Opportunities for developing value-added brassica seed

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Abstract
New Zealand produces approximately 5,500 tonnes of brassica seed per year, two thirds of which, valued at $13M, is exported. Black rot caused by *Xanthomonas campestris* pv. *campestris* is a common disease of brassicas, and while crop losses are not extensive in New Zealand, internationally total crop losses have been reported. Seeds are the primary source of inoculum and the ease with which this inoculum spreads means that even small traces can cause severe epidemics. Genetic resistance to black rot is a complex trait which makes breeding for resistance in brassicas challenging. The effectiveness of chemical and cultural practices is variable. Biological control with natural antagonistic microbes may provide a more effective means of controlling black rot and other pests and diseases, and create opportunities for increasing the export value of brassica seed. Current cultural practices and the potential for biological control for the management of black rot are reviewed.
Keywords: biocontrol, Brassicaceae, crucifer

Introduction

Brassicas are cultivated throughout the world as vegetable, forage and oilseed crops (Rimmer *et al.* 2007). Common crops include *Brassica oleracea* (cabbage, cauliflower, kale), *B. rapa* (Chinese cabbage, turnip, turnip rape), *B. napus* (swede, forage rape, oilseed rape), *B. juncea* (brown mustard) and *Raphanus sativus* (radish). Worldwide production of brassicas exceeded 100 million metric tonnes in 2006, an increase of more than 30% from 1996 (FAOSTAT 2008). This increase in brassica production has resulted in increased disease and pest pressures. These pressures have been in some cases considerable, often exacerbated by the interrelatedness of weed and crop species and the high value of individual plants.

Black rot caused by the gram-negative bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dawson occurs in all cultivated brassicas and many cruciferous weeds, and is regarded as the most serious disease of brassicas worldwide (Williams 2007). This seed-borne disease is favoured by warm and wet conditions, and plant-to-plant spread of the pathogen leads to the destruction of whole plants (Williams 2007). The pathogen produces characteristic V-shaped chlorotic lesions at leaf margins and blackened veins that are rapidly invaded and destroyed by soft-rotting bacteria such as *Erwinia* or *Pseudomonas* species (Cook *et al.* 1952; Williams 1980).

New Zealand produces approximately 5,500 tonnes of brassica seed per year, 60% of which is vegetable and 40% forage species (J. McKay and A. Stewart pers. comm. 2008). Although in New Zealand crop losses due to black rot are not extensive, for hybrid cabbage seed producers they can amount to $1,500-$8,400/ha (J. McKay pers. comm. 2008). The relatively frequent summer rains and cooler temperatures in New Zealand provide an environment that has been associated with the undetected spread and production of latent infections in seed crops (Williams 1980; Dane & Shaw 1996). Two thirds of brassica seed produced in New Zealand is exported to Asia and Europe (primarily vegetable seed) and Australia, the United States of America and United Kingdom (primarily forage seed) (J. McKay and A. Stewart pers. comm. 2008). Seed infected with black rot can cause extensive crop losses in a number of these export markets. In the United States of America, for example, complete crop losses have been reported (L. du Toit pers. comm. 2007).

In this paper, we review strategies for the control of black rot, including current cultural practices and opportunities for the use of biological control.

Cultural control of black rot

Brassicas with resistance to black rot have been identified (Guo *et al.* 1991; Taylor *et al.* 2002). Responses differ, however, with plant age, growth conditions and inoculation methods (Staub & Williams 1972; Camargo *et al.* 1995). Resistance in brassicas is often controlled by a single dominant locus but quantitative and recessive resistance is also observed (Camargo *et al.* 1995; Vicente *et al.* 2002; Soengas *et al.* 2007). In general this resistance is race specific with at least nine different races of the pathovar known to exist (Fargier & Manceau 2007). For some species such as *B. oleracea*, resistance to the predominant pathogen races is rare or only partial (Taylor *et al.* 2002). Resistance to these races, however, is known to exist in other species (Taylor *et al.* 2002; Soengas *et al.* 2007). Introgression through interspecific hybridisation has been used to transfer resistance but reduced or loss of resistance during backcross generations is reported (Hansen & Earle 1995; Tonguç & Griffiths 2004).

