

VG204

**Control of black rot contamination in
Brassica seeds**

E J Minchinton

Agriculture Victoria, IHD



Know-how for Horticulture™

VG204

This report is published by the Horticultural Research and Development Corporation to pass on information concerning horticultural research and development undertaken for the Vegetable industry.

The research contained in this report was funded by the Horticultural Research and Development Corporation with the financial support of Victorian Farmers Federation Inc, Fairbanks Selected Seed, Arthur Yates, New World Seeds, W Bone & Sons and Woodlyn Nursery Wholesale Supplies.

All expressions of opinion are not to be regarded as expressing the opinion of the Horticultural Research and Development Corporation or any authority of the Australian Government.

The Corporation and the Australian Government accept no responsibility for any of the opinions or the accuracy of the information contained in this Report and readers should rely upon their own inquiries in making decisions concerning their own interests.

Cover Price \$20.00

HRDC ISBN 1 86423 151 3

Published and Distributed by:



Horticultural Research and Development Corporation
Level 6
7 Merriwa Street
Gordon NSW 2072

Telephone: (02) 418 2200
Fax: (02) 418 1352

Contents

1. Summary	3
1.1 Industry Summary	3
1.2 Technical Summary	4
1.3 Publication Schedule	5
2. Technical Report	6
2.1 Introduction	6
2.2 Materials and Methods	7
2.2.1 <i>Source and application of inoculum</i>	7
2.2.2 <i>Seed treatments</i>	7
2.2.3 <i>Isolation of <u>X. campestris</u> pv. <u>campestris</u></i>	7
2.2.4 <i>Seed germination</i>	8
2.2.5 <i>Statistical analysis</i>	8
2.3 Results	8
2.3.1 <i>Chemical seed treatments</i>	8
2.3.2 <i>Heat treatments-artificially infected seed</i>	8
2.3.3 <i>Heat treatments-naturally infected seed</i>	8
2.4 Discussion	10
2.5 References	11
3. Recommendations	13
3.1 Extension/Adoption by Industry	13
3.2 Directions for Future Research	14
3.4 Financial/Commercial Benefits	14
Acknowledgments	15

Table

Table 1. Efficacy of seed treatments for control of Black rot of artificially and naturally infected cabbage seed of cultivar "Ballhead"	9
------------------------------------------------------------------------------------------------------------------------------------------------------	---

1. Summary

1.1 Industry Summary

Black Rot disease of brassicas is caused by the bacterium *Xanthomonas campestris* pv. *campestris*. It is a disease which causes blackening of veins, yellowing and death of leaves. Contaminated seed is considered to be the main source of infection. Very low levels of seed infection, more than 1 infected seed in 10,000 seeds for direct drill and less than 1 infected seed in 10,000 seeds for transplants, can lead to out-breaks of the Black Rot disease in the field. Tests showed that the natural levels of the bacterium in the cultivar "Ballhead" were very high. They were high enough to cause epidemics of Black Rot in the field or in transplants, given suitable environmental conditions. It is therefore imperative that seed is treated appropriately prior to planting.

A project recently completed at the Institute for Horticultural Development assessed the effectiveness of several chemicals, hot water and steam-air treatments to control Black Rot contamination of cabbage seeds. It was funded by the Horticultural Research and Development Corporation, selected seed companies (Yates, Fairbanks and New World Seeds), nurseries (W. Bones & Sons and Woodlyn Nursery) and the Gippsland Vegetable Group.

Seeds of the cabbage cultivar "Ballhead", were artificially infected with the bacterium, as levels of natural infection, although high, are too variable for routine testing. Treatments were evaluated for their ability to kill the bacterium in the seed. The bactericides ("Chinosol", "Bronopol" and "Natriphene") and a disinfectant ("Agribrom") were applied to seeds by infiltration under vacuum. Alternatively, seeds were treated with hot water, hot water and sodium hypochlorite (NaOCl) or steam-air. Treatments were assessed by extracting bacteria with seed washings and suspect colonies were identified by pathogenicity tests. Germination of seeds was assessed under the protocols of the International Seed Testing Association and vigour of seedling growth was examined visually.

None of the chemical treatments were successful in controlling Black Rot. Rates of germination were unaffected by "Agribrom", lowered by "Bronopol" and raised by "Chinosol" and "Natriphene".

All the heat treatments reduced the levels of the bacterium recovered from seeds. So any heat treatment is better than none at all. The most effective treatment was hot water and hypochlorite which apparently killed all bacteria in seed. Hot water treatments alone (for 20 or for 25 min) and steam air treatments did not eliminate the bacterium. Further work is required on the steam-air treatment to refine the times and temperatures of treatments for *Brassica* seed necessary to kill the Black Rot bacterium.

The efficacy of hot water treatments can be enhanced and the time required to treat "Ballhead" cabbage can be reduced by 5 minutes if chlorine (100ppm sodium hypochlorite) is added to the hot water bath. The heat treatments did not reduce germination of seeds when tested within a month after treatment. In fact germination was generally raised. The effect on other cultivars is unknown.

