Efficacy of Seed Treatments for Eradication of *Pyrenopeziza brassicae* from Mustard (*Brassica juncea*) Seed

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Introduction:

Brassicas in Washington: Brassica crops are grown in the Pacific Northwest USA for many markets and purposes, including conventional and organic fresh market and processing crops, cover crops, seed crops, oilseed crops, and biofuel crops (Inglis et al. 2013).

***Pyrenopeziza brassicae*:** In 2014, light leaf spot (caused by *Pyrenopeziza brassicae*) was detected in multiple types of brassica crops and on Brassicaceae weeds in western Oregon (Ocamb 2015). Light leaf spot had never been reported before in North America. In spring 2016, light leaf spot was found in *Brassica juncea* (mustard) cover crops and *B. rapa* (bird's rape mustard) weeds in northwestern Washington (Fig. 1). *P. brassicae* can be seedborne and seed transmitted on brassica seed (Carmody 2017). The potential impacts of this pathogen on the diverse brassica cropping systems in the Pacific Northwest USA not yet well understood, including the risk of introducing the pathogen on infected brassica seed and the risk of the fungus infecting brassica seed crops.

Objective: ***Assess the efficacy of seed treatments for eradicating Pyrenopeziza brassicae from brassica seed.***

Materials & Methods:

The mustard seed lot used (cv. Caliente 199) was infected with *P. brassicae* at 11 to 20%. Each of four seed treatment trials was completed, and all trials were repeated:

1. **Hot water:** 50°C for 15 and 30 minutes (Gabrielson et al. 1977; Miller & Lewis 2005).
2. **Chlorine:** 1.2% NaOCl for 10, 20, 30, and 40 minutes (Babadoost et al. 1996).
3. **Steam:** Proprietary steam treatment for 90 s at 62.8, 65.6, 68.3, and 71°C (Neergaard 1977; Tom Stearns, High Mowing Seed Co., VT).
4. **Fungicides:** (Gabrielson et al. 1977; Pesticide Information Center Online 2016)
   - i. Metlock = metconazole [Fungicide Resistance Action Committee (FRAC) Group 3]
   - ii. Vibrance = sedaxane (7)
   - iii. Rovral 4 Flowable = iprodione (2)
   - iv. Mertect 340F = thiabendazole (1)
   - v. Maxim 4FS = fludioxonil (12)
   - vi. Dynasty = azoxystrobin (11)
   - vii. Coronet = boscalid (7) + pyraclostrobin (11)
   - viii. Farmore I400 = azoxystrobin (11) + fludioxonil (12) + metalaxyl (4) + thiamethoxam (IRAC 4)
   - ix. Obvius = fluxapyroxad (7) + pyraclostrobin (11) + metalaxyl (4)
   - x. Helix Vibrance = difenoconazole (3) + fludioxonil (12) + metalaxyl (4) + sedaxane (7) + thiamethoxam (IRAC 4)

For each type of treatment, seed were subjected to:

1) A **seed germination assay** to assess the effects of treatments on seed quality: 4 replications of 100 seed/treatment were subjected to the mustard blotter germination assay of the Association of Official Seed Analysts (Yaklich 1985).
2) A **seed health assay** to quantify the incidence of seed infected with *P. brassicae*: For the chlorine, hot water, and steam seed treatments, 4 replications of 100 seed/treatment were plated onto Sorensen's NP-10 agar medium (Sorensen et al. 1991). For fungicide seed treatments, 4 replications of 100 seed were plated using a freeze-blotter assay (ISHI-Veg 2015). Seed were incubated at 4 ± 2°C in the dark, and examined microscopically after 21, 28, and 35 days (Carmody 2017).
3) A **seed transmission assay** (Fig. 2) to determine if treatments reduced or prevented seed transmission of *P. brassicae*: 4 replications of 200 seed/treatment were planted in 72-cell flats and misted for 30 s every 30 minutes to create extended periods of leaf wetness favorable for light leaf spot (Carmody 2017).