The control of black rot is difficult. Infection levels as low as 0.05% has been reported as leading to a high incidence of the disease in the field (Schaad *et al.* 1980a). The use of disease-free seed is critical in the control of the disease as seeds are the most important source of inoculum and the pathogen can persist for several years in seed as a surface contaminant or internal infection (Schaad *et al.* 1980a). Seed treatments involving hot water, hot air, ultraviolet light, chemicals and antibiotics have been evaluated for pathogen removal (Clayton 1924; Klisiewicz & Pound 1961; Humaydan *et al.* 1980; Schaad *et al.* 1980b; Schultz *et al.* 1986; Harman *et al.* 1987; Shiomi 1992; Brown *et al.* 2001). These treatments rarely completely remove the pathogen and often reduce seed germination and vigour. Tolerance of seed to these treatments varies between species and varieties and is affected by water content and age of seed. The black rot epidemics that occurred in the United States in 1972 and 1973 arose from the use of diseased seed that was not treated due to an intolerance to hot water treatment (Williams 1980).

Routine testing of seed lots for black rot is necessary to prevent the use of diseased seed. The International Seed Testing Association (ISTA) has recommended methods for the detection of the pathogen. There are, however, no internationally agreed regulations on acceptable thresholds. Recommended thresholds for direct-seeded crops are too high for preventing disease outbreaks in transplanted crops (Roberts *et al.* 2007).

Cultural practices are important in controlling the spread of the disease. The presence of multiple inoculum sources and/or favourable conditions for growth and dispersal are necessary for disease development over large areas. In 1980, a committee formed in the United States of America established guidelines that focused on sanitation practices to minimise the threat of black rot and black leg in brassicas (Williams 1980). Rotation with non-cruciferous crops is necessary. The pathogen can persist in the soil on incompletely decomposed plant residues for more than a year (Schaad & White 1974; Schultz & Gabrielson 1986). The planting of crops in fields close to other brassica crops is not recommended as the pathogen occurs in all cultivated brassicas and even though dispersal generally occurs over short distances, these can be increased by surface run-off and wind (Kocks *et al.* 1998).

Removal of cruciferous weeds is important for the control of black rot. In New Zealand the weeds *Capsella bursa-pastoris* and *Raphanus raphanistrum* are known to be naturally infected with the pathogen (Young 1969). The pathogen is also common in other weed species and transmission from weed to crop species is possible (Schaad & Dianese 1981; Ignatov *et al.* 2007). In addition, strains in weed species can elicit pathogenic responses in many crop species (Ignatov *et al.* 2007). Transmission, however, may be rare as strains in weeds from production or non-production areas appear to be genetically distinct from strains in crops.

Thorough cleaning and disinfecting of plant containers and equipment with steam and germicidal sprays is critical. The spread and severity of the disease is related to inoculum level (Kocks *et al.* 1999). In addition, to prevent dissemination of the pathogen, plants should not be sprayed or dipped in water prior to transplantation, or mechanically clipped (Williams 1980; Roberts *et al.* 2007).

The use of chemicals for black rot control has variable results. The pathogen is susceptible to some fungicides (Onsando 1987; Mochizuki & Alvarez 1996). These chemicals are most effective if plants are treated immediately prior to infection (Mochizuki & Alvarez 1996). Infections may occur throughout the growing season, making chemical control unsustainable. In addition, conditions favouring pathogen growth reduce the efficacy of chemical control. An alternative strategy involving the use of grass mulch to reduce water splash was found to be as effective as chemical control (Onsando 1987).

Biological control of black rot

Discovery of an effective means of preventing seed transmission of the pathogen is central in the control of black rot. Biological control using populations of one or more species to suppress the pathogen population may be a feasible alternative.

A small number of microbes have been investigated for potential biological control of black rot. A pathovar of *X. campestris* that causes bacterial blight in carrots (*X. campestris* pv. *carotae*) prevents infection of cabbage with *X. campestris* pv. *campestris* (Cook & Robeson 1986). This protection appears to result from the induction of defence responses but is dependent on the presence of threshold levels of *X. campestris* pv. *carotae*. Pre-treatment of cabbage with the pathovar *X. campestris* pv. *vesicatoria* or weakly pathogenic strains of *X. campestris* pv. *campestris* did not significantly reduce infection with a pathogenic strain of *X. campestris* pv. *campestris* (Dane & Shaw 1996). Other pathovars of *X. campestris* with distinct host specificities are known to exist (Fargier & Manceau 2007). However, the potential for these pathovars to cause disease in other crops species makes their release for biological control unlikely.

