Effects of chemicals on ascospore production by *Leptosphaeria maculans* on blackleg-infected canola stubble in Saskatchewan

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Chemical treatment of blackleg-infected stubble was often very effective in inhibiting or eliminating the production of ascospores by ***Leptosphaeria maculans.*** Among the most effective fungicides were the ergosterol biosynthesis inhibitors, nuarimol, propiconazole, and triadimenol, which at 0.1 g a.i./L reduced spore numbers by 99–100%. Prochloraz, imazalil, chlorothalonil, and benomyl also significantly reduced ascospore numbers at 0.1 g a.i./L. At 10 g a.i./L, the herbicide glyphosate completely prevented ascospore formation, whereas trifluralin increased sporulation. A role for chemical treatment of stubble for control of blackleg on canola is indicated in light of the trend toward minimum tillage practices by western Canadian producers.

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Les traitements chimiques du chaume contre la jambe noire ont permis très souvent d'empêcher ou d'éliminer la production d'ascospores par ***Leptosphaeria rnaculans.*** Parmi les fongicides les plus efficaces on compte les inhibiteurs de la synthèse des sterols, le nuarimol, le propiconazole et le triadimenol qui, a raison de 0,1 g de matière active par litre, ont reduit le nombre de spores de 99 à 100 %. Dans une proportion de 0,1 g de matiere active par litre, le prochloraz, l'imazalil, le chlorothalonil et le benomyl ont egalement reduit de façon importante le nombre d'ascospores. A raison de 10 g de matière active par litre, l'herbicide glyphosate a permis de prevenir complètement la formation d'ascospores, tandis que la trifluraline a, quant a elle, fait augmenter la sporulation. Le recours au traitement chimique pour combattre la jambe noire qui s'attaque au chaume du Canola est tout indique si on prend en consideration le fait que les producteurs de l'Ouest canadien somblent opter pour un travail réduit du sol.

Introduction

Work in Britain (3) has indicated that production of pseudothecia and ascospore release by ***Leptosphaeria maculans*** (Desm.) Ces. & de Not. (blackleg) are highly sensitive to some fungicides, herbicides, surfactants, or urea applied to naturally infected *Brassica* stems. Applications of these materials in the spring either before or after mature pseudothecia had formed were effective in suppressing further development of the pathogen.

To date, blackleg control strategies employed in western Canada have centred around the development of resistant cultivars, management practices such as crop rotation or burial of infected stubble, and fungicidal seed treatment. Recent work in Australia has revealed the presence of considerable genetic heterogeneity in populations of the blackleg pathogen (5), indicating a considerable potential for the appearance of new virulent pathotypes. In western Canada canola (***Brassica napus*** L. and ***6. rapa*** L.) production recently has increased dramatically in response to very favourable prices relative to those for wheat. The combined effects of increased canola production and shorter crop rotations will increase blackleg inoculum levels, and put pressure on our presently availlable "resistant" cultivars. Therefore, alternative control stirategies for blackleg may soon be required. The fungicide Tilt (propiconazole) has recently been approved for use on canola as an aerial spray at the rosette stage of growth for blackleg control **(6).** In western Canada, the pseudothecial stage of L. *maculans* is initiated in the spring or early summer on infected stubble of the previous year's canola crop. Chemicals applied as a stubble treatment in autumn or spring as part of more traditional chemical applications for weed control might be both effective and economically attractive.

Materials and methods

Canola stubble infected by the virulent strain of *L. maculans* was collected annually in April between 1984 and 1993 from blackleg-infested crops sown the previous year or two years earlier (one- and two-year-old stubble, respectively). Examination of the samples in May showed that while the

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one-year-old stubble was free of ascocarps, they were present on the two-year-old material. The harvested one- and two-year-old stubble was divided into lots each consisting of 15 basal stem segments, Four replicate stem lots were dipped for 10 seconds in a solution or suspension of each test compound, placed in 8 cm high by 11 cm. dia. plastic pots and transferred outdoors. The one-year-old samples were tested for sporulation in September; the two-year-old samples were tested before chemical treatment and again in mid-July or later. Estimates of sporulation were made using ascospore liberation tunnels (2) with the air flow adjusted to 13,000 cc/min using a Rotameter (Brooks Rotameter Co., Lansdale, PA). Ascospores were collected for one hour on microscope slides coated with vaseline. LSDs were calculated using log-transformed spore numbers. The chemicals used in the present study are listed in Table 1. Several had been tested earlier by Humpherson-Jones and Burchill (3), but prochloraz, nuarimol, imazalil, propiconazole, triadimenol, and chlorothalonil were not included in their study. Concentrations of 1.0, 0.5, and **0.1** g a.i./L were generally used for fungicides, **10** g a.i./L for herbicides, and 50 g product/L for surfactants and urea.

