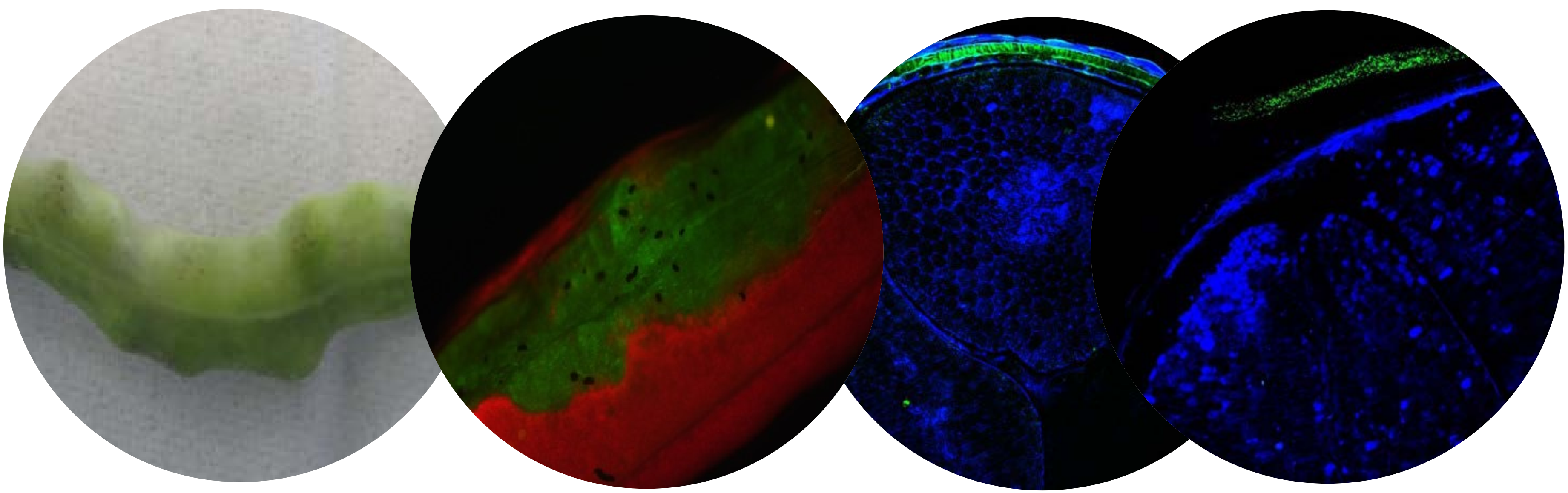
Testa

Colonization routes of *Xanthomonas campestris pv. campestris* in Brassica plants that can result in seed infection

Jan van der Wolf, Pieter Kastelein, Tadeu Antônio Fernandes da Silva Junior and Flávia Vieira Lelis



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Xanthomonas campestris pv. *campestris* – black rot

Mature plants

Transplants

V-shaped chlorotic and necrotic lesions along leaf margins, vein blackening

http://ohioline.osu.edu/hyg-fact/3000/3125.html

Objectives

- Study the seed infection process on Brassica plants after flower inoculation with GFP-tagged Xcc strains
- Study the transmission of Xcc by bumble bees from infected to Xcc-free plants

Flower infection of Brassica plants with Xcc

Flowers (5 days after beginning flowering) of Rapid Cycling Brassica plants grown at 24^{o}C, inoculated by spraying* strain GFP-tagged strain Xcc 3555

Experiment	Nr. inoculations	Period (2013)	Interval	Nr. plants 3555	Nr. plants water	Boxes (ca. 40 bumble bees)
1	11	May	2-3 days	64	12	3x
2	8	December	2-3 days	50	12	6x

* Experiment 1: 10^{8} cfu ml^{-1} suspension; Experiment 2: 10^{7} cfu ml^{-1} suspension

