 1995; Kocks *et al.*, 1998)

Aerosols were taken from a field with Xcc infected *Brassica campestris* weed plants using a particle sampler, both during rainy and dry periods. The number of cfu per cubic meter of air ranged from ca. 14 during rain to ca. 1 during dry periods indicating that Xcc can be dispersed from infected weeds (Kuan *et al.*, 1986).

Several weeds, in particular cruciferous weeds, have been found to host Xcc (Schaad and Dianese, 1981; Kuan *et al.*, 1986; Dane and Shaw, 1996). During a survey in the USA (Georgia and California) it was found that Xcc is readily transmitted from infected weeds to cabbage under field conditions. Infection of weeds was not always associated with cultivated crop of crucifers. They were even found 32 km from the nearest cultivated crucifer crop. Epiphytic populations on weeds were lower after periods of heavy rainfall and low temperatures at night (0.6-6 ^{o}C).

The role of insects is largely unknown. Only some transmission studies were done with flea beetles (*Phyllotreta cruciferae*) (Shelton and Hunter, 1985). Beetles infected by feeding them for 48 h on cabbage plants showing black rot symptoms, were able to transfer Xcc to broccoli plants in greenhouse experiments with a high efficiency. However, black rot did not develop when immigrating flea beetles were collected in cabbage fields and transferred to healthy broccoli plants in the green house. Insects are attracted by the nectar of blooming plants and may easily infect the flowers and from there the developing seed.

2.2 Dispersal of the pathogen

During germination of infected seeds, the pathogen invades the mesophyll and the vascular tissues of the epicotyl, infects foliage and is released through guttation droplets at leaf margins. During the cropping period, inoculum is spread by watersplash, wind-driven rain, aerosols, possibly insects and by mechanical injury during cultivation (Williams, 1980; Kuan *et al.*, 1986). In particular Xcc will rapidly spread in misted seedbeds from infected seedlings (Roberts *et al.*, 1999; Shigaki *et al.*, 2000).

After bacteria are deposited on the leaf surface, the epiphytic populations may build up, such as have been demonstrated for other Xanthomonads (Rudolph *et al.*, 1994). They can remain on the leaf surface at relatively high densities (> 100 cfu/cm^2) for more than 40 days (Dane & Shaw, 1993; Arias *et al.*, 1996). Xcc is protected from photobiological damage by the yellow pigment, xanthomonadin (Poplawsky and Chun, 1998). Xcc colonizes plant surfaces in aggregates.

Plants are mainly invaded through hydathodes and occasionally via leaf and root wounds and other natural openings such as stomates and the openings at root junctions. Hydathodes (water pores) provide a continual path of water from vessels to leaf margins. The water contains minerals, carbohydrates and amino acids (glutamine) and has a pH of 7.0, which is optimal for growth of Xcc. Cells probably loss motility when they colonize the leave surface, but as soon as free water becomes available, they shift to a motile phase to reach the leaf margins, due to chemotaxis. When they have colonized the guttating leaf margins they become non-motile again (Kamoun and Kado, 1990). Stomatal infections are rarely seen in the field, but occasionally are observed in seed beds under conditions that reduce hydrophobicity of the cells, e.g. after treatment with crop protection agents. Stomatal infection may result in

a leaf spot disease. Roots may become colonized from infected plant debris in soils. Root infections have resulted in extensive colonization of plants and typical V-shaped lesions on the leaves, indicating that this infection route is also possible (McElhaney *et al.*, 1998). Bacterial growth and symptom expression is strongly favored by temperatures of 25-30 °C and they are masked at 15-20 °C.

3. Seed infections

Cabbage seed is produced primarily in temperate regions, where cool mild winters are found for vernalization. Seed production starts in midsummer in seed beds and seedlings are transplanted to production fields roughly after a month. In spring, plants bolt to flower. In summer seeds ripen and are harvested. The seed production is most susceptible to infection with Xcc during the late summer in seed beds, before transplanting and just before flowering, because in these periods the temperatures are most favorable for bacterial growth.

3.1 Invasion of the seed via systemic movement of Xcc through the vascular tissue

On infected seed plants grown under optimal conditions for seed production, black rot symptoms develop in general after the flowering stage is well advanced (Cook at al, 1952b). At that time the vascular system of branches, pedicels and pods is systemically infected. Xcc moves through the infected pods to the funiculi. From there, the invasion of the seed coat is likely, although it has not been demonstrated histologically. In seeds with infected funiculi and seed coats Xcc likely is able to resist chemical- and hot water treatments.

