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).
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). Introggression 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).
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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 1976). 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).

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Seed germination (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Reduction of black rot incidence (%)&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>96 a</td>
<td>45.5 ab</td>
</tr>
<tr>
<td>76</td>
<td>82 a</td>
<td>40.9 ab</td>
</tr>
<tr>
<td>29C</td>
<td>89 a</td>
<td>68.2 a</td>
</tr>
<tr>
<td>69</td>
<td>98 a</td>
<td>68.2 a</td>
</tr>
<tr>
<td>80</td>
<td>73 ab</td>
<td>36.4 ab</td>
</tr>
<tr>
<td>29A</td>
<td>93 a</td>
<td>31.1 ab</td>
</tr>
<tr>
<td>8</td>
<td>87 a</td>
<td>77.3 a</td>
</tr>
<tr>
<td>17A</td>
<td>91 a</td>
<td>68.2 a</td>
</tr>
<tr>
<td>58</td>
<td>86 a</td>
<td>40.9 ab</td>
</tr>
<tr>
<td>74</td>
<td>93 a</td>
<td>27.3 ab</td>
</tr>
<tr>
<td>101</td>
<td>98 a</td>
<td>77.3 a</td>
</tr>
<tr>
<td>103</td>
<td>93 a</td>
<td>65.5 a</td>
</tr>
<tr>
<td>68</td>
<td>96 a</td>
<td>0.0 b</td>
</tr>
<tr>
<td>15</td>
<td>80 ab</td>
<td>68.2 a</td>
</tr>
<tr>
<td>17C</td>
<td>59 b</td>
<td>68.2 a</td>
</tr>
<tr>
<td>73</td>
<td>93 a</td>
<td>45.5 ab</td>
</tr>
<tr>
<td>71</td>
<td>86 a</td>
<td>77.3 a</td>
</tr>
<tr>
<td>84</td>
<td>89 a</td>
<td>58.6 ab</td>
</tr>
<tr>
<td>Control</td>
<td>100&lt;sup&gt;2&lt;/sup&gt; a</td>
<td>0.0&lt;sup&gt;3&lt;/sup&gt; b</td>
</tr>
</tbody>
</table>

<sup>1</sup>Means followed by the same letters were not significantly different

<sup>2</sup>Seeds dipped in sterile saline water

<sup>3</sup>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 \( \textit{Bacillus} \) 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 \( \textit{Bacillus} \) 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 \( \textit{Pseudomonas} \), \( \textit{Streptomyces} \), \( \textit{Trichoderma} \) and \( \textit{Coniothyrium} \) (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**


Cook, D.R.; Robeson, D.J. 1986. Active resistance of cabbage (\( \textit{Brassica oleracea} \)) to \( Xanthomonas \) \textit{campestris} pv. \textit{campestris}.
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.


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