Isolates of several different *Bacillus* species found on brassicas, including *B. amyloliquefaciens*, *B. pumilus* and *B. subtilis*, display antagonism against *X. campestris* pv. *campestris* (Pichard & Thouvenot 1999; Luna *et al.* 2002; Wulff *et al.* 2002a; Wulff *et al.* 2002b; Massomo *et al.* 2004; Monteiro *et al.* 2005). Of the listed species, *B. amyloliquefaciens* appears to be most effective but antagonistic activity differs between isolates (Table 1) (Wulff *et al.* 2002a). The mechanisms underlying the antagonistic activity of *Bacillus* isolates against black rot are unknown (Wulff *et al.* 2002a). The production of secondary metabolites with antibiotic and/or haemolytic properties may be important (Pichard & Thouvenot 1999; Monteiro *et al.* 2005). Some *Bacillus* isolates have the

Table 1 Effects of *Bacillus amyloliquefaciens* isolates on cabbage seed germination and black rot incidence *in vivo* (Wulff *et al.* 2002a).

Isolate number	Seed germination (%)[1]	Reduction of black rot incidence (%)[1]
55	96 a	45.5 ab
76	82 a	40.9 ab
29C	89 a	68.2 a
69	98 a	68.2 a
80	73 ab	36.4 ab
29A	93 a	31.1 ab
8	87 a	77.3 a
17A	91 a	68.2 a
58	86 a	40.9 ab
74	93 a	27.3 ab
101	98 a	77.3 a
103	93 a	65.5 a
68	96 a	0.0 b
15	80 ab	68.2 a
17C	59 b	68.2 a
73	93 a	45.5 ab
71	86 a	77.3 a
84	89 a	58.6 ab
Control	100[2] a	0.0[3] b

[1]Means followed by the same letters were not significantly different

[2]Seeds dipped in sterile saline water

[3]Seeds preinoculated with *X. campestris* pv. *campestris* and dipped in sterile saline water

ability to colonise plants endophytically (Wulff *et al.* 2002a, b) and potentially provide systemic protection against *X. campestris* pv. *campestris*.

The efficacy of *Bacillus* species for biological control of black rot under nursery and field conditions has been variable. Seed application of an isolate of *B. polymyxa* reduced disease incidence in cauliflower (Pichard & Thouvenot 1999) but in cabbage, seed application of various *Bacillus* species appeared to lower seed germination and be less effective than root application (Wulff *et al.* 2002a; Massomo *et al.* 2004). Biological control with an isolate of *B. subtilis* was poor in brassicas highly susceptible to black rot such as cabbage and rape, when conditions were optimal for the spread of the pathogen (Wulff *et al.* 2002b). The pathogen was effectively controlled by this isolate in broccoli.

Some yeasts with antagonism against *X. campestris* pv. *campestris* have been reported (Assis *et al.* 1999) and there are a number of other bacterial and fungal species that are known to have biological activity, including species of *Pseudomonas*, *Streptomyces*, *Trichoderma* and *Coniothyrium* (Alabouvette *et al.* 2006). Several of these are reported to confer biological control against other brassica diseases (Rabeendran *et al.* 2005). These studies demonstrate the potential for biological control of *X. campestris* pv. *campestris* and need for further research.

In summary growers of vegetable and forage brassicas can suffer large financial losses from black rot. There is currently no effective control mechanism for the pathogen. Biological control, through microbes added to the surface of brassica seeds or incorporated endophytically within the seed, has the potential to provide effective control of seed-borne inoculum. Delivery of such a value-added product would significantly increase the export value of brassica seed for New Zealand producers.

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References

Alabouvette, C.; Olivain, C.; Steinberg, C. 2006. Biological control of plant diseases: the European situation. *European Journal of Plant Pathology 114*: 329-341.

Assis, S.M.P.; Mariano, R.L.R.; Michereff, S.J.; Silva, G.; Maranhão, E.A.A. 1999. Antagonism of yeasts to *Xanthomonas campestris* pv. *campestris* on cabbage phylloplane in field. *Revista De Microbiologia 30*: 191-195.

Brown, J.E.; Lu, T.Y.; Stevens, C.; Khan, V.A.; Lu, J.Y.; Wilson, C.L.; Collins, D.J.; Wilson, M.A.; Igwegbe, E.C.K.; Chalutz, E.; Droby, S. 2001. The effect of low dose ultraviolet light-C seed treatment on induced resistance in cabbage to black rot (*Xanthomonas campestris* pv. *campestris*). *Crop Protection 20*: 873-883.

Camargo, L.E.A.; Williams, P.H.; Osborn, T.C. 1995. Mapping of quantitative trait loci controlling resistance of *Brassica oleracea* to *Xanthomonas campestris* pv. *campestris* in the field and greenhouse. *Phytopathology 85*: 1296-1300.

Clayton, E.E. 1924. Control of black-rot and black-leg of cruciferous crops by seed and seed bed treatments. *Phytopathology 14*: 24-25.

