Transmission and spread of *Xanthomonas campestris* pv. *campestris* in brassica transplants: implications for seed health standards

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Summary

Xanthomonas campestris pv. *campestris* is well known as an important seed-borne pathogen of brassicas. Seed health assays should be designed to have a high probability of detecting unacceptable seed lots. Mathematical models have been developed both for transmission of the pathogen from seed to seedling and subsequent spread in module-raised brassica transplants. Using these models, with different initial parameters, the potential for development of disease epidemics can be explored for negative results obtained by seed health assays with different sensitivities (detection limits) and tolerance standards. Examples of different scenarios will be presented, and suggest that the greatest risk arises when negative test results are obtained from seed lots with a relatively high proportion of infested seeds but low number of bacteria per seed.

Introduction

Xanthomonas campestris pv. *campestris* (*Xcc*) is well known as an important seed-borne pathogen of brassicas. Seed health assays should be designed to have a high probability of detecting unacceptable seed lots. The problem is to define an 'unacceptable seed lot', and in recent years there has been much dispute over the value of the most sensitive seed health assays and the tolerance standards required to achieve satisfactory control of *Xcc* in brassicas. Schaad *et al.* (1990) suggested a tolerance standard of 0.01% for a direct-drilled brassica crop, but that this was inadequate for transplant production. Most vegetable brassicas are grown as transplanted crops, but most seed is still tested to a tolerance standard of 0.01%. This paper will present the results of work done over several years to develop models to describe the transmission and spread of *Xcc*, which have then allowed us to examine the potential development of disease epidemics for seed lots with different seed health scenarios and the likelihood of their detection in seed health assays with different sensitivities.

Models

A model for the transmission of *Xcc* from seed to seedling was devised using data from glasshouse experiments. Seed was inoculated with different concentrations of bacteria, sown in commercial module trays, and subjected to different watering regimes (Roberts *et al.*, 1999). Visible symptoms were recorded, and leaf washings were carried out to detect

the pathogen on symptomless plants. The results were consistent with a one-hit model for infection/transmission:

$$P = 1 - \exp(-w.d^x)$$

where P is the probability of transmission, w is the 'one-hit' probability, d is the dose (number of *Xcc* per seed) and x is a dose coefficient.

A model for the spread of *Xcc* in brassica transplants was developed using data from a series of glasshouse experiments designed to simulate a typical commercial module plant raising system with overhead gantry irrigation (Roberts *et al.*, 2007). Primary inoculum was introduced as inoculated seeds in one or more cells. Disease symptoms were mapped and the presence of the pathogen on samples of plants was monitored by leaf washing, dilution and plating on a selective medium. Spread of symptoms and spread of contamination followed a similar pattern, but the proportion of plants contaminated was much greater than the proportion showing symptoms, approaching 100% after 6 weeks in the gantry-watered trays within 50 plants distance from a single primary infector. Models relating the proportion of plants with symptoms, or contaminated, to the distance from primary infector and time since sowing were fitted to the data:

$$\ln[p/(1-p)] = \ln(a) + b\ln[c + (k.x^2 + y^2)^{½}] + r.t$$

where p is the proportion of plants contaminated, a is an intercept parameter, b is the gradient, c is a truncation parameter, k is a directional scaling parameter, x,y are the distance from the primary infector in the x and y directions, r is the relative contamination rate, and t is time.

These models were used to explore the potential for development of disease epidemics in commercial-scale blocks of transplants for seedlots with different proportions of seed infested and different numbers of bacteria on those infested seeds. Using model parameters from different spread experiments, the expected proportions of contaminated transplants were calculated for a block of approximately 100,000 transplants, assuming uniform distribution of infested seedlings and assuming 100% transmission.

The average percentage contamination of transplants was then calculated by multiplying the expected proportion obtained from the spread models above by the probability of transmission obtained from the transmission model for the different seed infestation scenarios.

For each seed infestation scenario, the probability of detection was also calculated for seed health assays with different sensitivities (detection limits; resulting from the inclusion/omission of a centrifugation step). The probability of at least one infested seed being contained in the sample is given by:

$$P_{cont} = 1 - (1 - \theta)^n$$

where θ is the true proportion of infested seeds in the lot and n is the total number of seeds in the sample. Then, if present, the probability of detecting an infested seed in a sub-sample is given by:

$$P_d = 1 - e^{-\lambda v}$$

where λ is the mean density of bacteria in the suspension (the number of bacteria per infested seed divided by the volume in which the sub-sample is suspended) and v is the effective volume plated. Thus the probability of a positive result for the test is given by:

$$P_{+} = P_{cont} \times P_{d}$$

Arbitrarily, an unacceptable seed lot was defined as one in which the expected average contamination of transplants was greater than 10% at the time of planting (6 weeks after sowing), and an unacceptable test was indicated when the probability of detection was less than the probability of transmission for an unacceptable lot.