3.2 Invasion of seeds via flowers

For several plant pathogens flowers have been recognized as an important port of entry. *Erwinia amylovora*, the fire blight pathogen, can enter unwounded blossoms through natural openings in the stigma, nectary, anthers and sepals of pear (Van der Zwet *et al.*, 1988). Pollinating bees and other insects play an important role in these flower infections. Flower infections have also been proven for bacterial blossom blight of kiwifruit caused by a *Pseudomonas* sp., for *Pseudomonas syringae* in pear and for *Acidovorax avenae* subsp. *citrulli* in watermelon (Ercolani, 1970; Morita, 1995; Walcott *et al*, 2005).
Surprisingly, no literature on the role of flower infections in the epidemiology of Xcc is available. In most cabbage varieties, insects play a crucial role in pollination. Therefore it can be hypothesized that contaminated bees, bumble bees, flies but also pest insects may readily spread the Xcc when visiting flowering cabbage plants. The potential of insects to transmit bacteria may also be used for delivering of biocontrol agents, as have been demonstrated for honey bees contaminated with *Bacillus subtilis*, which pollinated blueberry flowers (Dedej *et al.*, 2004).

3.3 Seed contamination during harvest and storage

External seed contamination probably occurs mainly during threshing, during which contact with infected pods, stems and leaves may result in seed contaminations. Further smearing may occur during seed grading and storage handlings. In general this will result in an external seed contamination. It is not exactly known to what extent external contaminations contribute to seedling infections. Experiments at Bejo Seeds with surface sterilized seeds and untreated seeds indicated that mainly seeds internally colonized result in disease expression and transmission (V.d Heijden, personal communication). Bacterial populations superficially present on seed coats will decrease during storage and in particular after sowing in soil, due to physical factors, predation and antagonism. Bacteria that persist may however enter and colonize the germinating seeds and emerging seedlings. The exact role of the externally located seed populations is unknown.

3.4 Population in and on seed

According to Clayton (1925), Xcc can survive for a period of 3 years in seed, although the percentage of seeds with viable bacteria decreased with age of the seed. However, for many other bacteria, including other *Xanthomonas* species a survival period of 2-3 years have been described for populations on the seed surface, but prolonged survival times for bacteria in the seed (Neergaard, 1979). It is therefore likely that in the seed, Xcc may survive for longer periods than 3 years. It is expected that the Xcc internally present will survive better than on the outside. The percentage of infected seed is generally small (<0.1%) and rarely exceeds 1%. In the initial stages of infection, the percentage infected seeds per seed lot relates relatively well with the number of infected plants (Schaad *et al.*, 1980).

Under favorable conditions for disease expression, a percentage of 0.03% seed infection can cause black rot in the field already (Schaad *et al.*, 1980). At initial stages of an epidemic, symptom expression is mainly dependent on the percentage of seed infection and the densities per seed (Roberts *et al.*, 1999). In later stages the environmental conditions, under which the water regime, determines disease expression more.

The distribution of pathogenic bacteria in seeds seems to follow a lognormal or a Poisson distribution (Gitaitis *et al.*, 2005). Densities of *X. axonopodis* pv. *vignicola* in cowpea, *Clavibacter michiganensis* subsp. *michiganensis* in tomato and *Acidovorax avenae* subsp. *citrulli* in watermelon seed ranged from 10 to 10^6 cfu per seed, but a high percentage (> 95%) of the infected seed contained relative low cell densities. It was concluded that there is a risk for an over estimation of the mean populations of the pathogen and thus potentially an under estimation of the seed sample size required for a desired level of confidence.

4. Xcc in organic seed production

At a workshop held at Ryton Organic Gardens (Ryton, UK, 1 November, 2001), it was black rot was listed as one of the two important seedborne pathogens in organic cabbage production. Further, no specific literature on the risks for Xcc infections in organic seed production is known, but overall an increased risk on seed infections can be expected. It can be hypothesized that the use of organic manures in stead of fertilizers reduces the risk on oversized succulent transplants, which result in extended periods of leave wetness and which are more susceptible for Xcc infections (Williams, 1980). On the other hand, it was found that high levels of nitrogen slowed down the colonization of cabbage plants by Xcc (McElhaney *et al.*, 1998).

High microbial activity in soils may favor rapid decomposting of crucifer residues which reduces risks for introduction of Xcc in organic seed production compared to conventional systems. The lack of efficient means for weed control, however will increase the risks for survival of Xcc in the absence of Brassica's.

Increased risks for damage by pests and diseases due to the avoidance of chemical pesticides will enhance risks on wounding and infection. The relative high number of insects in organic cabbage plants may also increase the risks for dissemination of Xcc and flower infections.

5. Literature

Remark. Literature was collected from the following sources: Biological Abstracts, Current Contents, CABI abstracts, IBL kennisbank, the FiBL data base, and literature collections from the former RPVZ (Wageningen, the Netherlands).

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