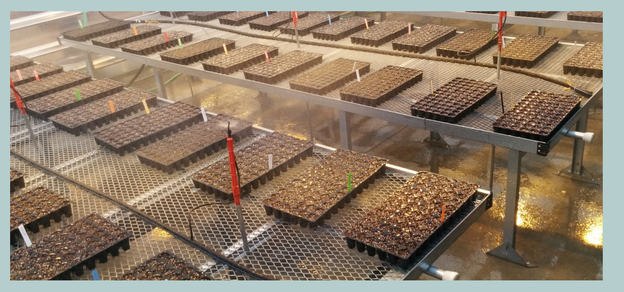

Fig. 2. *Pyrenopeziza brassicae* seed transmission assay.

Fig. 1. Counterclockwise from top left: Light leaf spot symptoms in a mustard cover crop; *Pyrenopeziza brassicae* infected cabbage siliques; *P. brassicae* infected cabbage seed coat; symptoms on a mustard seedling as a result of seed transmission of *P. brassicae*.

Abstract:

Light leaf spot of brassicas is caused by *Pyrenopeziza brassicae,* a new disease to the USA. The fungus can be seedborne and seed transmitted. A seed lot of 'Caliente 199' mustard (*B. juncea*) infected with *P. brassicae* was used to assess the efficacy of chlorine (1.2% NaOCl for 10, 20, 30, and 40 minutes), hot water (50°C for 15 and 30 minutes), steam (62.8, 65.6, 68.3, and 71.1°C), and 10 fungicide treatments to manage seedborne *P. brassicae*. Each seed treatment was compared to non-treated seed, and fungicide treatments were also compared to seed treated with a polymer colorant (seed coating) added to each product. All treatments reduced the incidence of seed infected with *P. brassicae*, from an average of 13.5% for non-treated seed to 0 to 4.3%, based on seed health assays. Likewise, all treatments, including the seed colorant control treatment, reduced seed transmission of *P. brassicae* from an average of 3.4% for non-treated seed to 0 to 0.4%. Seed transmission was not observed for the hot water, steam, and six of the fungicide treatments (azoxystrobin, fludioxonil, iprodione, thiabendazole, pyracostrobin + boscalid, and difenoconazole + fludioxonil + mefenoxam + sedaxane + thiamethoxam). The hottest steam treatment reduced seed germination from 98.0% for non-treated seed to 90.0 and 93.8% in Trials 1 and 2, respectively. The results demonstrate there are effective organic and conventional seed treatments for management of *P. brassicae*.

Results & Discussion:

All seed treatments evaluated reduced the incidence of seed infection and seed transmission of *P. brassicae* (as did the polymer dye coating control treatment). Repeat trials had very similar results.

1. Hot Water & Chlorine Seed Treatments (Fig. 3): Non-treated control seed had 98% germination, 16.5% seed infection, and 3.9% seed transmission rate by *P. brassicae*. Germination was not affected by any duration of chlorine seed treatment, but 30 min of 50°C water treatment reduced seed germination. Hot water treatment for 15 or 30 min eliminated *P. brassicae* on seed and prevented seed transmission. All durations of chlorine treatment reduced seed infection and transmission, but did not eradicate the pathogen, with seed transmission detected at 0.25 and 0.13% for the shorter durations of chlorine treatment (10 and 20 min, respectively).

2. Steam Seed Treatments (Fig. 4): Non-treated control seed had 98% germination, and 14.5% seed infection and 1.5% seed transmission rate by *P. brassicae*. Seed germination was reduced to 90% at the hottest steam treatment (71.1°C), but was not affected by steam treatments at 62.8, 65.6, or 68.3°C. Steam seed treatments largely eradicated *P. brassicae* on the seed, and prevented seed transmission.