Results and conclusions

Some example scenarios are shown in Table 1, starting with seed infestation levels ranging from 1 in 5,000 to 1 in 50,000 seeds and mean numbers of *Xcc* per infested seed from 10 to 1000. The remaining columns show the results of running the transmission and spread models, together with the probabilities of obtaining a positive seed test result with and without a centrifugation step.

The transmission and spread models suggested that the high levels of disease incidence often seen in the field can be explained by rapid rates of pathogen spread during plant-raising, and

Table 1 Example scenarios for different proportions of infested seed and numbers of *Xanthomonas campestris* pv *campestris* per infested seed, together with the probability of a positive test result with (Cent) and without (No cent) centrifugation to improve analytical test sensitivity

One infested seed in:	% infested	CFU per infested seed	Probability of transmission	Average % contamination of transplants	Probability of positive seed test	
					Cent.	No cent.
50,000	0.002	10	0.06	0–5	0.08	0.01
		100	0.12	1–11	0.39	0.08
		1000	0.23	1–21	0.45	0.39
25,000	0.004	10	0.14	1–13	0.13	0.01
		100	0.26	3–26	0.60	0.13[1]
		1000	0.47	5–46	0.70	0.60
10,000	0.01	10	0.25	7–25	0.17[1]	0.02[1]
		100	0.46	12–45	0.82	0.17[1]
		1000	0.72	19–71	0.95	0.82
5,000	0.02	10	0.44	20–44	0.33[1]	0.04[1]
		100	0.71	32–70	0.98	0.33[1]
		1000	0.92	42–91	0.99	0.98

[1]Unacceptable tests.

that the widely used tolerance standard for seed health testing (0.01%) is inadequate and should be revised to 0.004%. Given the potential difficulty of achieving this standard (it requires 75,000 seeds to be tested), in addition to seed health testing, control should focus on raising transplants under conditions that minimise the rate of disease/pathogen spread.

The results also indicated that omitting the centrifugation step (as in the current ISTA method) gives a greater risk of unacceptable tests. The greatest danger of detection failures occurs with seed lots with a relatively high percentage infestation but low numbers of bacteria per seed, and highlights the importance of both the detection limits and analytical sensitivity when designing effective seed health assays.

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Occurrence and importance of seed-borne *Bipolaris sorokinana* in Norwegian barley

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Summary

During recent years *Bipolaris sorokiniana* has occurred at high frequencies in seed lots of the barley cvs Annabell and Edel in Norway. In seed treatment experiments with two heavily infected barley lots the infection level was reduced by chemical treatment and field emergence and yields were significantly increased. The best effect was seen with an imazalil + flutriafol compound which increased the yield by approximately 35% compared with untreated. A healthy untreated seed lot of the same cultivar showed approximately the same level of emergence and yield as the best fungicide treatment of a heavily infected seed lot.

Introduction

Bipolaris sorokinana (teleomorph *Cochliobolus sativus*) is a widespread pathogen of cereals and many grasses. It can infect seeds, roots and leaves, causing seedling blight, common root rot, foot rot and spot blotch. Inoculum of *B. sorokiniana* may be seed-borne or arise from infected plant debris in the field and from conidia in the soil. Under favourable conditions infections may result in severe yield losses, due to reduced stand establishment, reduced tillering and shrivelled kernels with reduced size and weight. The pathogen has been considered to be most important in barley and wheat in warm temperate areas. However, it has also been reported to be important in cool climates of Northern Europe (Olofsson, 1976; Kurppa, 1984; Jørgensen, 1986).

In Norway, all cereal seed lots are tested for seed-borne pathogens (Brodal, 1993) and for many years *B. sorokinana* has been observed only sporadically in barley, oats and wheat seeds. However, during recent years the pathogen has been recorded at rather high frequencies in seed of barley, especially the cultivars Edel and Annabell. It was decided to include routine testing for *B. sorokinana* in all seed lots of these two cultivars from 2004. Cultivars Frisco and Helium were also included from 2006. The incidence of *B. sorokinana* recorded in these barley cultivars is presented below. In order to evaluate the importance of the seed-borne inoculum in barley and to test the effect of seed treatment fungicides against the pathogen, field and laboratory experiments have been carried out. Results from experiments in 2007 are presented.

Materials and methods

Occurrence of B. sorokinana in seed

The number of seed lots tested for *B. sorokinana* from 2004 to 2008 is shown in Table 1, and included both seed intended for certification and farm-saved seed. The presence of the fungus was determined after 4 days incubation at 10°C followed by 4 days at 20°C on moist filter paper, according to a modified version of the Doyer method (Jørgensen, 1971). After incubation, the seeds were examined individually under a stereomicroscope (6–25×) and the number of seeds showing sporulation of *B. sorokinana* recorded as infected. Of each sample, 100 seeds were tested.

