

Transmission of *Xanthomonas campestris* pv. *campestris* by insects

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Introduction

Xanthomonas campestris pv. *campestris* (Xcc) is the causal organism of black rot, a destructive disease of a wide range of plants within the crucifer family *Brassicaceae*, including cabbage. Black rot often starts with infected seed as the initial inoculum, even though infected seeds often appear healthy. There are strong indications that disease outbreaks are mainly caused by internal seed infections. Hence, the use of seed free from internal infections is one of the most important ways to avoid disease problems in organic agriculture. The epidemiology of Xcc should be known in order to identify critical control points. During the cropping period, it is established that inoculum is spread by water splashes, wind-driven rain, aerosols and by mechanical injury during cultivation. In particular Xcc will rapidly spread in misted seedbeds from infected seedlings (Köhl and Van der Wolf, 2005).

The role of insects in transmission of Xcc is largely unknown. Insects may play a role both in short- and long distance dissemination of Xcc. Contaminated insects pose a threat for organic seed production as insects cannot be controlled with synthetic pesticides. Only some transmission studies were done with flea beetles (*Phyllotreta cruciferae*), which showed that they were able to transfer Xcc from diseased to pathogen-free cabbage plants with a high efficiency (Shelton and Hunter, 1985). No information is present if pollinating insects can transmit Xcc to flowers and if this can result in internal seed infections.

The objective of our study was to determine the potential role of pollinating insects in (internal) seed infection of *Brassica oleracea* with Xcc. The survival of *Xanthomonas* on flies was determined. It was investigated if infection of plants with Xcc-contaminated flies results in (internal) seed infections. By brush inoculation of flowers with Xcc it was determined if flower infections can result in internal seed infections.

Survival of *Xanthomonas* on flies

Flies (*Calliphora vomitoria*) were inoculated with Xcc by adding flies in boxes with YDC plates grown with Xcc for 24 h (Fig. 1A). YDC plates were removed and seven boxes with 3 flies were sampled at each day. Flies were shaken in PBS + 0.1% Tween20 (PBST) for 30 min. Extracts were plated on the semi-selective medium FS. The Xcc population on flies declined gradually in 5 days to 1-10 cfu per fly (Fig. 1B). No Xcc was detected after 7 days.

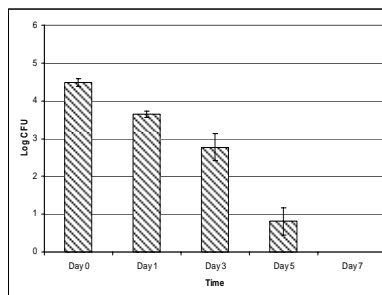


Fig. 1. Survival of *X. campestris* pv. *campestris* on flies (*Calliphora vomitoria*). A. Experimental set up. Flies were fed for 2 h on a medium with Xcc. B. Population of Xcc on flies were determined 0, 1, 3, 5 and 7 days after inoculation.

Transmission by flies to cabbage plants

For the tunnel experiments, ten week old seedlings of an open-pollinating cauliflower cv. Opaal (RZ) (Cv. A) and a breeding line of a summer cauliflower (cv. B) were placed in tunnels in Wageningen (the Netherlands). Per treatment 20 plants were used, randomized over 5 tunnels. Plants were covered per pair by a nylon insect proof cage. Flies were inoculated with Xcc as described above. Inoculated and non-inoculated flies were released 8 times from 14 June till 8 July 2005, during blooming of the plants. Flowers of plants were also inoculated manually with a brush dipped in a Xcc suspension in water. Before new flies were released, the flies present on plants were killed by fumigation. Plants were observed for symptoms in August and suspected black rot, V-shaped lesions were checked for the presence of Xcc by plating on FS. Seeds were harvested at 22 September 2005. Seeds of two plants, growing together under a insect proof cage were pooled. Seeds were cleaned and analysed for the presence of Xcc. For seed analysis by dilution plating on FS, 100 – 1000 seeds per sample were extracted; per 100 seeds 1 ml of PBST was used. Seeds were tested before and after disinfection with hot water. For each sample, the identity of a suspected colony on FS was confirmed by PCR, using both primer pairs described by Rijlaarsdam et al. (2004).

After brush- and fly-inoculation, a few plants developed typical black rot symptoms in August. The presence of Xcc in the lesions was confirmed by dilution plating and characterization of suspected colonies by PCR. Probably insects or contaminated blown flowers that dropped down have infected (guttating) leaves. Infection of blooming cauliflower plants with contaminated flies or by brush-inoculation of flowers resulted for cultivar A in internal seed infection (Table 1). For cultivar B, internal infections were only found after brush-inoculation. All suspected colonies on FS were positive with both primer sets in PCR. The identity of the strains was also confirmed with BOX-PCR. The experiments will be repeated in 2006. Also the frequency in which insects become contaminated with Xcc when present on an infected cabbage crop will be determined in field experiments.

Table 1. Seed infections with *X. c. pv. campestris*

	Number of seed samples positive (n=10)	
	Before disinfection	After disinfection
Cv. A.		
Fly-inoculated	8	5
Brush-inoculated	5	3
Control	2*	0
Cv. B		
Fly-inoculated	5	0
Brush-inoculated	8	4
Control	1*	0

*Only low densities of 10-100 cfu/ml were found

Conclusions

Xcc can survive for up to 5 days on flies. Within this period Xcc can be disseminated within a field, but flies can also migrate for a distance of more than 20 km, thus infecting cabbage plants at a more distant location. The use of contaminated flies for pollination resulted in internal seed infections. It is likely that Xcc moved via style and ovary into developing seeds. It cannot be entirely excluded, however, that internal seed infection was a result from movement and transport via the vascular tissue and funiculi after leaf infection. Due to the risk for transmission of Xcc by flies it is strongly recommended to locate Brassica seed production fields at a distance of preferably more than 20 km from other cabbage fields.

Acknowledgement

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References

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