Cook, A.A.; Walker, J.C.; Larson, R.H. 1952. Studies on the disease cycle of black rot of crucifers. *Phytopathology 42*: 162-167.

Cook, D.R.; Robeson, D.J. 1986. Active resistance of cabbage (*Brassica oleracea*) to *Xanthomonas* *campestris* pv. *carotae* and protection against the causal agent of black rot *Xanthomonas campestris* pv. *campestris*, by co-inoculation. *Physiological and Molecular Plant Pathology 28*: 41-52.

Dane, F.; Shaw, J.J. 1996. Survival and persistence of bioluminescent *Xanthomonas campestris* pv. *campestris* on host and non-host plants in the field environment. *Journal of Applied Bacteriology 80*: 73-80.

FAOSTAT 2008. Retrieved 24 July, 2008 from http://faostat.fao.org/site/567/default aspx

Fargier, E.; Manceau, C. 2007. Pathogenicity assays restrict the species *Xanthomonas campestris* into three pathovars and reveal nine races within *X. campestris* pv. *campestris*. *Plant Pathology 56*: 805-818.

Guo, H.; Dickson, M.H.; Hunter, J.E. 1991. *Brassica napus* sources of resistance to black rot in crucifers and inheritance of resistance. *Hortscience 26*: 1545-1547.

Hansen, L.N.; Earle, E.D. 1995. Transfer of resistance to *Xanthomonas campestris* pv. *campestris* into *Brassica oleracea* L. by protoplast fusion. *Theoretical and Applied Genetics 91*: 1293-1300.

Harman, G.E.; Norton, J.M.; Stasz, T.E.; Humaydan, H.S. 1987. Nyolate seed treatment of brassica spp. to eradicate or reduce black rot caused by *Xanthomonas campestris* pv. *campestris*. *Plant Disease 71*: 27-30.

Humaydan, H.S.; Harman, G.E.; Nedrow, B.L.; Dinitto, L.V. 1980. Eradication of *Xanthomonas campestris*, the causal agent of black rot, from *Brassica* seeds with antibiotics and sodium-hypochlorite. *Phytopathology 70*: 127-131.

Ignatov, A.; Sechler, A.; Schuenzel, E.L.; Agarkova, I.; Oliver, B.; Vidaver, A.K.; Schaad, N.W. 2007. Genetic diversity in populations of *Xanthomonas campestris* pv. *campestris* in cruciferous weeds in central coastal California. *Phytopathology 97*: 803-812.

Klisiewicz, J.M.; Pound, G.S. 1961. Studies on control of black rot of crucifers by treating seeds with antibiotics. *Phytopathology 51*: 419-494.

Kocks, C.G.; Zadoks, J.C.; Ruissen, T.A. 1998. Response of black rot in cabbage to spatial distribution of inoculum. *European Journal of Plant Pathology 104*: 713-723.

Kocks, C.G.; Zadoks, J.C.; Ruissen, M.A. 1999. Spatio-temporal development of black rot (*X. campestris* pv. *campestris*) in cabbage in relation to initial inoculum levels in field plots in The Netherlands. *Plant Pathology 48*: 176-188.

Luna, C.L.; Mariano, R.L.R.; Souto-Maior, A.M. 2002. Production of a biocontrol agent for crucifers black rot disease. *Brazilian Journal of Chemical Engineering 19*: 133-140.

Massomo, S.M.S.; Mortensen, C.N.; Mabagala, R.B.; Newman, M.-A.; Hockenhull, J. 2004. Biological control of black rot (*Xanthomonas campestris* pv. *campestris*) of cabbage in Tanzania with *Bacillus* strains. *Journal of Phytopathology 152*: 98-105.

Mochizuki, G.T.; Alvarez, A.M. 1996. A bioluminescent *Xanthomonas campestris* pv. *campestris* used to monitor black rot infections in cabbage seedlings treated with fosetyl-Al. *Plant Disease 80*: 758-762.

Monteiro, L.; Mariano, R.L.R.; Souto-Maior, A.M. 2005. Antagonism of *Bacillus* spp. against *Xanthomonas campestris* pv. *campestris*. *Brazilian Archives of Biology and Technology 48*: 23-29.

Onsando, J.M. 1987. Management of black rot of cabbage (*Xanthomonas campestris* pv. *campestris*) in Kenya. *Tropical Pest Management 33*: 5-6.

Pichard, B.; Thouvenot, D. 1999. Effect of *Bacillus polymyxa* seed treatments on control of black-rot and damping-off of cauliflower. *Seed Science and Technology 27*: 455-465.

