Research

Efficacy of Fungicide Seed Treatments in Controlling Blackleg of Canola

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Abstract

The efficacy of five fungicide seed treatments as a management tool against blackleg on spring canola was evaluated under greenhouse and field conditions in North Dakota. Blackleg, caused by *Leptosphaeria maculans*, inflicts the greatest yield losses when infecting seedlings before they reach the six-leaf growth stage. In greenhouse studies, 10-day-old seedlings were inoculated with *L. maculans* spore suspensions and evaluated 12 days later and at maturity or inoculated 12, 20, or 28 days after planting and evaluated at maturity. In field trials conducted in 2017 and 2018, severity was assessed at maturity. In the greenhouse, all fungicide seed treatments reduced ($P = 0.05$) disease severity at the seedling stage, but only the protection provided by Obvius

(fluxapyroxad + pyraclostrobin + metalaxyl) and Helix Vibrance (mefenoxam + fludioxonil + sedaxane + difenoconazole + thiamethoxam) reduced ($P < 0.05$) severity at the adult stage; however, none of them provide effective protection when plants were inoculated 20 days after planting or later. In field trials, none of the treatments significantly ($P > 0.05$) improved plant stand and yield or reduced disease incidence and severity. Although fungicide seed treatment is a valuable tool, it should not be used as the only method to manage blackleg disease.

Keywords: chemical control, soil-borne pathogens, seed-borne pathogens, *Brassica napus*, *Leptosphaeria maculans*, oil seed rape

Blackleg of canola (Fig. 1) is caused by *Leptosphaeria maculans* (Desm.) Ces. & de Not (anamorph = *Phoma lingam* [Tode:Fr.] Desm.) and is one of the major production constraints in North Dakota as well as worldwide (del Río Mendoza et al. 2012; Howlett et al. 2001; Rimmer 2006; West et al. 2001). In North Dakota, the largest canola producing state in the United States, the first blackleg epidemic was detected in 1991 (Lamey and Hershman 1993). Since then, the virulence profile of its *L. maculans* populations have changed (Bradley et al. 2005; Nepal et al. 2014), and currently prevalent strains are capable of infecting cultivars that carry resistance genes *Rlm1*, *Rlm2*, or *Rlm3*. Generally, in North Dakota, yield loss owing to blackleg disease ranges between 5 and 20%; however, under severely infected conditions, estimated yield losses of >45% have been observed (del Río Mendoza et al. 2012).

Protecting canola seedlings from emergence until they reach the six-leaf growth stage is critical to avoid formation of yield-robbing blackleg cankers (Marcroft et al. 2005). This susceptible period could last, depending on temperature, between 5 and 7 weeks

(Miller et al. 2018). To protect the plants, canola growers in North Dakota are advised to make fungicide foliar applications when seedlings are between the two- and four-leaf growth stages (Markell et al. 2008), which means seedlings could be exposed without protection to blackleg inoculum for 3 to 4 weeks. Some researchers believe the use of fungicide-treated seed could eliminate the need for fungicide foliar applications during the early growth stages (Mancini and Romanazzi 2014). The heightened threat of blackleg to the canola industry in the region has prompted the registration in recent years of several fungicide seed treatments for its management (Friskop et al. 2017). Fungicide seed treatments, in general, reduce the risk of seedborne inoculum being introduced to an area and protect seedlings from pathogens that could reduce germination and plant emergence from soil (Gugel and Petrie 1992; Mancini and Romanazzi 2014). However, their impact on chronic diseases, such as blackleg, is less clear and seems to be influenced by the environment. Studies conducted in Australia indicated that under high disease pressure conditions, seed treatments reduced disease severity and plant mortality and increased yields in susceptible and moderately resistant canola cultivars but were not economical when used on a resistant cultivar (Khangura and Barbeti 2004; Marcroft and Potter 2008). In contrast, Sprague et al. (2007) noted that treating seeds with Maxim (fludioxonil + metalaxyl) and Jockey (fluquinconazole) occasionally reduced blackleg severity and rarely increased yields. Interestingly, canola seeds imbibed with flutriafol (6 g/kg of seed) reduced disease severity at cotyledon, two-leaf, and four-leaf stages (Sprague and Burgess 2001). The objectives of this research were to evaluate the efficacy of five fungicide seed

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treatments registered for use in North Dakota against blackleg of canola and to evaluate the longevity of the protection they provide.