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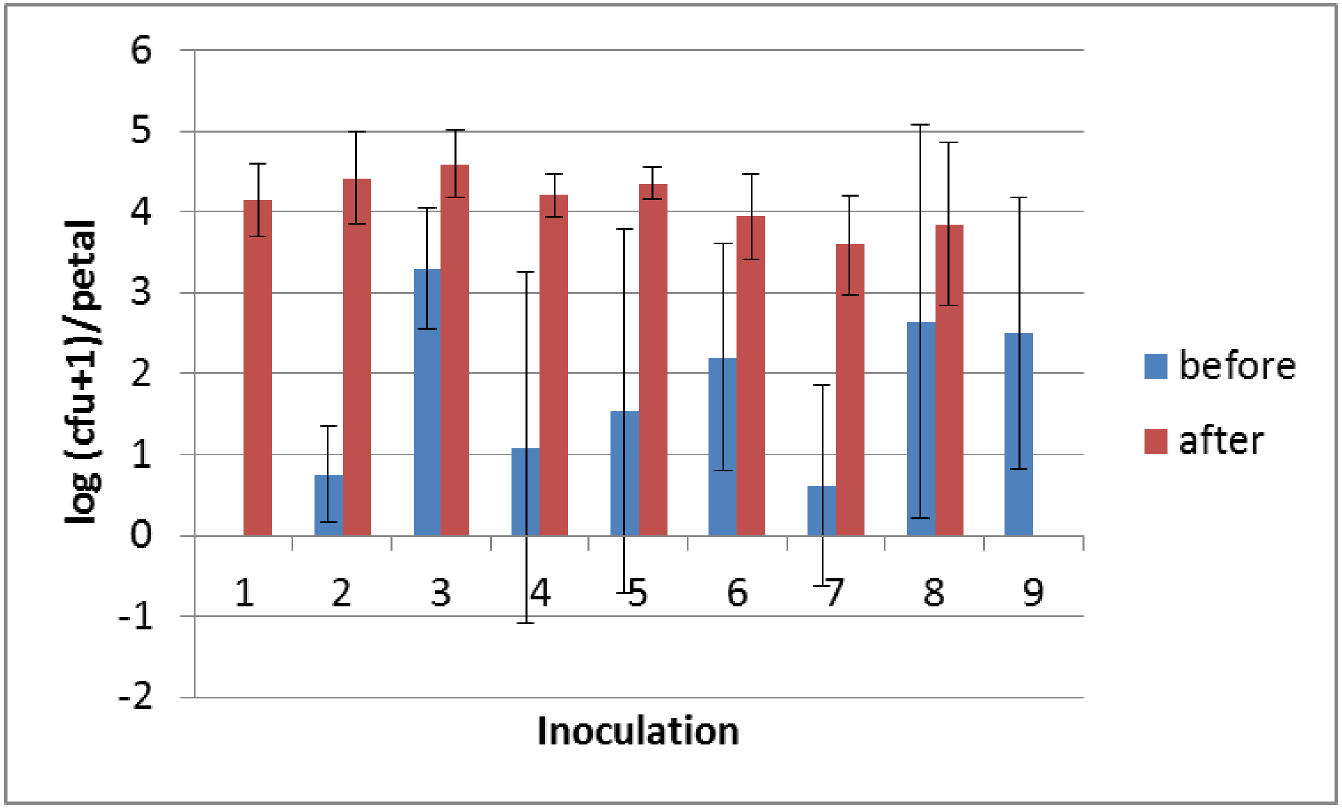
Flower Infection of Brassica Plants by Xcc

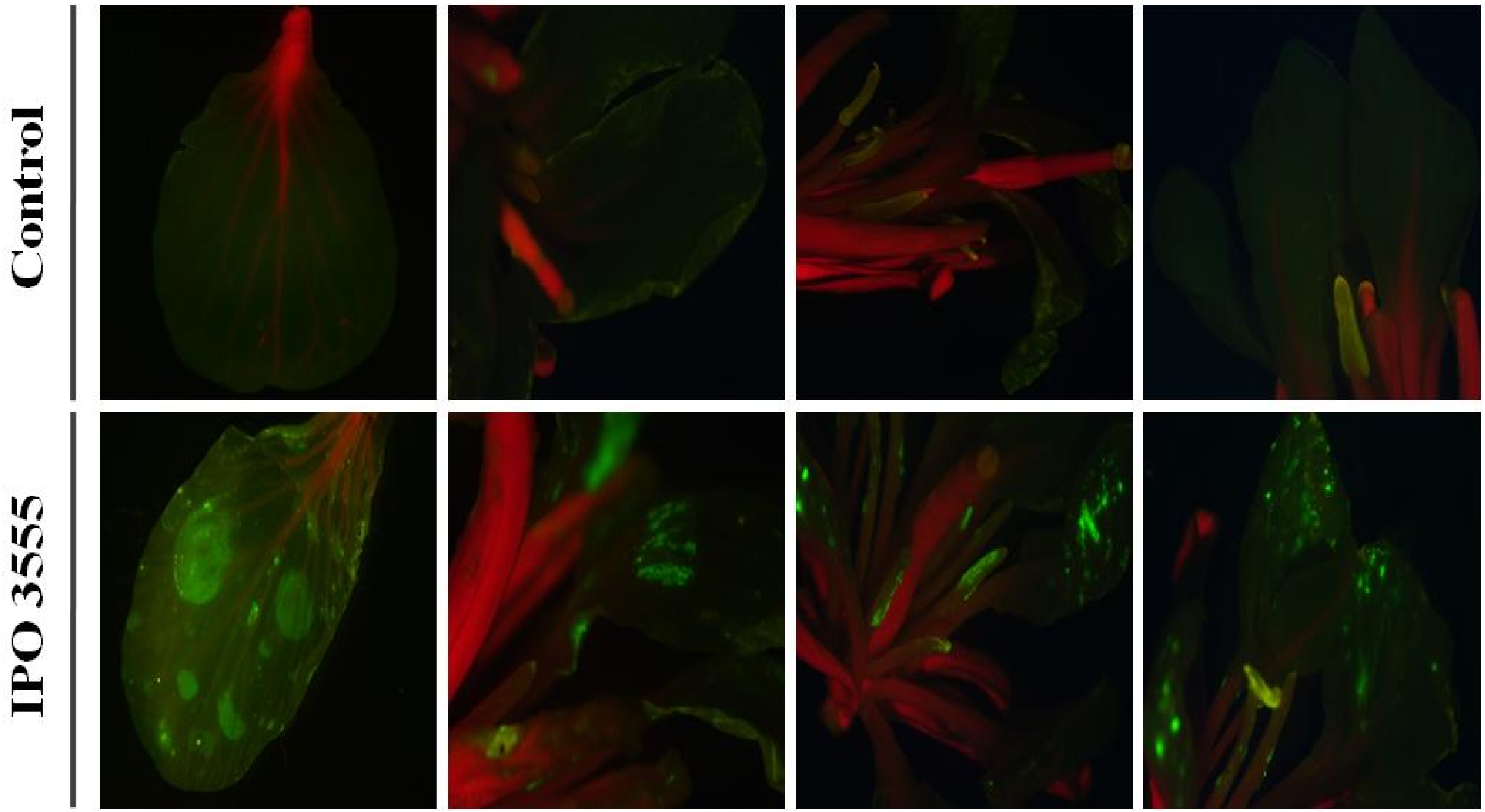
- Petals
  - Population dynamic studies by dilution plating
  - Localization studies by epifluorescence microscopy (ESM)
- Siliques
  - Black rot incidence on siliques – analysis 11 days after the last inoculation
  - Localization studies with ESM
- Seed
  - Incidence of external and internal infections by dilution plating on mFS(kan)
  - Localization studies with ESM and confocal laser scanning microscopy (CLSM)

