FINAL REPORT TO THE AGRICULTURAL RESEARCH FOUNDATION
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TITLE: Seed Treatments for Controlling Seedborne Black Leg in *Brassica* Crops

RESEARCH LEADER: Cynthia M. Ocamb

YEARS AT OSU: 20

PHONE NUMBER: (541) 737-4020
E-MAIL ADDRESS: ocambc@science.oregonstate.edu
FAX NUMBER: (541) 737-3573

COLLABORATORS: Morgan Schneider, Graduate Research Assistant in Botany and Plant Pathology, OSU; Carol Mallory-Smith, Professor of Weed Science, Crop and Soil Science, OSU

Executive Summary: Black leg (caused by *Phoma lingam*; teleomorph = *Leptosphaeria maculans*) has occurred widely in cruciferous crops across western Oregon since recent outbreaks began in spring 2014. Plants known to be susceptible to black leg include species of *Brassica* (broccoli, Brussels sprouts, cabbage, canola, cauliflower, Chinese *Brassica* vegetables, collards, kales, mizuna, mustards, oilseed rape, oilseed turnip rape, rutabaga, turnip, etc.), *Sinapis* (white and yellow mustard), and *Raphanus* (daikon or radish). *Phoma lingam* may be seedborne, and the presence of black leg on seed can result in seed unsuitable for marketing. Many areas, including Oregon and Washington, require that crucifer seed to be planted in the region be accompanied by an official certificate showing that seeds are black leg-free based on an official black leg seed health test. Infected seeds can result in disease at the seedling stage, which can spread by rain or irrigation water splashing plant to plant, potentially leading to epidemic levels within a given field. Fungicide seed treatments are critical for combatting black leg seed infections, especially those that occur below the seed testing detection threshold. Hot water seed treatment has been the only seed treatment option for growers of organic crucifer crops. Seed disinfestation with chemical treatments offers an alternative to the hot water treatment, and potentially better control of seedborne black leg can be accomplished.

Objectives for 2015-2017:(1) evaluate chemical disinfestation treatments for seed of *Brassica* crops for reduction in seedborne *Phoma lingam* as well as effect on seedling emergence and seed storability; and (2) evaluate fungicide seed treatments for seedborne *Phoma lingam* as well as effect on seedling emergence.

Procedures: Seeds of eight different cruciferous crops were obtained during the summer of 2015, assayed for seedborne black leg levels, and confirmed to be black leg-free. These commercial, non-infected seed lots were inoculated with *Leptosphaeria maculans* (syn. *Plenodomus lingam*) in order to evaluate chemical disinfestation as well as fungicide applications to seed for management of seedborne *Leptosphaeria*. Seeds were soaked in *L. maculans* spore suspensions ($1x10^7$) for 6 hr and then were dried overnight at ambient room temperature. The *Leptosphaeria*-inoculated seed lines were assayed three times for *Leptosphaeria* according to the Association of Official Seed Analysts (AOSA) freeze-blotter seed assay to determine seed infection levels.

Subsamples of infected seeds were disinfested in the laboratory with household bleach or hydrogen peroxide for various time periods and germinated in laboratory chambers to ensure seed viability; subsets of chemically-disinfested seeds of cabbage and mustard greens were stored for evaluation of seed viability during 12 months of storage. The presence of seedborne *Leptosphaeria* was determined after chemical disinfestation treatments to evaluate the effect on seedborne *Leptosphaeria*. For the fungicide seed treatments, seeds were added to fungicide slurries and were stirred to coat seeds evenly. Fungicide treated seeds were air dried overnight prior to sowing in the greenhouse. *Leptosphaeria*-inoculated, non-fungicide-treated seeds were used as a control. Seeds were planted individually in each cell of 180-cell styroblock containers; the 70-ml cells were filled with Metro-Mix 840 planting medium. Styroblock containers were placed on trays and bottom watered. In each run of the greenhouse assay, 90 replicate seeds were sown per seed treatment. Greenhouse assays were conducted twice for each crop and fungicide seed treatment combination. Emerged seedlings were examined weekly up to 28 days after sowing for leaf spots or stem lesion symptoms consistent with black leg; stand counts were taken seven days after planting. Diseased seedlings were removed weekly, placed in moist chambers, and incubated at 5°C in the dark for up to three weeks to induce sporulation. Diseased seedlings were microscopically examined for pycnidia to confirm the presence of *L. maculans.*

Significant Accomplishments:

In a commercial, non-infected seed lot of radish cv 'NitroRadish' that was inoculated, 74% of the seeds were infected with *L. maculans*, and when this infected seed lot was evaluated in the greenhouse, 27% of the seedlings had black leg. Although none of the fungicides investigated completely inhibited *L. maculans*, all four fungicide treatments evaluated resulted in lower percentages of diseased radish seedlings compared to the non-treated control (Table 1). There were no significant differences in the percentage of diseased radish plants among the four fungicides evaluated. There were no significant differences in the mean plant emergence percentages between the various seed treatments and the non-treated control.