1.2 Technical Summary

Black Rot of *Brassica* is caused by the bacterium *Xanthomonas campestris* pv. *campestris*. Contaminated seed is considered to be the major source of Black Rot epidemics in seedlings and in the field. The natural levels of *X. campestris* pv. *campestris* in the cultivar "Ballhead" were very high, however, they were too variable to rely on for routine testing of treatments. Further, the recommended hot water treatment of 50°C for 25 minutes does not completely eliminate the Black Rot causing bacterium. It is therefore imperative that seed is treated appropriately prior to planting because even low levels of bacteria remaining after treatment can cause epidemics of Black Rot in the field or in transplants, given appropriate environmental conditions. Treatments, including chemicals, hot water, hot water and hypochlorite and steam-air, were evaluated for their ability to kill the bacterium in seed without affecting germination and vigour.

In preliminary tests, seeds were artificially inoculated with *X. campestris* pv. *campestris*. Seeds were infiltrated with the bactericides, "Chinosol", "Bronopol", "Natriphene" and the disinfectant "Agribrom". Alternatively seeds were treated with hot water at 50°C for 20 or 25 minutes or with hot water and NaOCl (100ppm chlorine) at 50°C for 20 minutes. The steam-air treatment was applied at 56°C for 30 minutes on artificially infected seeds and at 51°C for 40 minutes on naturally infected seeds. The hot water hypochlorite treatment was the most effective treatment and so was tested on naturally infected seeds. Treatments were assessed by extracting bacteria from seed by soaking, centrifugation and plating onto selective media containing antibiotics. Identification of *X. campestris* pv. *campestris* was confirmed on cultural morphology and by pathogenicity tests. Germination was assessed under the protocols of the International Seed Testing Association standards. Vigour was assessed visually as there are no internationally recognised standard vigour tests for brassicas.

Chemicals were ineffective as treatments for eradication of *X. campestris* pv. *campestris* from seed. Rates of germination were unaffected by "Agribrom", lowered by "Bronopol" and raised by "Chinosol" and "Natriphene".

The hot water and hypochlorite treatment (50°C, 20 min, 100ppm Cl) apparently killed *X. campestris* pv. *campestris* in naturally and artificially infected seed because no bacteria were isolated from treated seed. Hot water treatments at 50°C for 20 and 25 min and steam-air treatments at 56°C for 30 min and 51°C for 40 min did not eliminate *X. campestris* pv. *campestris* from seed. All reduced levels of bacteria cultured from seed with hot water at 50°C for 25 min reducing it by a factor of 10⁴. Steam-air at the higher temperature was more effective than at the lower temperature. None of the heat treatments affected germination or vigour, in fact most raised germination.

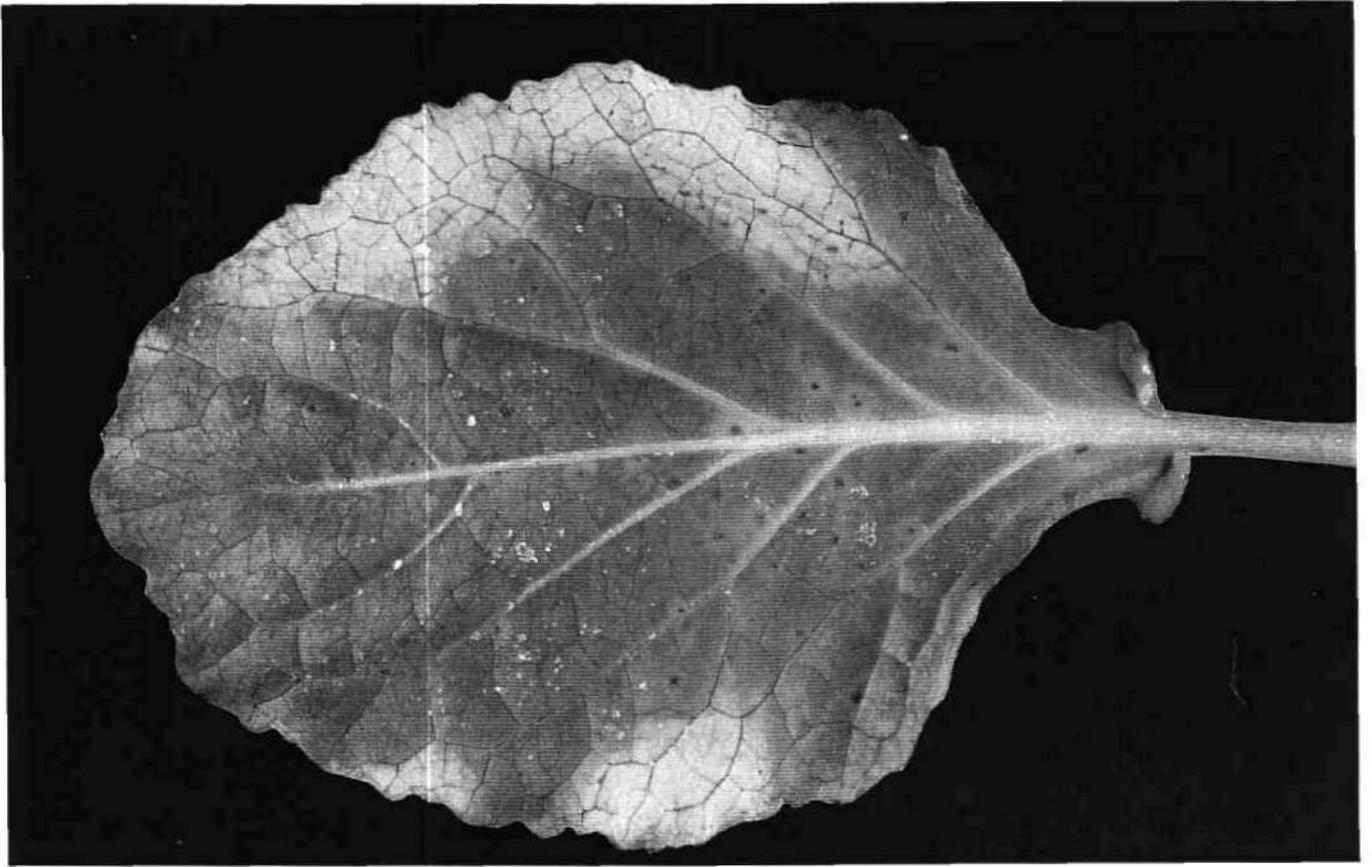
Further work is recommended on the steam-air treatment because if successful this method will deliver seed in a dry state ready for sowing. This would be a significant advantage to growers.

