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Effects of fungicide, seeding date and seedling age on clubroot severity, seedling emergence and yield of canola

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is a devastating soil-borne disease of the cultivated crucifers worldwide. In recent years, *P. brassicae* has become a severe constraint to the production of canola (*Brassica napus* L., *B. rapa* L.) in central Alberta, Canada (Tewari et al. 2005; Strelkov et al. 2006).

A canola hybrid resistant to clubroot was released for commercial production in Canada in 2009, and several other resistant cultivars have since been released, but the number of clubroot resistance genes available for deployment is limited (Hirai 2006), and single gene resistance to clubroot has broken down quickly in winter canola and other crops (Kuginuki et al. 1999). Also, substantial genetic and pathotype variation exists in the *P. brassicae* population in western Canada (Strelkov et al. 2006; Xue et al. 2008; Cao et al. 2009). Production of breeding lines with single-gene resistance against a genetically diverse pathogen on a large acreage will surely impose strong selection pressure for pathogen genotypes that are able to overcome this resistance, and so the eventual breakdown of this resistance seems likely. Therefore, genetic resistance should be considered as just one component in the management of clubroot of canola on the Canadian prairies.

Several clubroot management strategies are used in the production of cruciferous vegetables, including drench application of fungicides and amendment with lime to increase soil pH (Donald and Porter 2009). However, these options are not practical or cost effective for use in the production of field crops such as canola. Reduction in clubroot severity is also possible with the application of fungicides (Donald et al. 2001; Mitani et al. 2003) and soil amendment products (Hwang et al. 2008), but the rates used for horticultural crops are too costly for canola. As a result, a range of alternative management strategies were studied for their usefulness in canola production, including the timing of seeding (Gossen et al. 2009), biological control (Peng et al. 2009), and use of bait crops (Kroll et al. 1984; Ikegami 1985; Murakami et al. 2001; Ahmed et al. 2011).

The field-to-field spread of clubroot is usually initiated from the field entrance, and is likely related to the soil movement on farming equipment (Cao et al. 2009). Warne (1943) pointed out that the clubroot pathogen could be seed-borne, which could account for the long distance spread of the disease. A recent study indicated the possible dissemination of *P. brassicae* resting spores as external contaminants of the seeds of canola and other crops, including wheat (*Triticum aestivum* L.), as well as of potato (*Solanum tuberosum* L.) tubers harvested from clubroot-infested fields (Rennie et al. 2011). While seed cleaning seemed to reduce the risk associated with seed-borne dispersal of *P. brassicae* (Rennie et al. 2011), a prudent clubroot containment strategy should also include pre-seeding seed sanitization treatments with chemical fungicides if the seeds were harvested from a clubroot-infested field. Moreover, any delay of infection reduces the severity of clubroot symptoms on affected plants, supporting the idea of seed treatment with appropriate fungicides as a way to reduce infection from inoculum that is already present in a field (Rod 1992).

Studies on the impact of inoculum density indicated greater clubroot severity and reduced plant height and seed yield of a susceptible canola cultivar with increasing *P. brassicae* resting spore concentration in the soil (Hwang et al. 2011a, b). These studies also revealed that the infection of younger seedlings resulted in higher clubroot severity, shorter plants and lower yield than infection of older seedlings. Since clubroot has been detected, albeit at generally low levels, in fields sown to clubroot-resistant canola cultivars (Strelkov et al. 2011), the effects of seedling age on disease development, plant growth parameters and yield in resistant cultivars grown in clubroot-infested soil warrants investigation. The objectives of this study were to examine the efficacy of fungicide seed treatments on suppressing clubroot in canola, and to evaluate the effects of seeding date on seedling emergence, plant height, clubroot severity and yield of canola under greenhouse and field conditions.

This article is adapted from the research paper "Effects of fungicide, seeding date and seedling age on clubroot severity, seedling emergence and yield of canola" by S.F Hwang, T Cao, Q Xiao, H.U Ahmed, V.P Manolii, G.D Turnbull, B.D Gossen, G Peng and S.E Strelkov published in the Canadian Journal of Soil Science (Vol. 91: 1-12, 2012)

MATERIALS AND METHODS

Efficacy of Fungicide in Eliminating Seed-borne Inoculum

Pathogen, Inoculum Preparation, and Soil Inoculation

All experiments were performed using the single-spore isolate SACAN-ss1 of *P. brassicae*, which was originally obtained from a canola field in St. Albert, AB, and was classified as pathotype 3 (Xue et al. 2008) on the differential hosts of Williams (1966). Pathotype 3 is the most virulent pathotype found on canola in Alberta (Cao et al. 2009).