Table 1. Mean percentage of radish seedlings with black leg in greenhouse assays

Fungicide treatment (rate/100 lb seed)	Mean % of seedlings emerged[z]	Mean % of seedlings infected with *L. maculans*[y]
Non-treated control	99 a[x]	27 a[x]
Coronet (6.1 fl oz)	96 a	3 b
Mertect 340-F (3.5 fl oz)	100 a	7 b
Rovral 4F (16 fl oz)	99 a	5 b
Tilt (8 fl oz)	96 a	8 b

[z] Plant emergence was determined seven days after sowing.
[y] Plants were assessed for leaf spots weekly up to 28 days after sowing.
[x] Means within a column followed by the same letter are not significantly different at $P = 0.05$ by Fisher's F-protected Least Significant Difference (LSD) test.

When a commercial seed lot of mustard greens 'Mighty Mustard Pacific Gold' was inoculated with *L. maculans*, it resulted in 99.5% of the seeds infected, and when planted in the greenhouse, 7% of the seedlings developed black leg. All four fungicides evaluated resulted in fewer diseased seedlings compared to the non-treated control treatment. Coronet or Tilt treatments completely inhibited the development of diseased mustard green seedlings and both fungicides resulted in fewer diseased plants compared to Mertect-treated seeds. Rovral-treated seeds were not different from Coronet, Mertect, or Tilt. Seedling emergence was not different among Coronet, Rovral, Mertect, or the non-treated control treatment while the percentage of seedlings emerged was significantly less when seeds were treated with Tilt.

Table 2. Mean percentage of mustard greens seedlings with black leg in greenhouse assays

Fungicide treatment (rate/100 lb seed)	**Mean % of seedlings emerged**[z]	**Mean % of seedlings infected with *L. maculans***[y]
Non-treated control	77.5 a[x]	7.0 a[x]
Coronet (6.1 fl oz)	75.5 a	0 c
Mertect 340-F (3.5 fl oz)	75.0 a	2.5 b
Rovral 4F (16 fl oz)	80.5 a	1.5 bc
Tilt (8 fl oz)	40.0 b	0 c

[z] Plant emergence was determined 14 days after sowing.
[y] Plants were assessed for leaf spots weekly up to 28 days after sowing.
[x] Means within a column followed by the same letter are not significantly different at $P = 0.05$ by Fisher's F-protected Least Significant Difference (LSD) test.

Chemical disinfestation studies developed a set of optimum treatment times for soaking seed in hydrogen peroxide (3.0% H_2O_2) or Clorox bleach (0.9% NaOCl) for the eight seed lines examined (Table 3). Broccoli and radish seed did not tolerate treatment with hydrogen peroxide, seed coats were shed while seeds were soaking in the chemical disinfectant.

Table 3. Chemical disinfestation of crucifer seed (corresponding optimum seed treatment)

Crucifer crops evaluated		**Disinfestation time (min)**[x]	
Common name	**Scientific name and plant variety**	**3.0% H_2O_2**	**0.9% NaOCl**
mustard greens	*Brassica juncea* 'PacificGold'	30	30
canola	*B. napus* 'Baldar'	30	30
broccoli	*B. oleracea* (sprouting broccoli)	-[y]	30
cabbage	*B. oleracea* 'Golden Acre'	40	20
collards	*B. oleracea* 'Vates'	20	20
kale	*B. oleracea* 'Dwarf Blue Curled Scotch'	20	60
turnip	*B. rapa* 'Purple Top White Globe'	40	40
radish	*Raphanus sativus* 'NitroRadish'	-	40

[x] Time based on germination means determined seven days after sowing.
[y] Seed did not tolerate disinfestation with this chemical treatment.

Seed lines of cabbage and mustard greens were treated with a chemical disinfestation treatment did not exhibit a decrease in seed germination up to 12 months after treatment (Table 4) when stored at room temperature or under refrigeration. The only significant change was a slight but significant decline in the germination rate of non-treated mustard seeds after the 12 months in storage under refrigeration.

Table 4. Effect of chemical seed disinfestation on seed viability in long-term storage

	% Seed germination of cabbage 'Golden Acre'					
	24° C storage temperature			**4° C storage temperature**		
Treatment	**6 mo[y]**	**9 mo**	**12 mo**	**6 mo**	**9 mo**	**12 mo**
Control	93 a	94 a	90 a	94.0 a	93 a	90 a
0.9% NaOCl	94 a	94 a	92 a	93.0 a	93 a	92 a
3% H_2O_2	96 a	94 a	94 a	95.0 a	97 a	94 a

	% Seed germination of mustard greens 'Mighty Mustard'					
	24° C storage temperature			**4° C storage temperature**		
Treatment	**6 mo[y]**	**9 mo**	**12 mo**	**6 mo**	**9 mo**	**12 mo**
Control	98 a	99 a	99 a	99 a	99 a	97 b
0.9% NaOCl	98 a	99 a	99 a	99 a	100 a	99 a
3% H_2O_2	99 a	99 a	99 a	99 a	99 a	99 a

[y] Means within a column followed by the same letter are not significantly different at $P = 0.05$ by Fisher's F-protected LSD test. Each mean represents 100 seeds in each of 3 runs (300 seeds).