Xcc does not colonize petals after spray-inoculation



% infected petals before inoculation: 66 (n=32)
% infected petals after inoculation: 100 (n=32)

ESM analysis of flowers direct after inoculation



GFP-signals on petals, sepals and stamens

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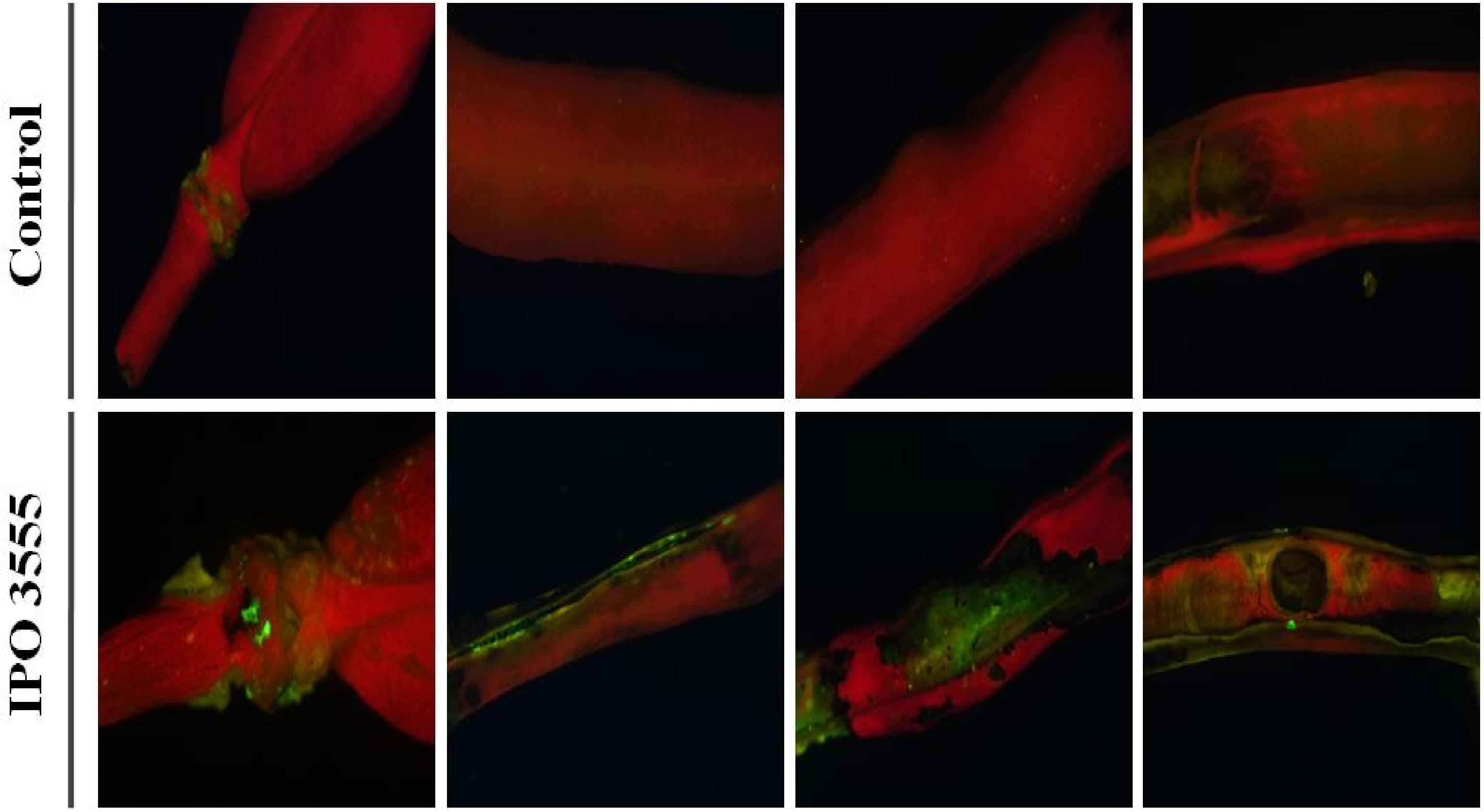
% symptomatic siliques