Seed Infestation and Fungicide Seed Treatment

The clubroot susceptible canola cv. 34-65RR was used in all of the fungicide seed treatment experiments. Seed infestations were carried out with a mixture of 30% *P. brassicae*-inoculated soil: 70% non-inoculated soil (wt/wt), or a concentration of 1×107 spores g−1 soil. The soil-coated canola seeds were then vortexed in 500 µL solution containing one of the following fungicides at the rates indicated in parentheses: Dynasty 100 FS (azoxystrobin, 20 g a.i. 100 kg−1 seed), Nebijin™ 5SC (flusulfamide 0.2 g a.i. 100 kg−1 seed), Vitavax RS (carbathiin+thiram, 125 g a.i. 100 kg−1 seed), Prosper FX (carba thiin+trifloxystrobin+metalaxyl, 87.5 g a.i. 100 kg−1 seed), and Helix Xtra (difencazo le+metalaxyl+fludioxonil, 122.5 g a.i. 100 kg−1 seed). The control was vortexed in 500 µL water.

Efficacy of Fungicide in Reducing Disease Under Field Conditions

Seed of canola (*B. napus*) cvs. 45H28 (susceptible) and 45H29 (resistant) was treated with Cruiser 5FS (thiamethoxam 47.5%) at 400 g a.i. 100 kg−1 seed as an insecticide control, Prosper FX (clothianidin, 21.75%, trifloxystrobin, 0.54%, carboxin, 3.81%, metalaxyl, 0.41%) at 485 g a.i. 100 kg−1 seed, or Helix Xtra (difenconazole, 1.25%+fludioxonil, 0.13%+metalaxyl-M, 0.39%+thiamethoxam, 20.7% FS) at 434 g a.i. 100 kg−1 seed alone or combined with Sedaxane at 5 g a.i. 100 kg−1 seed, or with Dynasty (azoxystrobin, 75 g L−1+metalaxyl-M, 37.5 g L−1+fludioxonil, 12.5 g L−1 FL) at 5, 10 or 20 g a.i. 100 kg−1 seed. Clubroot symptom severity was rated on a 0–3 scale as noted above. The data sets consisted of the following variables: treatment, replication, severity rating (0–3), and subject (a unique identifier that is needed for calculating confidence intervals) (Shah and Madden 2004).

Effect of Seeding Dates on Clubroot of Canola – Field Experiments

Field trials to assess the impact of seeding date on disease severity were conducted at two sites in naturally infested soil. Small plots (6 m×1.5 m) of canola cv. 34-65RR were seeded on 2008 May 11, May 19 and May 28 at St. Albert and 2008 May 16, May 29 and Jun. 04 at Leduc, in a four-replicate RCB design. Seed of 45H26 (clubroot-susceptible) and 45H29 (clubroot resistant) was seeded on 2010 May 11, May 25 and Jun. 07 at Edmonton and 2010 May 12, May 25 and Jun. 13 at Leduc.

Effect of Seedling Age on Clubroot of Canola – Greenhouse Experiment

Experiments were conducted under greenhouse conditions with a 16-h photoperiod (22±5oC day/16±2oC night) to test the effect of different canola cultivars and inoculation of canola seedlings of different ages on the clubroot severity, canola plant height and seed yield. Canola cultivars 45H29 and 45H26 were used in the study. Both cultivars were seeded on five different dates: 2009 Jul. 15, Jul. 20, Jul. 25, Jul. 30 and Aug. 04.

RESULTS

Efficacy of Fungicide in Eliminating Seed-borne Inoculum – Greenhouse Experiment

Pairwise comparisons indicated that all five fungicide seed treatments significantly decreased the index of disease (ID) relative to the control, in which ID=68.1% (data not shown). Dynasty 100 FS (ID=13.2%) and Nebijin™ 5SC (ID=16.4%)

Table 1. *Effects of fungicidal seed treatment on seedling emergence, clubroot severity and yield of canola in clubroot-infested soils in Alberta in 2010*

		Resistant (45H29)		Susceptible (45H28)	
Treatment	Rate (mL 100 kg^{1} seed)	Plants m^{-2}	Yield (t ha^{-1})	Plants/m^{-2}	Yield (t ha^{-1})
Edmonton Cruiser 5 FS	400	31.5a	4.05a	39.7a	2.42a
Helix XTRA 289 FS	434	35.9a	3.94a	43.7a	1.85b
Helix XTRA 289 FS+Sedaxane	434+5	32.4a	3.94a	37.2a	1.87b
Helix XTRA 289 FS+DYNASTY	434+5	36.2a	4.29a	38.9a	2.00ab
Helix XTRA 289 FS+DYNASTY	434+10	34.3a	4.67a	37.7a	1.86b
Helix XTRA 289 FS+DYNASTY	434+20	33.9a	4.45a	40.4a	1.95ab
Prosper FX	485	34.9a	4.21a	38.8a	2.21ab
Leduc Cruiser 5 FS	400	54.3a	3.28a	55.4ab	3.11a
Helix XTRA 289 FS	434	58.1a	3.85a	58.4ab	2.67ab
Helix XTRA 289 FS+Sedaxane	434+5	57.4a	3.94a	60.7a	2.82ab
Helix XTRA 289 FS+DYNASTY	434+5	56.3a	3.71a	61.3a	2.74ab
Helix XTRA 289 FS+DYNASTY	434+10	57.7a	3.68a	50.2b	2.54ab
Helix XTRA 289 FS+DYNASTY	434+20	58.3a	3.33a	53.1ab	2.43b
Prosper FX	485	61.2a	3.57a	54.1ab	2.43b