1.3 Publication Schedule

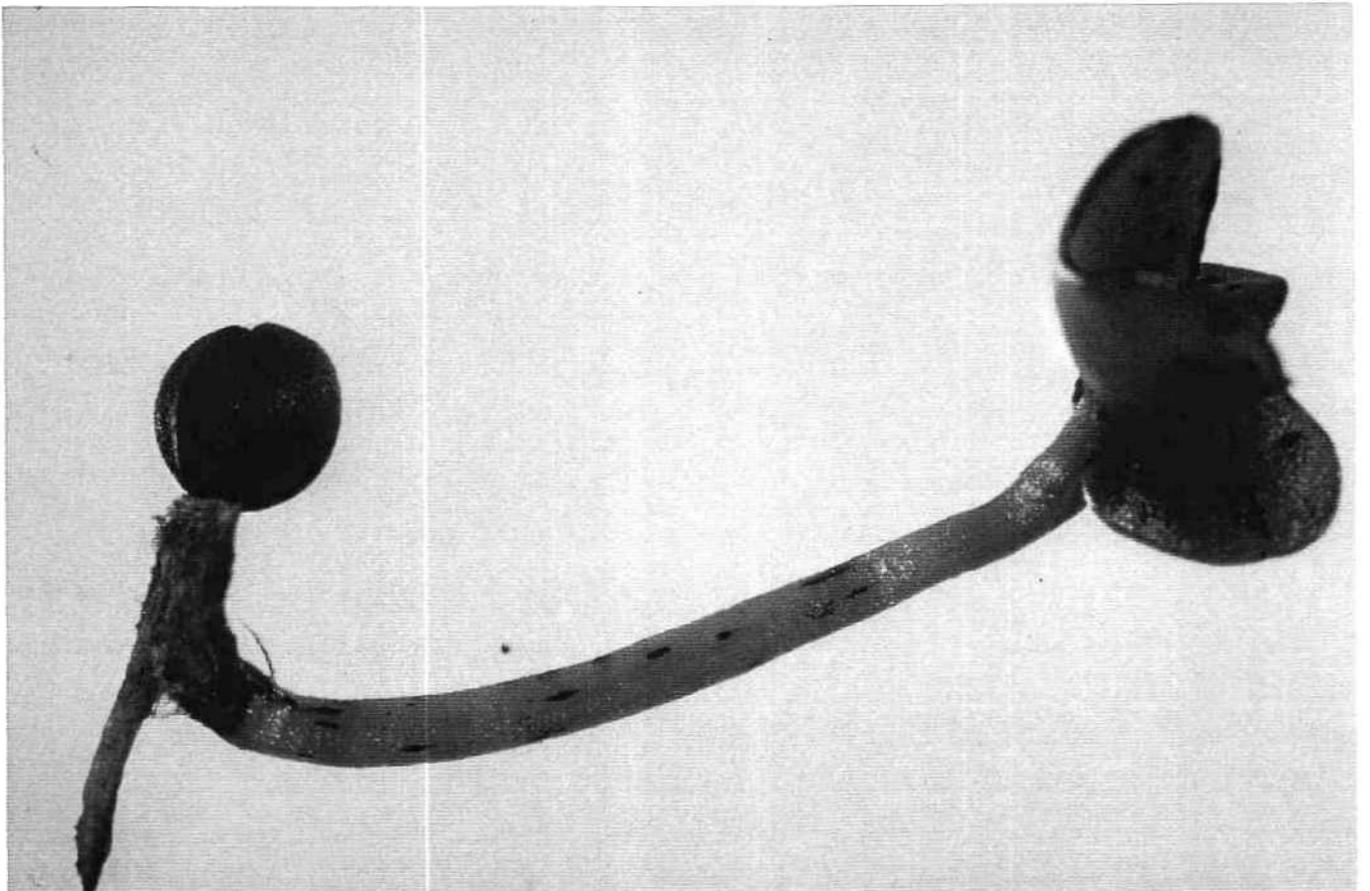
Minchinton, E. (1992). "Control of black rot contamination in *Brassica* seeds". The Victorian Vegetable Grower, No 7, 9. Vegetable Growers Association of Victoria.