Greenhouse Studies

Greenhouse studies were conducted to evaluate five commercial seed-treatment products (Table 1) for their efficacy and longevity of protection under controlled conditions. The seeds of blackleg-susceptible cultivar Westar were treated with each product separately at their recommended dosage and compared with the nonprotected seeds. All products used in this study are registered in North Dakota for control of blackleg of canola (Friskop et al. 2017). Cultivar Westar was used because it does not have known major blackleg-resistance genes (Balesdent et al. 2005) and would in this way provide us with a worst-case scenario that is plausible given the status of blackleg in the state.

Fungicide-treated seeds were planted in plastic pots filled with a soilless potting mix (PRO-MIX BX, Premier Tech Horticulture, Quakertown, PA), and the pots were arranged in randomized complete blocks. The plants were grown in greenhouse rooms maintained at 20 ± 2°C with a 16-h photoperiod regulated by 600-W high-pressure sodium lamps (P.L. Light Systems, Beamsville, ON, Canada). Canola seedlings were inoculated at the cotyledon stage using mixtures of five *L. maculans* isolates selected for being highly aggressive (Franceschi 2015). These isolates were cultured separately on dishes containing V8-agar medium. After 12 days, the dishes were flooded with 5 ml of sterile distilled water and gently scraped with a sterile glass rod. The resulting spore suspensions were collected in plastic vials, and their concentrations were estimated with the help of a hemocytometer and adjusted to 10⁷

FIGURE 1
A, Blackleg lesion on a canola leaf with pycnidia; and **B**, girdling of an adult canola plant owing to blackleg stem canker.

pycnidiospores per milliliter. Equal amounts of these suspensions were mixed and used immediately for inoculations.

Efficacy study. The protective activity of the seed treatments was evaluated using four replications per trial and five plants per replication. Each trial was conducted twice, and each time the cotyledon leaves of 10-day-old plants were slightly wounded once with a sharp tweezer and 10 µl of the spore suspension was deposited on each wound (Fig. 2A). Plants were incubated in dark mist chambers for 24 h at 20°C and 98% relative humidity and returned to the greenhouse room. Reactions to inoculations were evaluated at the seedling stage 12 days after inoculation (Fig. 2B) and when plants reached maturity. At the seedling stage, disease severity was recorded using a modified 0 to 9 scale of Williams and Delwiche (1979). In the modified scale, a 0 represents no lesion development and 3, 5, 7, and 9 represent lesions of up to 3, up to 5, up to 7, and >7 mm in diameter, respectively. At maturity, plants were cut at the stem crown region, and the percentage of internal discolored tissues was estimated between 0 and 100. An illustration of the severity scales used in this study is presented in Mansouripour and del Río Mendoza (2017).

Seedling data were analyzed using the nonparametric approach suggested by Shah and Madden (2004), whereas data from adult plants were evaluated using parametric analysis of variance (ANOVA). Levene’s test of homogeneity of variances performed across the trials confirmed that both trials were homogenous (*P* > 0.05) and could be combined for analysis. For seedlings, the median disease severity was calculated for each replication and treatment using PROC MEANS of SAS 9.4 (SAS Institute, Cary, NC). The medians were ranked using PROC RANK, and ANOVA-type analysis was performed using PROC MIXED. Treatment relative effects and their 95% confidence intervals were calculated using SAS macro LD_CI.sas (Brunner et al. 2002). The relative effect ranges from 0 to 1, with treatments providing the best protection

FIGURE 2
A, Inoculation of cotyledon leaves of 10-day-old plant (picture courtesy of K. Chittem); and **B**, blackleg symptoms on a cotyledon leaf in a greenhouse trial 12 days after inoculation.

TABLE 1 Details on seed treatments, active ingredients, and application rates used in field and greenhouse studies			
Fungicide trade name	Manufacturer	Active ingredients	Application rate (liters/100 kg of seed)
Dynasty	Syngenta	Azoxystrobin (9.6%)	0.24
Prosper EverGol	Bayer	Clothianidin (22.32%) + penflufen (0.82%) + trifloxystrobin (0.55%) + metalaxyl (0.55%)	1.4
Obvius	BASF	Fluxapyroxad (1.58%) + pyraclostrobin (1.58%) + metalaxyl (1.26%)	0.59
Helix Vibrance	Syngenta	Sedaxane (0.26%) + difenoconazole (1.25%) + mefenoxam (0.4%) + fludioxonil (0.13%) + thiamethoxam (20.7%)	1.5
Maxim 4FS	Syngenta	Fludioxonil (40.3%)	0.01

having it closer to zero. For adult plants, Levene's test of homogeneity of variances was performed to determine whether trials could be combined for analysis. Upon confirmation, a combined ANOVA was performed considering treatments as fixed effects and replications, trials, and their interactions with treatments as random effects. The least significant difference (LSD) test was conducted to compare treatment means at $P = 0.05$.