Experiment	Inoculum	Nplants	Overall		Range per plant
1	10^8 cfu ml^{-1}	20	76.5	a	15 - 100
2	10^7 cfu ml^{-1}	19	57.6	b	35 - 95

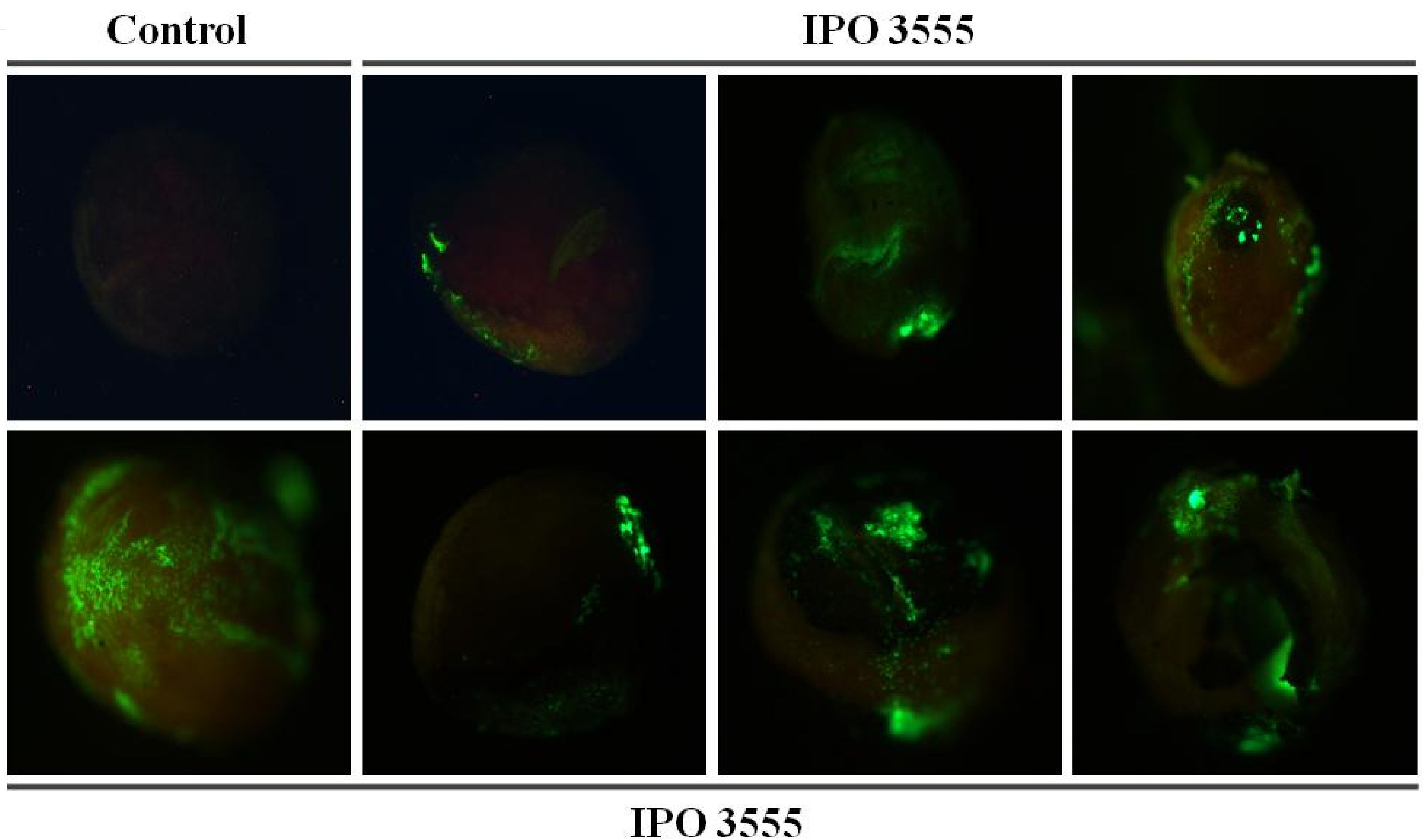
Per randomly selected plant, 20 siliques were assessed