Minchinton, E. (1995). "Control of black rot contamination in *Brassica* seeds - research findings. Draft of the industry summary to be submitted to "The Victorian Vegetable Grower" by end of January.

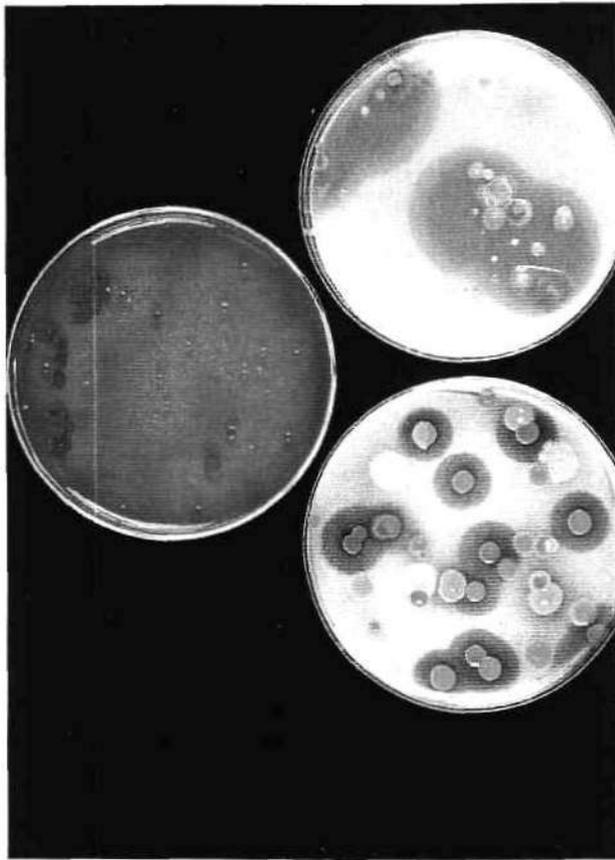
Minchinton, E. J., Hepworth, G. and Mebalds, M. Control of black rot contamination in *Brassica* seeds. Draft of final report to be submitted to "Plant Protection Quarterly", by end of February.



Symptoms of Black Rot on a cabbage leaf cultivar "Ballhead" showing lesions with black veins surrounded by chlorotic (yellow) V-shaped areas at the leaf margins.



Symptoms of Black Rot on young seedlings of cultivar "Ballhead", showing black lesions on hypocotyl and cotyledon of the seedling.



Isolates of *Xanthomonas* isolated onto selective media BSCAA (top right), NSCAA (bottom right) and SX (left), showing haloes around starch hydrolysing colonies.



Assessment of seed germination in seed germination trays, each of which holds 400 seeds.

2. Technical Report

2.1 Introduction

Black Rot of *Brassica*, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel 1895) Dowson 1939 is a world wide problem (Williams, 1980). Seed is a major source of inoculum (Walker, 1941; Hunter *et al.*, 1975). Infected seedlings start to turn black and die about 8 days after germination (Shackleton, 1962). On more advanced plants, symptoms initially appear as a V-shaped chlorosis at leaf margins, then later veins in this region blacken and the leaf starts to shrivel and turn necrotic. Very low levels of seed infection, greater than 1 infected seed in 10,000 seeds, can result in epidemics in the field (Schaad *et al.*, 1980). The bacteria are located both externally and internally on seeds, the latter occurring after invasion of the vascular system through the funiculus (Walker, 1952; Cook *et al.*, 1952).

The disease can spread easily in plant beds because of the conditions under which seeds are germinated and the close proximity of plants (Hunter *et al.*, 1975; Schaad and Alvarez, 1993). Optimal conditions for bacterial growth are high temperatures (25-30°C) and high relative humidities (80-100%), whilst lower temperatures (10-18°C), are conducive to the growth of the host (Schaad and Alvarez, 1993). Spread can also occur through any damage to seedlings, e.g. clipping of foliage and soaking of transplants prior to planting (Williams, 1980). Once the bacterium is present, warm wet conditions in the field can lead to the rapid development of epidemics (Walker, 1952).