When inoculated seeds of the eight lines underwent a hot water or a chemical disinfestation treatment, the levels significantly decreased compared to the control. The effect of the chemical disinfestation treatments were similar to hot water for most crops tested, except that hot water was better in mustard greens and worse in broccoli, and bleach was less effective in turnip.

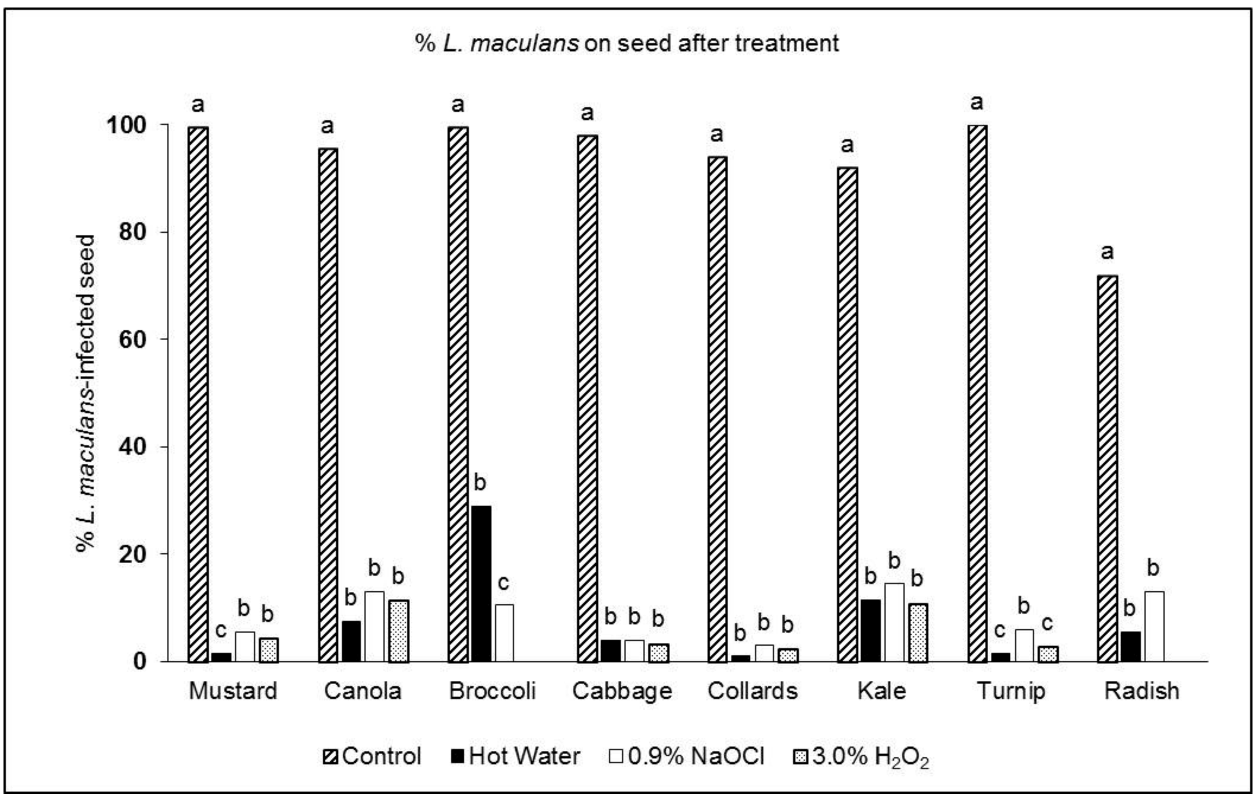

Figure 1. Mean % of seed with *L. maculans* on seed following hot water or chemical disinfestation treatment. For each crop type, bars with the same letter are not significantly different (P=0.05) by Fisher's F-protected LSD test.

Benefits and Impact: We have shown that fungicide seed treatments are effective to control for seedborne black leg in radish and mustard greens crops. Results from this grant enabled the development of data for labeling of these seed fungicides for these crop groups. An alternative to hot water or fungicide seed treatments is possible with chemical disinfestation but the length and efficacy of chemical disinfestation treatment varies for the crop type. Following an optimized treatment protocol there were no detrimental effects on seed storability and the treatments were very effective on reducing seedborne black leg. Further work should be conducted with additional varieties and other members of crucifer crops that have not yet been examined. Information generated by this grant funding aided in understanding seed treatments for black leg on crucifer seeds in the Pacific Northwest.

Future funding: At this time, additional funding from WSARE is being directed to further pursue additional seed treatments for black leg in crucifers.