Xcc does colonize siliques after spray-inoculation



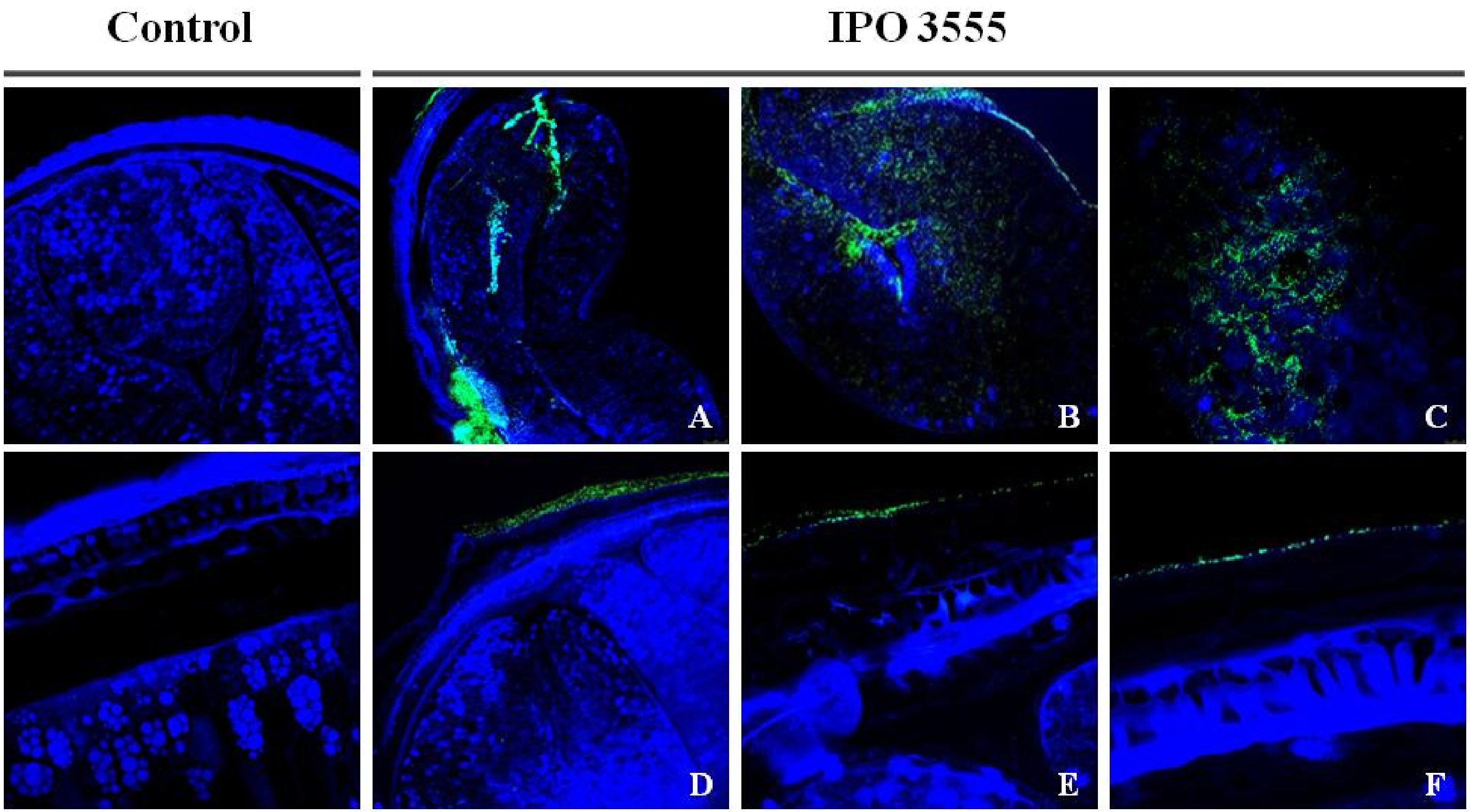
Colonization of pedicels, septum and valves

Xcc can colonize the seed coat after spray inoculation often in low densities



2[nd] experiment: colonization seed coat in 5.2% seeds (n=174, 23 siliques (not visible before incubation on blotters)

Xcc can cause internal seed infections



Colonization seed coat in 5.2% seeds (n=174, 23 siliques
(not visible before incubation on blotters)

% Xcc-positive subsamples

Exp.	*N*		*% external contaminations*			*% internal infections**		
			Total	*Low densities ($0-10^3$)*	*High densities ($>10^3$)*	*Total*	*Low densities ($0-10^3$)*	*High densities ($>10^3$)*
1	*32*	*Xcc*	*84*	59	25	66	28	38
	32	*Kan^r Xcc*	*12*	9	3	6	0	6
2	*100*	*Xcc*	*85*	16	69	32	32	0
	100	*Kan^r Xcc*	*9*	9	0	1	1	0