Seed treatments, with hot water, chemicals, antibiotics or steam-air have been used to control seed infections with varying degrees of success. The original hot water treatment of *Brassica* seed (cauliflower) by Clayton (1924) recommended 50°C for 30 minutes. Hot water treatments for cabbage seed, are now generally recommended at 50°C for 20 to 25 minutes (Sutherland, 1987; Sherf and MacNab, 1986), whilst those for other brassicas are generally 50°C for 15 to 20 minutes (Smith, 1962; Chapman and Sivapalan, 1991; Floyd and Melvin-Carter, 1993; Sutherland, 1987). These treatments often reduced seed viability (Schaad *et al.*, 1980) without eliminating the pathogen (Humayda *et al.*, 1980). Other treatments including hot acidified zinc sulphate (Huang and Lee, 1988), a calcium hypochlorite slurry (Schults *et al.*, 1986), Nyalate (Harman *et al.*, 1987), hot acidified cupric acetate (Schaad *et al.*, 1980), were unsatisfactory either because of inconsistencies in eliminating the bacterium or they had adverse effects on germination. Antibiotics (Humaydan *et al.*, 1980), eradicated *X.campestris* pv. *campestris* without any phytotoxic effects, however, this treatment is unacceptable for use in Australia. A steam-air treatment of 54°C for 30 minutes (Navaratnam *et al.*, 1980) reportedly removed *X. campestris* from seeds.

This study reports on the assessment of the efficacy of several chemicals, hot water and steam-air treatments to control Black Rot contamination of cabbage (*Brassica*) seeds and their effect on seed germination.



Inoculations of cabbage seedlings to test pathogenicity of starch hydrolysing, *Xanthomonas*-like isolates obtained from seed washings.



Assessment for Black Rot on inoculated seedling of cabbage cultivar "Ballhead", in the pathogenicity tests.

2.2 Materials and Methods

2.2.1 Source and application of inoculum

Virulent isolates of the bacterium were isolated from diseased seedlings (cv "Ballhead") and from the Biological and Chemical Research Institute, NSW (DAR 25606, cauliflower; DAR 26922, cabbage; DAR 30538, broccoli) courtesy of Dr. P. Fahy. Cultures of the 4 isolates were grown for 2-3 days on Nutrient agar at 25°C and made into a suspension of approximately 10^7 - 10^8 cfu/ml with sterile distilled water, 0.85% NaCl and 1 drop of Tween 80/l (Schaad, 1989). Seeds of the cabbage cultivar "Ballhead", which contained natural populations of the bacterium, were artificially infected with a bacterial suspension by infiltration under vacuum, following the method of Bashan and Assouline (1983), 3 days prior to treatment applications. Concentration of inoculum was determined by 'lawn plating' 0.1ml of suspension onto nutrient agar or onto medium B of King *et al.*, 1954 and incubating as previously described. Seeds of the cabbage cultivar "Ballhead", were used as a source of naturally infected seed.

2.2.2 Seed treatments

In each replicate of each treatment, 10,000 seeds were treated (Schaad *et al.*, 1980). The chemicals "Agribrom" (Great Lakes Chemical Corp.) at 20mg/l, 40mg/l, 60mg/l, 80mg/l and 100mg/l; "Bronopol" (Schering Pty. Ltd.) at 0.05g/l, 0.1g/l, 0.5g/l and 1.0g/l; "Chinosol" (Hoechst Aust. Ltd.) at 0.5g/l, 1.0g/l and 1.5g/l; and "Natriphene" (Natriphene Company) at 0.5g/l and 1.0g/l; were applied to seeds under vacuum, as previously described, followed by a 1 hour soak. The steam-air treatments were applied by a recently commissioned steam-air machine (HRDC Project NY209, M. Mebalds pers. comm.). Hot water treatments were applied by immersing seeds in a sealed flask of sterile deionised water pre-heated in a hot water bath. The hot water sodium hypochlorite treatment was applied by adding sodium hypochlorite to the flask of heated water just prior to addition of seeds, after which the flask was resealed. Seeds, commercially treated with "Thiram", were also tested for presence of the bacterium. The bactericidal treatments were not replicated. The heat treatments were replicated 3 times.

2.2.3 Isolation of *X. campestris* pv. *campestris* and calculation of its concentration

Bacteria were recovered from infiltrated treated seed, from naturally infected seed and from the commercially treated "Thiram" seed, following the method of Schaad (1989). A total of 10,000 seed were soaked in 75 ml of sterile 0.85% NaCl containing 1 drop of Tween 80/l, 5mg "Benlate" 50WP and 0.5 ml of a 1:10 dilution of "Bravo" 500, on a shaker for 1.5 hr at 4°C. The soakate was filtered off, centrifuged at 12,000g for 20 min and the pellet resuspended in 3ml 0.85% NaCl. Bacterial suspensions were dilution plated in triplicate onto the media NSCAA and BSCAA of Schaad (1989) with the anti-biotic amendments of Randhawa and Schaad (1984) and SX media (Schaad and Stall, 1988). Identity was confirmed by pathogenicity tests. Suspected *Xanthomonas* isolates were each made into a suspension of 10^{8-10} cfu/ml and atomised onto 3 young leaves of a cabbage seedling which had been cut with scissors prior to inoculation. Plants were covered with a plastic bag for 2 days and assessed for Black Rot symptoms 10 to 14 days after inoculation. Control plants were atomised with sterile distilled water and treated as above. The estimated concentration of *X. campestris* pv. *campestris* was determined by multiplying the number of pathogenic isolates by the mean number of starch hydrolysing colonies detected on the selective media.