a, b Means within a column followed by the same letter are not significantly different using Duncan's New Multiple Range Test ($P < 0.05$). Dynasty 100 FS (azoxystrobin), Helix Xtra (th iamethoxam+difenconazole+metataxyal+fludioxonil), Prosper FX (clothianidin+carbathiin+ trifloxystrobin+metalaxyl), Cruiser (thiamethoxam), Helix Xtra, Dynasty, Prosper and Sedaxane (pyrazole anilide).

reduced the ID the most, whilst Helix Xtra (ID=53.9%) had the smallest impact on this parameter.

Pairwise contrasts indicated that all dosages of Nebijin™ 5SC significantly decreased the ID relative to the control (data not shown). The ID values decreased as the Nebijin™ 5SC dosage was increased from a 1-fold to an 80-fold application of the recommended rate. All higher concentrations significantly reduced ID values in comparison to the 1-fold concentration.

Efficacy of Fungicide in Reducing Disease Under Field Conditions

In the case of the susceptible cultivar, none of the fungicide treatments differed in seedling emergence from the insecticide control at either site; yield in the insecticide control exceeded that of Helix Xtra alone, Helix Xtra+Sedaxane and Helix Xtra+Dynasty at the 2× rate at the Edmonton site and of Prosper FX and Helix Xtra+Dynasty at the 4× rate at the Leduc site (Table 1). In the case of the resistant cultivar, emergence and yield did not differ among any of the treatments at either site. In the susceptible cultivar at the Leduc site, the insecticidal control exhibited less severe disease relative to every fungicide treatment except Helix Xtra+Sedaxane (data not shown). Helix Xtra+Sedaxane reduced clubroot severity relative to Helix Xtra+Dynasty at the 2× rate. The insecticidal control and Helix Xtra+Dynasty at the 2× rate showed lower disease severity compared with Helix Xtra alone at the Edmonton site. As expected, the clubroot severity was greater on the susceptible cultivar compared with the resistant canola cultivar at both sites.

Effect of Seeding Date on Clubroot of Canola – Field Experiment

The analysis of variance indicated a significant effect of seeding date on seedling emergence and yield of canola at St. Albert and Leduc in 2008 (Table 2). Seedling emergence was significantly higher for the late seeding (Jun. 04) at Leduc and for the mid- to late seedings (May 19 and May 28) at St. Albert than for the other seeding dates. The canola yield at both sites was significantly higher in the early-seeded plots than the other seeding dates. Clubroot severity was greater in the late seeding compared with the two earlier seeding dates at St. Albert, since the median ranking and the relative effect of estimate were greater in the late seeding (data not shown). At Leduc, the early seeding date had a lower disease rating than the mid-seeding date, but not the late seeding date.

In the 2010 trial, no significant effect of seeding dates on the clubroot severity was evident (data not shown).

Effect of Seedling Age on Clubroot of Canola – Greenhouse Experiment

As expected, the resistant canola cultivar 45H29 developed few disease symptoms. In the susceptible cultivar 45H26, the median disease ratings for the early seeding dates (Jul. 15 and 20) were zero, and moreover, the median ranks and estimated relative effects for early seeding dates were significantly lower than those for the later seeding dates (Jul. 25, 30 and Aug. 04). Furthermore, the effect of seeding date (Jul. 15 and 20) on clubroot severity was similar, and likewise there was no significant difference in clubroot severity among the plants seeded at the later dates.

The height of both cultivars gradually declined from the earlier to the later seeding dates (data not shown). For seed yield, a similar pattern of results was observed, with a gradual decline associated with later seeding (Fig. 1). In the susceptible canola cultivar, the seed yield was not significantly different between the first two seeding dates, but was reduced to a significantly lower level in the later three seeding dates.