(25-seed subsamples)

* Positive after hot-water treatment

% Xcc-positive seeds

Experiment	*N*		% external contamination	% internal infection
1	*32*	*Xcc*	7.2	4.2
2	*100*	*Xcc*	7.0	2.0

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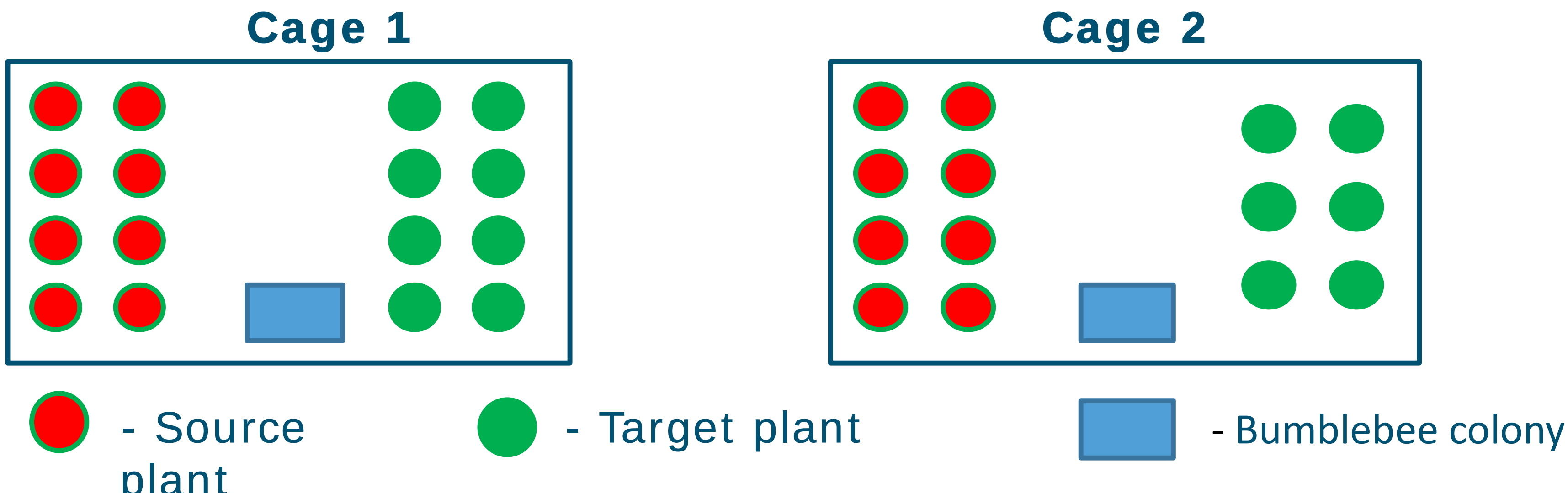
$$I = \{1 – [(N – p)/N]1/n\}*100$$

Transmission of Xcc by bumblebees

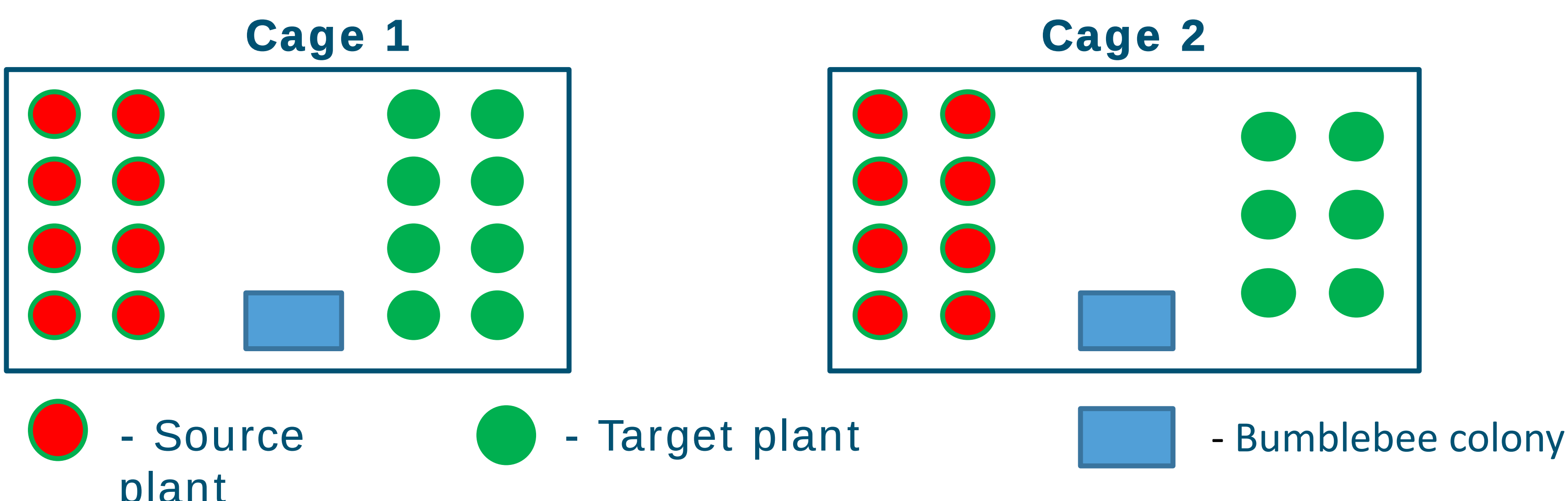
- Two cages with flowering RCB plants
  - One cage with 8 inoculated source plants and 8 non-inoculated target plants
  - Another cage with 8 inoculated source plants and 6 non-inoculated target plants