At the seedling stage, all seed treatments significantly reduced the disease severity ($P = 0.05$) compared with the nonprotected controls and had median disease severities of <5, whereas the nonprotected controls had median disease severity of 7.3 (Table 2). Obvius had the strongest effect on disease severity, whereas Prosper EverGol had the lowest (Table 2). Disease severity on mature plants ranged between 39 and 100% on fungicide-protected plants and between 72 and 100% on nonprotected plants. Although Obvius and Helix Vibrance were the only products whose protection extended into significantly ($P < 0.05$) lower levels of disease severity at the adult plant stage compared with the nonprotected controls (Table 2), the average severity for all treatments was 68% or higher.

Timing of inoculation study. To identify the length to which the protection provided by seed treatments extended, 12-day-old canola seedlings were inoculated with *L. maculans* on the cotyledons as described (Fig. 2A) or on the petioles at 20 and 28 days after planting. Inoculated plants were incubated in a mist chamber as described and then returned to the greenhouse room. The study was conducted twice using a randomized complete block design with 6×3 factorial arrangement. At maturity, disease severities were assessed based on the percentage of internal discoloration of the stem crown region.

Single-degree-of-freedom orthogonal linear contrast analyses were conducted in SAS 9.4 to make independent comparisons between treatments. Overall, the seed treatments reduced disease severity compared with the nonprotected controls. However, the protective effect of the treatments was evident only on plants inoculated 12 days after planting. In later inoculations, all seed treatments behaved in the same way as the nonprotected controls. Among seed treatments, only Obvius and Helix Vibrance significantly ($P < 0.047$) reduced the mean disease severity compared with the nonprotected control inoculated 12 days after planting (Table 3).

TABLE 2
Blackleg disease severity observed in greenhouse trials at seedling and adult stage

Seed treatments	Seedling stage			Adult stage mean severity (%) ^y
	Median ^w	Mean relative effect ^x	95% confidence interval	
Dynasty	4.5	0.48	0.36–0.60	78 abc
Helix Vibrance	4.3	0.45	0.30–0.60	74 bc
Maxim 4FS	4.0	0.44	0.32–0.57	91 a
Obvius	2.8	0.12	0.09–0.23	68 c
Prosper EverGol	5.0	0.64	0.52–0.74	83 ab
Control ^z	7.3	0.88	0.79–0.90	89 a

^w Medians based on the disease severity on two cotyledons per plant, five plants per replication, four replications per trial, and two trials. Disease severity ratings based on modified 0 to 9 scale of Williams and Delwiche (1979).

^x Treatment with mean relative effect closest to 0 was the most effective.

^y Mean severities based on the percentage discoloration of internal stem tissues at the crown region. Means with the same letter are not significantly different based on Fisher's protected least significant difference test ($P = 0.05$).

^z Control = Westar seeds without fungicide seed treatment.

TABLE 3
Linear contrast analyses of the effect of time of inoculation and of seed treatments on blackleg severity on canola cultivar Westar evaluated under greenhouse conditions

Linear contrasts	Mean 1 ^z	Mean 2	Significance (P)
All seed treatments versus nontreated seeds			
Inoculated 12 days after planting	76	84	0.0244
Inoculated 20 days after planting	78	82	0.2481
Inoculated 28 days after planting	60	66	0.0796
Effect of time of inoculation on nontreated seeds			
12 days versus 20 days	84	82	0.7402
20 days versus 28 days	82	66	0.0003
Obvius versus no treatment			
12 days after planting	69	84	0.0010
20 days after planting	77	82	0.2461
28 days after planting	59	66	0.1221
Helix Vibrance versus no treatment			
12 days after planting	75	84	0.0470
20 days after planting	82	82	0.9120
28 days after planting	60	66	0.2040

^z Means 1 and 2 represent the values to the left and right of the "versus" term in the contrast.