Rabeendran, N.; Jones, E.E.; Moot, D.J.; Stewart, A. 2005. Evaluation of selected fungal isolates for the control of *Sclerotinia sclerotiorum* using cabbage pot bioassays. *New Zealand Plant Protection 58*: 251-255.

Rimmer, S.R.; Shattuck, V.I.; Buchwaldt, L. 2007. Compendium of brassica disease. APS Press, St. Paul. St. Paul, Minnesota, United States of America.

Roberts, S.J.; Brough, J.; Hunter, P.J. 2007. Modelling the spread of *Xanthomonas campestris* pv. *campestris* in module-raised brassica transplants. *Plant Pathology 56*: 391-401.

Schaad, N.W.; White, W.C. 1974. Survival of *Xanthomonas campestris* in soil. *Phytopathology 64*: 1518-1520.

Schaad, N.W.; Sitterly, W.R.; Humaydan, H. 1980a. Relationship of incidence of seedborne *Xanthomonas campestris* to black rot of crucifers. *Plant Disease 64*: 91-92.

Schaad, N.W.; Gabrielson, R.L.; Mulanax, M.W. 1980b. Hot acidified cupric acetate soaks for eradication of *Xanthomonas campestris* from crucifer seeds. *Applied and Environmental Microbiology 39*: 803-807.

Schaad, N.W.; Dianese, J.C. 1981. Cruciferous weeds as sources of inoculum of *Xanthomonas campestris* in black rot of crucifers. *Phytopathology 71*: 1215-1220.

Schultz, T.; Gabrielson, R.L. 1986. *Xanthomonas campestris* pv. *campestris* in western Washington crucifer seed fields: Occurrence and Survival. *Phytopathology 76*: 1306-1309.

Schultz, T.; Gabrielson, R.L.; Olson, S. 1986. Control of *Xanthomonas campestris* pv. *campestris* in crucifer seed with slurry treatments of calcium hypochlorite. *Plant Disease 70*: 1027-1030.

Shiomi, T. 1992. Black rot of cabbage seeds and its disinfection under a hot-air treatment. *Japan Agricultural Research Quarterly 26*: 13-18.

Soengas, P.; Hand, P.; Vicente, J.G.; Pole, J.M.; Pink, D.A.C. 2007. Identification of quantitative trait loci for resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica rapa*. *Theoretical and Applied Genetics 114*: 637-645.

Staub, T.; Williams, P.H. 1972. Factors influencing black rot lesion development in resistant and susceptible cabbage. *Phytopathology 62*: 722-728.

Taylor, J.D.; Conway, J.; Roberts, S.J.; Astley, D.; Vicente, J.G. 2002. Sources and origin of resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica* genomes. *Phytopathology 92*: 105-111.

Tonguç, M.; Griffiths, P.D. 2004. Development of black rot resistant interspecific hybrids between *Brassica oleracea* L. cultivars and *Brassica* accession A 19182, using embryo rescue. *Euphytica 136*: 313-318.

Vicente, J.G.; Taylor, J.D.; Sharpe, A.G.; Parkin, I.A.P.; Lydiate, D.J.; King, G.J. 2002. Inheritance of race-specific resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica* genomes. *Phytopathology 92*: 1134-1141.

Williams, P.H. 1980. Black rot: A continuing threat to world crucifers. *Plant Disease 64*: 736-742.

Williams, P.H. 2007. Black rot. pp. 60-62. *In*: Compedium of brassica diseases. Eds. Rimmer, S.R.; Shattuck, V.I.; Buchwaldt, L. APS Press, St. Paul, Minnesota, United States of America.

Wulff, E.G.; Mguni, C.M.; Mansfeld-Giese, K.; Fels, J.; Lübeck, M.; Hockenhull, J. 2002a. Biochemical and molecular characterization of *Bacillus amyloliquefaciens*, *B. subtilis* and *B. pumilus* isolates with distinct antagonistic potential against *Xanthomonas campestris* pv. *campestris*. *Plant Pathology 51*: 574-584.

Wulff, E.G.; Mguni, C.M.; Mortensen, C.N.; Keswani, C.L.; Hockenhull, J. 2002b. Biological control of black rot (*Xanthomonas campestris* pv. *campestris*) of brassicas with an antagonistic strain of *Bacillus subtilis* in Zimbabwe. *European Journal of Plant Pathology 108*: 317-325.

Young, J.M. 1969. An alternative weed host for *Xanthomonas campestris*. *Plant Disease Reporter 53*: 820-821.