Transmission of Xcc by bumblebees

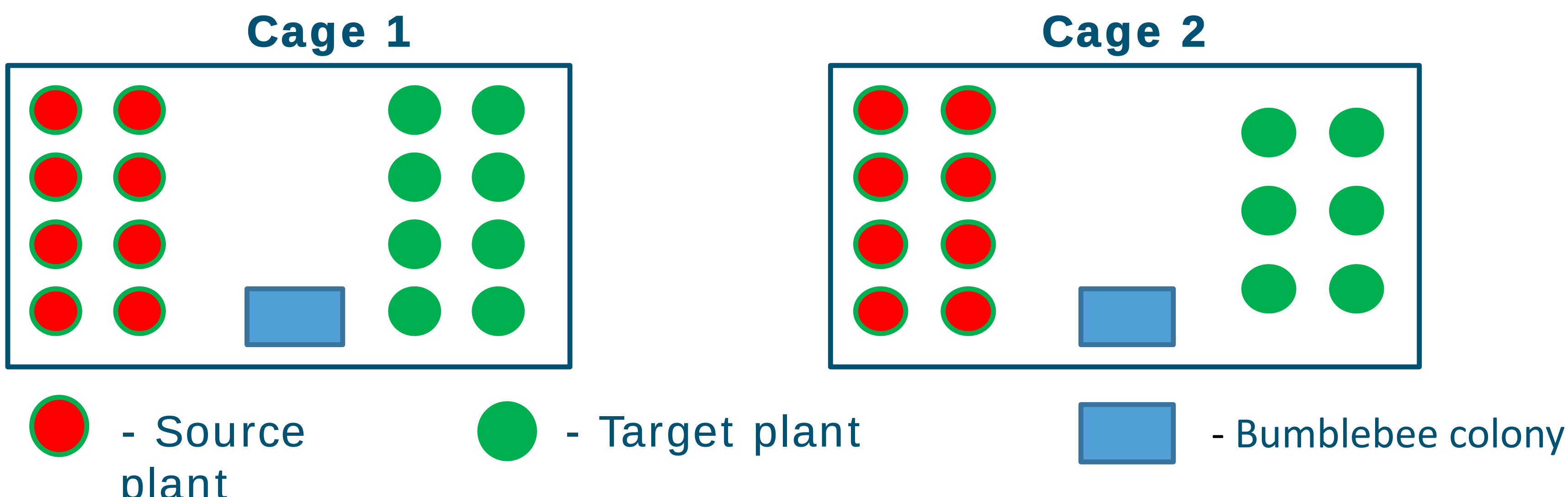
- Source plants and target plants were kept separately until starting the experiments
- Source plants were inoculated 4 times with WT strain IPO 3078 (10^8 cfu/ml)
  - Leaves and flowers at 40 days after sowing
  - Flowers at 42, 45 and 47 days after sowing

  (Leaves developed black rot symptoms)



Transmission of Xcc by bumblebees

- Source plants, target plants and bumblebees were placed in the cages directly after the 4th inoculation of the target plants
- Source plants and target plants without direct contact
- Bumblebees in the cages during 7 days
- Source plants and bumblebees removed from the cages at the same time



Transmission of Xcc by bumblebees

- Assessments on target plants
  - % siliques with black rot symptoms
  - Xcc infections in seed
- Siliques with black rot
  - Cage 1
    - Total over 8 plants: 12.0%; Range 2 – 22%
  - Cage 2
    - Total over 8 plants: 14.6%; Range 3 – 31%
- Seed infections
  - Cage 1
    - Xcc detected by plating in seed from 5 of the 8 plants
  - Cage 2
    - Xcc detected by plating in seed from 3 of the 6 plants