2.2.4 Seed germination

Assessment of germination followed protocols of the International Seed Testing Association. In all cases 400 seeds were tested for each replicate.

2.2.5 Statistical analysis

Comparisons between treatments were made using analysis of variance. A logarithmic transformation was applied to bacterial concentrations to make the variation more stable across the range of values observed.

2.3 Results

2.3.1 Chemical seed treatments

Preliminary non replicated screening, showed that none of the chemical treatments ("Agribrom", "Bronopol", "Chinosol" or "Natriphene"), when infiltrated into *Brassica* seed under vacuum, eliminated the bacteria or reduced incidence of Black Rot. Consequently they were not tested further. Rates of germination were unaffected by "Agribrom", lowered by "Bronopol" and raised by "Chinosol" and "Natriphene".

X. campestris pv. *campestris* was recovered from "Thiram" treated cabbage seeds at the rate of 2.8×10^3 cfu/ml.

2.3.2 Heat treatments - artificially infected seed

The hot water with chlorine (100ppm NaOCl, 50°C, 20min) treatment apparently eliminated the Black Rot bacterium from artificially infected seed. The remaining treatments in order of decreasing efficiency were hot water (50°C, 25min), hot water (50°C, 20min) and steam (56°C, 30min). All treatments were significantly ($P < 0.01$) different from the control (untreated, artificially inoculated) seeds. Germination was greater for the hot water treatments than the steam treatments, which in turn exceeded the germination of the control seeds. However, the differences were not significant at $P = 0.05$.

2.3.3 Heat treatments - naturally infected seed

The hot water with chlorine (100ppm NaOCl, 50°C, 20min) treatment apparently eliminated the Black Rot bacterium from naturally infected seed. Whilst the steam-air treatment (51°C, 40 min) was only marginally better than the control. A conclusive demonstration of the significant effects of the hot water treatment with sodium hypochlorite was not possible because of the variation in levels of seed contamination caused by *X. campestris* pv. *campestris*. In one replicate the bacterium was not detected. Germination was significantly enhanced ($P=0.05$) with the steam-air treatment.

Table 1. Efficacy of seed treatments for control of Black Rot in artificially and naturally infected cabbage seed of cultivar "Ballhead"

Seed infection	Seed treatment	Estimated concentration of <i>X. campestris</i> pv. <i>campestris</i> (mean ^a cfu/ml)	log _e transformed mean	Germination (mean ^b %)
Artificial	Control (no treatments)	3.28x10 ⁵	(12.7) ^c	73.7 ^d
	Hot water 50°C 20min	2.56x10 ¹	(3.3)	86.7
	Hot water 50°C 25min	3.5	(1.5)	87.3
	Hot water 50°C 20min + NaOCl (100ppm)	0	(0)	89.7
	Steam 56°30min	5.70x10 ¹	(4.1)	79.3
	lsd		(4.9)	17.1
Natural	Control (no treatments)	3.66x10 ³	(5.4)	69.7 ^e
	Hot water 50°C 20min + NaOCl (100ppm)	0	(0.0)	69.1
	Steam 51° 40min	2.33x10 ²	(3.7)	80.7
	lsd		(6.7)	7.0

^a, mean of 3 replicates, each of 10,000 seeds. ^b, mean of 3 replicates, each of 400 seeds. ^c, log e^(x+1) transformed means.

^d, germination assessed at 11 days. ^e, germination assessed at 6 days.

2.4 Discussion

The plethora of treatments reported in the literature to control Black Rot contamination of *Brassica* seed is an indication of the seriousness of the problem. Treatment failure is probably attributable to its internal location within the seed (Cook *et al.*, 1952). The numerous seed treatments available, are successful to various degrees, some are difficult for growers to perform, others require trained professionals, a few are unacceptable, legally in Australian. Growers are often reluctant to treat seed as it is perceived to lower germination.

Heat treatments alone (hot water at 50°C for 20 or 25 min) reduced the levels of *Xanthomonas* recovered from artificially infected seed, which contained natural populations of the bacterium, by a factor of 10⁴ cfu/ml. Low levels of bacteria, however, were still detectable in seed. The hot water treatments currently recommended to growers are not good enough to control Black Rot, as numbers of bacteria recovered would still be high enough to lead to an epidemic in the field (Schaad *et al.*, 1980). In fact Hunter *et al.* (1975), attributed an epidemic of Black Rot in cabbages in New York to infected, untreated seed.