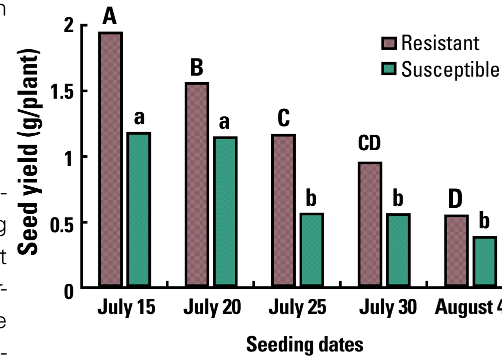

Fig. 1. *Effect of seeding date on seed yield of 45H29 (resistant) and 45H26 (susceptible) canola cultivars as grown in clubroot-infested soil-less mix under greenhouse conditions. The bars with the same letters are not significantly different for the same cultivar according to LSD at P ≤ 0.05. Bars with upper case letters represent the resistant cultivar and those with lower case letters represent the susceptible cultivar.*

Table 2. *Effect of seeding date on emergence, clubroot severity and yield of susceptible canola seeded into clubroot-infested soils near Leduc and St. Albert, AB, in 2008*

		Emergence		*Yield (t ha^{-1})*	
Treatment		*Leduc*	*St. Albert*	*Leduc*	*St. Albert*
Early	May 11		58.6b		1.18a
Late	May 19		167.8a		0.78b
Mid	May 28		145.4a		0.91b
Early	May 16	50.0b		0.81a	
Late	May 29	78.1b		0.42b	
Mid	June 04	165.3a		0.14b	

a, b Data are the means of four replications. Data followed by the same letters are not significantly different at the P<0.05 level as determined by Fishers' Protected Least Significant Difference test.

DISCUSSION

All five fungicides tested in the seed treatment experiment significantly decreased clubroot disease severity (ID) relative to the untreated control. The greatest reductions in ID were obtained with Dynasty 100 FS and Nebijin™ 5SC. Application of Nebijin™ 5SC at 20- to 80-fold the rate recommended for the control of powdery scab of potato gave the best results with this chemical. However, since Dynasty 100 FS is already registered as a canola seed treatment while Nebijin™ 5SC is not, the former represents the most logical tool for the control of seed-borne clubroot. Considering the relatively low levels of *P. brassicae* infestation that occur in naturally infested seedlots (Rennie et al. 2011), it is likely that any of the registered treatments assessed in this study may be sufficient to eliminate any risk of seed-borne transmission of clubroot.

There were no differences between the ID values obtained with 20-, 40-, and 80-fold applications of Nebijin™ 5SC, suggesting that the effect of 80-fold Nebijin dosages could be achieved by an application of 20-fold dosage. Nebijin may also keep clubroot disease severity index to about 10% or lower at rates above 20 mL 100 kg−1 seed.

Clubroot severity was relatively low when canola was seeded early, and yield also was higher when the crop was seeded earlier. Previous research indicated that younger canola seedlings are more susceptible to infection than older seedlings (Hwang et al. 2011a) and clubroot development is highly favored by high soil moisture (Karling 1968) and temperatures near 25°C (Feng et al. 2010; Sharma et al. 2011). Crops that were seeded early were able to establish in high soil moisture at low temperatures. Later, when high soil moisture occurred at higher temperatures, the plants were at a much less vulnerable growth stage. In contrast, plants that were seeded later faced both high soil moisture and high temperatures at a younger and more vulnerable growth stage.

In the seedling age study, the resistant cultivar exhibited few or no symptoms of clubroot. However, resistant plants still became infected when they were planted at the late seeding dates (i.e., when they were inoculated as young seedlings). This study also revealed that canola cultivar 45H29 is not completely resistant to clubroot, or that there may be a small amount of susceptible seeds or off-types mixed with the 45H29 seeds. Moreover, repeated cultivation of this resistant cultivar likely would select for virulent *P. brassicae* pathotypes able to overcome the resistance. The plants at the earlier seeding dates were relatively older for both the resistant and susceptible cultivars and more resistant to clubroot, as well as relatively taller, and also produced greater yields compared with the plants in the later seeding dates. The susceptibility of canola roots to infection by *P. brassicae* declines with increasing age, perhaps due to the thickening of cell walls (Mellano et al. 1970) and formation of other barriers that limit pathogen colonization. In addition, there may have been some environmental differences in the greenhouses as a result of the 1-h reduction/day in the amount of natural sunlight received by seedlings planted on Jul. 15 (16.5 h) compared with those planted on Aug. 04 (15.5 h).

While seed treatments may be effective in eliminating any risk associated with seed-borne dissemination of *P. brassicae*, it appears that they are, on their own, insufficient to adequately reduce the impact of clubroot in heavily infected fields. Similarly, manipulation of seeding dates alone is not sufficient to manage this disease. A combination of approaches, including seed treatments and early seeding, in conjunction with the deployment of resistant cultivars, will be required for the sustainable management of clubroot of canola.

References available upon request.

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