Survival of Xcc in bumblebee colonies

- Two experiments with survival of Xcc bumblebee colonies in the laboratory
  - Xcc WT strain IPO 3078
  - 48 hr cultures on TSA plates
  - Bumble bees were brought in contact with bacterial slime
  - In each experiment 2 inoculated bumblebee colonies and 1 negative control
  - Xcc contamination assessed
    - 0, 2, 5, 7, 9, 12 and 14 dpi (experiment 1)
    - 0, 2, 5, 7, 9, 12, 14, 16, 19, 21 and 23 dpi (experiment 2)
  - 3 bumblebees/colony shaken for 10 min in 10 ml PBST
    - Dilution plating on mFS medium
    - 72h incubation 25^{o}C

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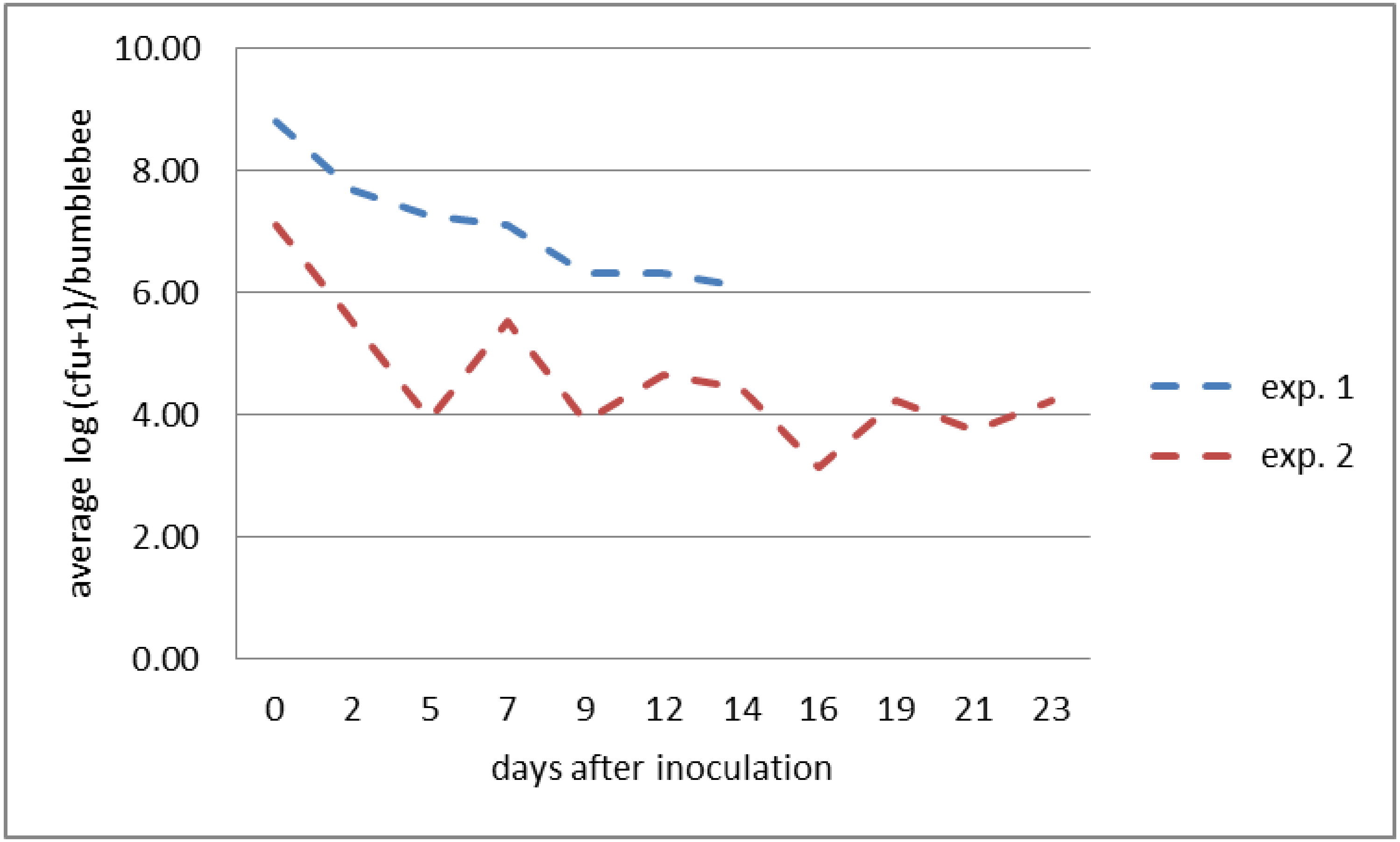
Survival of Xcc in bumblebee colonies



Average of 6 bumblebees per time point

Conclusions and discussion

- Flower inoculation of Brassica plants results in high rate of infected seed
- CLSM analysis strongly suggests internal infections of seeds
- The presence of Xcc in hot-water treated seeds also indicates the presence of internal infections
- It is very likely that bumble bees can transmit Xcc from symptomatic to Xcc-free plants
- Xcc can survive for a long period on bumble bees
- The lack of stability of the GFP-expression in transformed strains is still a problem

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