The addition of NaOCl (100ppm) to the 50°C, 20min treatment apparently eradicated *X. campestris* pv. *campestris* in artificially and naturally infected seed. Further, the recovery of other bacteria was also obviously reduced with the NaOCl treatment compared with the hot water treatments only. As the sodium hypochlorite was applied to a sealed container, there is little chance of volatilisation and as replicates were done in clean water with fresh chemical there would have been little breakdown of chemical with time. This treatment, however, requires growers to treat seed in a sealed container and to accurately measure small volume of sodium hypochlorite, which in some situations may not be user friendly. Further, there may be a health and safety issue with the use of sodium hypochlorite. The advantages of treating seeds are 2 fold; it removes or lowers bacteria and probably other diseases, as well as enhancing germination.

The lack of a significant difference between treatments for the naturally infected seed is due to variability in the recovery of the bacterium from the 3 replicates of naturally infected seeds. Our work suggests that it is imperative to do several replications. Some papers on the subject only mention that 1 or 2 replications were performed (Harman *et al.*, 1987; Humaydan *et al.*, 1980).

Navaratnam *et al.* (1980), successfully used steam air to eradicate *X. campestris* pv. *campestris* from *Brassica* seed. It is possible that the detection methods used here are far more sensitive than the plating method he used, as the bacterium was recovered from the steam air treatment at both times and temperatures tested. It appears that the time and temperature regimes need further refinement. The steam-air treatment is far more "user friendly" compared to the other treatments as seed is dried during the treatment cycle and useable or storable immediately. It also has the advantage of stimulating germination, possibly by removing other seed rotting organisms.

None of the heat treatments had detrimental effects on germination of seeds of the cultivar "Ballhead". Walker (1952) and Clayton (1924), however, reported that old or weakened seeds were intolerant of heat treatments. Whilst Lacasico (1963), showed that hot water and steam air treated seeds stored well for a year after treatment. There are no internationally recognised vigour tests prescribed for brassica. There was no evidence of reduced vigour in any of the germination tests.

2.5 References

- Bashan, Y. and Assouline, I. (1983). Complementary bacterial enrichment techniques for the detection of *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* in infested tomato and pepper seeds. *Phytoparasitica* 11: 187-193.
- Chapman, R. and Sivapalan, A. (1991). Broccoli seed fungi disease identified. *Good Fruit and Vegetables*, 13: 9.
- Clayton, E. E. (1924). Investigations of cauliflower diseases on Long Island. New York State Agriculture Experimental Station Geneva Bulletin 506, 31pp.
- Cook, A. A., Larson, R. H. and Walker, J. C. (1952). Relation of the black rot pathogen to cabbage seed. *Phytopathology*, 42: 316-320.
- Floyd, R. and Melvin-Carter, E. (1993). Vegetable seed treatments. *Victorian Vegetable Grower* No.3: 4.
- Harman, G. E., Norton, J. M., Stasz, T. E. and 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.
- Huang, T. C. and Lee, H. L., (1988). Hot acidified zinc sulphate as seed soaking agent for the control of crucifer black rot. *Plant Protection Bulletin, Taiwan*. 30: 245-258.
- Humaydan, H. S., Harman, G. E., Nedrow, B. L. and 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.
- Hunter, J. E., Abawi, G. S. and Becker, R. F. (1975). Observations on the source and spread of *Xanthomonas campestris* in an epidemic of black rot in New York. *Plant Disease Reporter*, 59: 384-387.
- King, E. O., Ward, M. K. and Raney, D. E. (1954). Two simple media for the demonstration of pyrocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine* 44, 301-317.
- Locascio, S. J. (1963). Hot water and aerated steam treatment of vegetable seed. *Florida Agricultural Experimental Stations Journal Series No. 1735*: 183-189.
- Navaratnam, S. J., Schuttleworth, D. and Wallace, D. (1980). The effect of aerated steam on six seed-borne pathogens. *Australian Journal of Agriculture and Animal Husbandary* 20: 97-101.
- Randhawa, P. S. and Schaad, N. W. (1984). Selective isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seeds. *Phytopathology* 74: 268-272.
- Schaad, N. W. (1989). Detection of *Xanthomonas campestris* pv. *campestris* in crucifers. In "Detection of Bacteria in Seed and Other Planting Material". Edts A. W. Saettler, N. W. Schaad and D. A. Roth. APS Press 68-75.