3. Fungicide Seed Treatments (Fig. 5): Non-treated control seed had 98% germination, and 8.5% seed infection and 2.6% seed transmission rate by *P. brassicae*. No fungicide treatment adversely affected germination, and all the treatments reduced the incidence of seed infection and seed transmission of *P. brassicae*, including control seed treated with only the dye (polymer based seed coating used to apply fungicides to seed). The dye appeared to affect the ability of *P. brassicae* to form acervuli. Four fungicide treatments, Coronet, Farmore I400, Helix Vibrance, and Mertect 340F eradicated *P. brassicae* on mustard seed and prevented seed transmission.

This study identified highly effective organic and conventional seed treatments for management of *P. brassicae* on mustard seed.

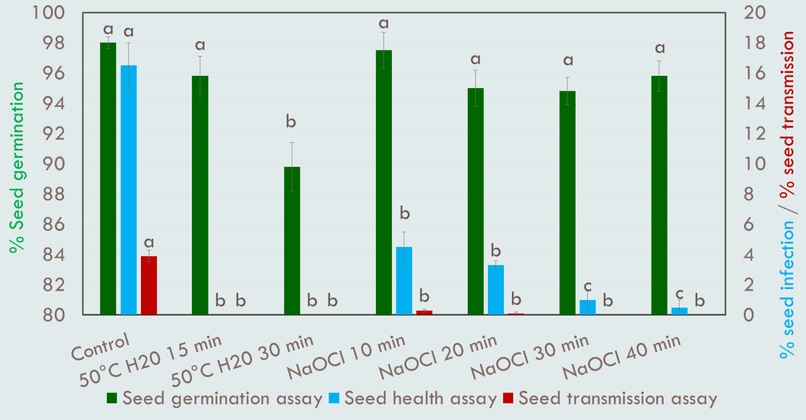

Fig. 3. Results of hot water & chlorine seed treatments.

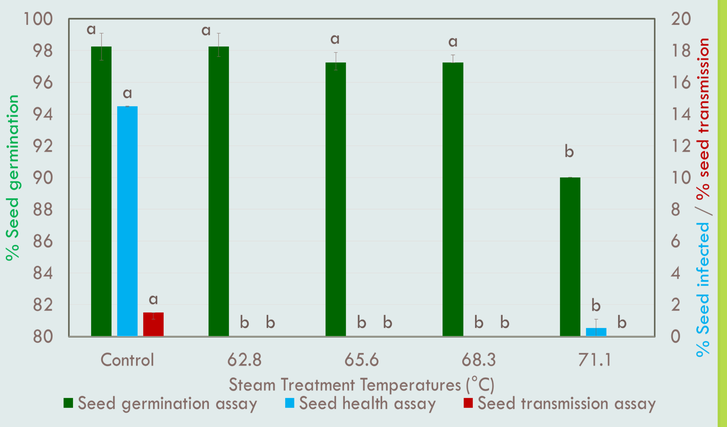

Fig. 4. Results of steam seed treatments.

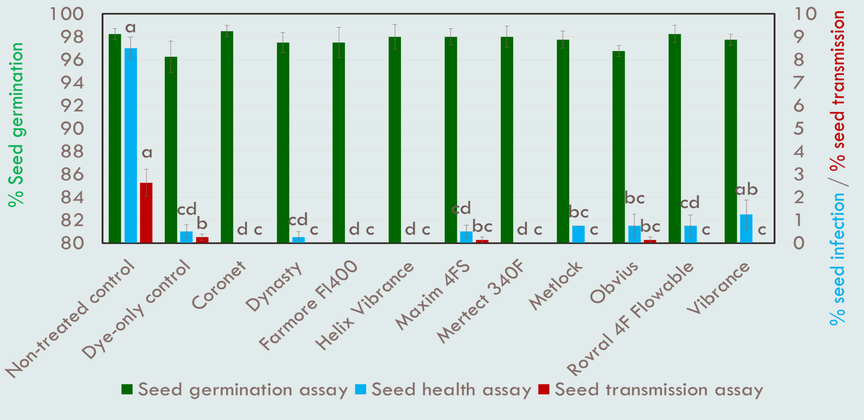

Fig. 5. Results of fungicide seed treatments.

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Acknowledgements & Funding:

seed matters

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