Seed treatment experiments

Two field experiments were established in 2007. Seed from two naturally infected seed lots (Annabell, 90% infection; Edel, 40% infection) were treated with fludioxinil (Celest 025 FS), guazatine + imazalil (Panoctine Plus), imazalil + flutriafol (Fungazil Gold) and *Pseudomonas chlororaphis* (Cedomon) at recommended doses. The four treatments and an untreated control were sown in field plots of 1.5 × 8 m in three replicates (randomised block design). In addition, healthy seed (as healthy as possible) of the same cultivar was included in each experiment as a healthy control. Samples from all treatments and controls were tested in the laboratory for germination and presence of *B. sorokiniana*. In the field, emergence was recorded at growth stage BBCH 12–13 by counting number of seedlings in 4 × 1 m row in the plot. Plots were harvested and the yield measured. Seed samples from the harvested yield of each plot were tested in the laboratory for the presence of *B. sorokiniana*.

Results

Occurrence of B. sorokinana in seed

A large proportion of the seed lots tested for *B. sorokiniana* were infected, and the average infection frequencies were rather high (Table 1). Despite this, the germination capacity (results not shown) were in general not severely affected. Most of the seed lots showed a germination percentage above the minimum requirements of 85% for certification.

Seed treatment experiments

In both experiments, chemical seed treatment reduced the infection level in the seed and increased emergence in the field (Tables 2 and 3). The best effect was found with compounds containing imazalil. Imazalil + flutriafol treatment showed better emergence than guazatine + imazalil with the most infected seed lot (Annabell, Table 2). Both imazalil + flutriafol and guazatine + imazalil significantly increased the yield compared with untreated seed in the most infected seed lot, Annabell. Only imazalil + flutriafol treatment showed significant yield effect in the seed lot of Edel (Table 3). No increased emergence or significant yield increase was found after treatment with *Pseudomonas chlororaphis*.

Emergence and yield in the healthy untreated seed was approximately the same as the most effective seed treatments.

Laboratory tests of seed harvested from all treatments showed a high and consistent level of between 92 and 97% seeds infected with *B. sorokiniana* (data not shown). This indicates

Table 1 Incidence of *B. sorokiniana* in seed lots of the barley cultivars Annabell, Edel, Frisco and Helium grown in Norway during 2004–08

Cultivar	Year of harvest	Number of samples tested	Percentage of samples infected	Average infection frequency
Annabell	2004	88	26	3.4
	2005	148	45	7.8
	2006	123	96	30.7
	2007	94	90	62.5
	2008	41	100	56.0
Edel	2004	382	97	13.6
	2005	527	94	17.0
	2006	444	98	27.9
	2007	362	99	52.3
	2008	243	99	20.2
Frisco	2006	13	84	38.9
	2007	9	100	55.9
	2008	6	83	8.1
Helium	2006	43	77	4.8
	2007	69	75	4.7
	2008	108	69	2.1

that the inoculum of this pathogen can easily spread from infected to healthy plots during the growing season.

Discussion

The high incidences of *B. sorokiniana* in certain cultivars indicate that there are rather clear differences in susceptibility among barley cultivars grown in Norway. The importance of resistant cultivars has been discussed by Piening (1997) and Steffenson (1997).

The investigations indicate that the use of healthy seed, or seed treated with an effective fungicide, is important to reduce the damage from *B. sorokiniana*.

Table 2 Germination (%), incidence of *B. sorokiniana* (%), emergence (number of seedlings/m row) and yield (kg/ha) in a seed treatment experiment in Norway 2007 using naturally infected seeds of barley cv. Annabell

Seed lot	Treatment	g a.i./kg seed	Dose (ml/kg)	Laboratory		Field		
				% germination	% infection	No. of seedlings	Yield (kg/ha)	Relative yield
1 (healthy)	Untreated			85	17	62	3480	100
2 (infected)	Untreated			95	89	44	2620	75
	Fludioxinil	0.05	2	95	33	54	2920	84
	Pseudomonas chlororaphis		7.5	94	86	44	2910	84
	Imazalil + guazatine	0.04 + 0.6	2	93	15	58	3180	91
	Imazalil + flutriafol	0.05 + 0.04	2	96	5	71	3630	104
Lsd 5%					5.4	11.4	450	

Table 3 Germination (%), incidence of *B. sorokiniana* (%), emergence (number of seedlings/m row) and yield (kg/ha) in a seed treatment experiment in Norway 2007 using naturally infected seeds of barley cv. Edel

Seed lot	Treatment	g a.i./kg seed	Dose (ml/kg)	Laboratory		Field		
				% germination	% infection	No. of seedlings	Yield (kg/ha)	Relative yield
1 (healthy)	Untreated			97	1	75	3330	100
2 (infected)	Untreated			89	40	53	2390	72
	Fludioxinil	0.05	2	97	15	60	2880	87
	Pseudomonas chlororaphis		7.5	92	37	55	2260	68
	Imazalil + guazatine	0.04 + 0.6	2	93	6	66	2490	75
	Imazalil + flutriafol	0.05 + 0.04	2	95	1	67	3110	93
Lsd 5%					3.2	7.3	490	

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