-
- Schaad, N. W. and Alvarez, A. (1993). *Xanthomonas campestris* pv. *campestris*: cause of black rot of crucifers. In "Xanthomonas", Edt J. G. Swings and E. L. Civerolo. Chapman and Hall, 51-55.
- Schaad, N. W., Gabrielson, R. L. and Mulanax, M. W. (1980). Hot acidified cupric acetate soaks for eradication of *Xanthomonas campestris* from crucifer seeds. Applied and Environmental Microbiology 39: 803-807.
- Schaad, N. W., Sitterly, W. R. and Humaydan, H. (1980). Relationship of incidence of seed borne *Xanthomonas campestris* to black rot of crucifers. Plant Disease 64: 91-92.
- Schaad, N. W. and Stall, R. E. (1988). *Xanthomonas*. In "Laboratory Guide for Identification of Plant Pathogenic Bacteria". Ed. N. W. Schaad. 2nd. edt. APS Press, 81-94.
- Schults, T., Gabrielson, R. L. and Olson, S. (1986). Control of *Xanthomonas campestris* pv. *campestris* in crucifer seed with slurry treatments of calcium hypochlorite. Plant Disease 70: 1027-1030.
- Shackleton, D. A. (1962). A method for detection of *Xanthomonas campestris* (Pammel 1895) Dowson 1939 in *Brassica* seed. Nature 193: 78.
- Sherf, A. F. and MacNab, A. A. (1986). Crucifers. In "Vegetable Diseases and their Control". John Wiley and Sons, 2nd. edt. 251-253.
- Smith, W. P. C. (1962). Black rot of cabbage, cauliflower and other related plants. Journal of Agriculture of Western Australia Ser. 4, 3, 3: 231-232.
- Sutherland, J. (1987). Control seed-borne diseases with a hot-water bath. Agnote, Order No. 3660/87, 2pp.
- Walker, J. C. (1941). Origin of cabbage black rot epidemics. Plant Disease Reporter 25: 91-94.
- Walker, J. C. (1952). Black rot. In "Diseases of vegetable Crops". McGraw-Hill Book Company, 529pp.
- Williams P. H. (1980). Black rot: A continuing threat to world crucifers. Plant Disease 64: 736-742.
- Worthing, C. R. and Walker, S. B. Edts. (1987). "The Pesticide Manual". The British Crop Protection Council. 8th edt, 1081pp.

3. Recommendations

3.1 Extension/Adoption by Industry

- 1993 The project was reviewed by HRDC on 25 November 1993.
- 1994 Seminar to seed company representatives at the Institute for Horticultural Development, 4 February 1994.
- 1995 Seminar/workshop to Gippsland Vegetable Growers Group, Bairnsdale, March 1995.
- 1995 Seminar/workshop to Cranbourne Vegetable Growers Group and Nurseries, Cranbourne, to be advised.
- 1995 Seminar/workshop to Werribee Vegetable Growers Group, Werribee, to be advised.
- 1995 A report on the project will be published in all relevant industry publications.

3.2 Directions for Future Research

There are several areas of future research for Black Rot contamination of *Brassica* seeds:

1. There is a need to refine the steam-air treatment in order to determine the best temperature and time combination to control Black Rot without reducing germination. Further, it has advantages over the hot water treatments as it is far more "user friendly".
2. The hot water with sodium hypochlorite treatment needs to be tested on natural populations of the bacterium from *Brassica* seeds, other than cabbage. Especially as the recommendations for hot water treatment of cauliflower, sprout and broccoli seeds is generally 5 min less than for cabbage seed due to smaller seed size.
3. It could be informative to try a thermo-chemical treatment developed for bacterial contamination of tomato seeds by Kritzman, G. (1993). A chemi-thermal treatment for control of seed borne bacterial pathogens of tomato. *Phytoparasitica* 21 (2):101-109.
4. Deficiencies of the hot water sodium hypochlorite treatment need to be defined.

3.3 Financial/Commercial Benefits

Seed borne inoculum is the main source of the Black Rot disease. Once established in seedlings, it is difficult to control with chemicals. It is therefore imperative that growers treat seed, or buy seedlings originating from treated seed. Further this project has indicated that hot water treatments currently in use may not be as effective as originally thought.

Benefits from treating seed are removal of bacterial contamination and stimulation of germination. Seed contamination of 0.0001% can lead to epidemics in the field, which reduce the productivity, quality and yield of the crop. Field infections often rapidly develop into epidemics and are difficult to control even with copper spays. Overall Black Rot in a crop could add an estimated 10% to the cost of production.

4. Acknowledgments

The author thank the following for their financial contribution to the project:

Arthur Yates & Co. Pty. Ltd.,
Fairbanks Selected Seed Co. Pty. Ltd.,
Horticultural and Research Development Corporation,
New World Seeds Pty. Ltd.,
Victorian Farmers Federation, Gippsland Vegetable Group,
W. Bone & Sons Pty. Ltd. Wholesale Nurserymen,
Woodlyn Nurseries Pty. Ltd.

The author thanks: Ms W. Morgan (Industry Manager Vegetables, IHD) and Mr R. Dimsey for assistance with extension; Mr G. Hepworth for assistance with the statistics and experimental design; Mr M. Meabald for use of his steam-air machine and Mr B. Henderson for operating the machine; Ms S. Isaac and Mr P. Pierce for assistance with germination tests; The Academy of Grain Technology, 260 Princes Highway, Werribee 3030 for conducting some of the germination tests and Dr P. Merriman for proof reading the report.