TABLE 4
Effect of seed treatments on plant stand, disease incidence, disease severity, and yield in Langdon in 2017 and 2018

Seed treatment	2017				2018			
	Plant stand ^x	Blackleg ^y		Yield (kg/ha)	Plant stand	Blackleg		Yield (kg/ha)
		Incidence (%)	Severity (%)			Incidence (%)	Severity (%)	
Dynasty	37	96	72	1,214	34	98	89	535
Helix Vibrance	44	97	70	1,426	35	98	88	608
Maxim 4FS	36	93	65	1,542	30	98	90	437
Obvius	40	95	74	1,331	26	97	88	530
Nonprotected control	30	97	68	1,584	36	99	91	673
LSD ^z	NS	NS	NS	NS	NS	NS	NS	NS

^x Average number of plants per meter of row at two- to three-leaf growth stage.

^y Blackleg incidence calculated as percentage of symptomatic plants based on 200 plants per treatment (50 in each of four replications) per year. Severity based on percentage of discolored internal stem tissues at crown region based on 200 plants per treatment (50 in each of four replications) per year.

^z LSD = least significant difference; and NS = not significant at $P = 0.05$.

None of the seed treatments reduced disease significantly ($P > 0.05$) when plants were inoculated 28 days after planting (Table 3).

Field Study

Field trials were conducted in Langdon (Research Extension Center, North Dakota State University), North Dakota, in 2017 and 2018 to evaluate the efficacy of the seed treatments under field conditions. Four treatments (Dynasty, Helix Vibrance, Maxim 4FS, and Obvius) were applied to seeds from cultivar Westar and evaluated against nontreated controls using randomized complete blocks with four replications. The trials were established during mid-May. To ensure high-disease-pressure conditions, *L. maculans* inoculum was produced as described earlier, and three spray inoculations of a suspension containing 10^5 pycnidiospores/ml were made when seedlings were between the cotyledon and three-leaf growth stages. In addition, the plots were supplemented with blackleg-infected canola stubbles collected from commercial fields.

Data collected from field trials included plant stand counts and disease severity at the adult stage. Plant stand counts were taken from the central two rows of each plot when plants were at the two- to three-leaf growth stage. At physiological maturity, the plants were swathed, and 50 stubbles were uprooted from each plot for disease severity rating. The stubbles were cut perpendicular to the stem at the crown region. The disease severity was assessed on a scale of 0 to 100, where 0 and 100 indicated no discoloration and complete discoloration of the internal tissue, respectively. In addition to disease severity, the yield data of each experimental unit were recorded.

Levene's test of homogeneity of variances, conducted using the general linear model procedure of SAS 9.4, indicated the trials could not be combined for analysis ($P < 0.05$). Therefore, the trials were analyzed separately (Table 4). Treatment mean separation was performed using Fisher's protected LSD at $P = 0.05$. Compared with year 2018, 2017 had lower blackleg severity and higher yield. Statistical analysis indicated that none of the seed treatments improved ($P = 0.05$) plant stand count and yield or reduced blackleg incidence and severity in both years.

Conclusions

Under controlled conditions, all seed treatments evaluated in this study reduced the severity of blackleg infections compared with the nonprotected control. However, none of them suppressed disease development completely or prevented the fungus from further colonizing the stems as the plants grew. At maturity, plants from all treatments had on average more than 50% of the stem internal

tissues at the crown region affected by the pathogen, a condition that would result in severe yield reductions (Hwang et al. 2016). Although incomplete, the protection offered by the seed treatments lasted only 2 weeks, because plants inoculated after that time had blackleg severities equal to that of the nonprotected controls. A similar observation was made by Kharbanda (1992), who also reported that iprodione and prochloraz protected canola seedlings against blackleg for 3 weeks under controlled conditions. Iprodione is no longer available in the U.S. market, and prochloraz is not registered for use on canola in North Dakota (Friskop et al. 2017).

The beneficial activity of the seed treatments observed in the greenhouse trials did not translate into lower disease severity or higher yields under field conditions. In contrast to greenhouse conditions, in the field there is no control over the frequency and timing of arrival of *L. maculans* inoculum; thus, it is likely that inoculum reached the plants after the protective action of the seed treatments had faded out. This would also explain in part the difference between results of these studies and those reported by Marcroft and Potter (2008) and Sprague et al. (2007), who saw yield advantages of seed treatments of susceptible cultivars planted under high-blackleg-pressure conditions. Using Westar, which is known to have neither quantitative nor qualitative resistance to blackleg (Balesdent et al. 2005), allowed us to isolate and evaluate the protective effect of fungicide seed treatments under a worst-case scenario. It is possible that materials that carry quantitative resistance may benefit more from the seed treatments, especially if moderate disease pressure is present.

Nevertheless, results from this study suggest that fungicide seed treatments as an only management tool is not enough for the control of blackleg and that, under high disease pressure, the application of foliar fungicides may be required.

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