Results

For at least five years between 1984 and 1993, adverse environmental conditions resulted in low spore numbers (Petrie, unpublished). Sporulation in 1992 and 1993 was at a higher level and results for these two years will be reported. Additional data for propiconazole were obtained in **1994** using one- and two-year-old blackleg-infected stubble.

Several fungicides significantly reduced or entirely prevented sporulation by ***L. maculans*** when applied to the one-year-old stubble samples before pseudothecial development. Among the most effective were the ergosterol biosynthesis inhibitors, nuarimol, triadimenol and propiconazole (Tables 2, 3 and 5). Among the herbicides, glyphosate (Roundup) at 10 g a.i./L prevented ascospore production entirely, whereas trifluralin (Treflan) increased it significantly. Triallate (Avadex BW) reduced sporulation substantially but the results were not statistically significant. Metribuzin (Sencor) had only a slight effect (Table 2). Propiconazole, urea, benomyl and triton X-100 suppressed ascospore production substantially when applied to samples that were already producing ascospores (2-year-old material) (Tables 4 and 5). There was a slight increase in ascospore numbers with trifluralin (Table 4). The mean number of ascospores discharged by the water controls in mid-July was 6.9 times greater than that in mid-May (Table 4). An estimate of potential ascospore production was calculated for the four chemical treatments by multiplying their mid-May values by 6.9. These four potential values for the treatments were less than 25% of the control value and showed a 77 to almost 100% reduction in potential ascospore production. In 1994 propiconazole at a concentration of 0.01 g a.i./L or higher produced significant reductions in ascospore numbers in both one- and two-year-old material (Table 5). Sporulation by ***L. rnaculans*** on the untreated one-year-old stems was much better than on the untreated 2-year-old stems, a reflection of the relative fecundity of the pathogen on the individual collections, which originated in different fields.

Discussion

The results support the conclusion of the British workers (3) that chemical treatment of blackleg-infected stubble may be effective in breaking the life cycle of ***L. maculans.*** Stubble treatments would appear to be more effective than applications of the same materials as seed dressings (7,8) or aerial sprays of field plantings (6).

Sporulation of ***L. maculans*** can continue on exposed stubble for seven or more years; complete burial of stubble reduces inoculum levels more rapidly. However, it has proven difficult if not impossible to entirely eliminate blackleg from a field by rotations and conventional tillage, or even by burial of crop residues (1,4, and Petrie, unpublished).

The profound effect upon ascospore production shown by some commonly used herbicides indicates a need for further studies in this area. With the movement toward minimum tillage on the part of producers, and conservation of plant debris on the soil surface, it would be timely to investigate selected chemicals on a field scale and to obtain estimates of their economic returns.

Literature cited

1. Alabouvette, C. and Brunin, B. **1970.** Recherches sur la maladie du colza due à ***Leptosphaeria*** maculans (Desm.) Ces. et de Not. I. - Rôle des restes de culture dans la conservation et la dissemination du parasite. Ann. Phytopathol. 2:463—475.

2. Hirst, J.M. and Stedman, O.J. **1962.** The Epidemiology of apple scab (***Venturia inaequalis*** (Cke.) Wint.). II. Observations on the liberation of ascospores. Ann. Appl. Biol. 50:525—550.

3. Humpherson-Jones, F.M. and Burchill, R.T. **1982.** Chemical suppression of the sexual stage of ***Leptosphaeria*** maculans on oil-seed rape and turnip seed crop straw. Ann. Appl. Biol. 100:281—288.

4. Mac Nish, G.C. **1979.** Survival of ***Leptosphaeria*** maculans in rapeseed root tissue. Australas. Plant Pathol. 8:23—24.

5. Plummer, K.M. and Howlett, B.J. **1993.** Major chromosomal length polymorphisms are evident after meiosis in the phytopathogenic fungus ***Leptosphaeria*** maculans. Curr. Genet. 21:1—7.

6. Séguin-Swartz, G. (ed.). **1994.** Fungicide 'Tilt" for the control of blackleg in canola. Blackleg News 2:4—5.

7. Verrna, P.R. and McKenzie, D.L. 1982. Evaluation of seed treatment fungicides for control of blackleg infection in canola, 1982. Pesticide Research Report, Agriculture Canada, Ottawa, Ontario. p. 233.

8. Verrna, P.R., McKenzie, D L. and Kaminski, D. 1983. Effectiveness of fungicide seed treatments in controlling blackleg infection in canola, 1983. Pesticide Research Report, Agriculture Canada, Ottawa, Ontario. p.232.

Table 1. Chemicals tested for their ability to suppress ascospore production in *Leptosphaeria maculans*.

Chemical	Active ingredient	Source
Fungicides		
Baytan	triadirnenol	Uniroyal
Benlate	benornyl	DuPont
Bravo	chlorothalonil	SIDS Biotech.
Fungaflor	irnazalil	Elanco
Mertect	thiabendazole	Clhiprnan
--	nuarirnol	Elanco
Ronilan	vinclozolin	BASF
Rovral	iprodione	Rhône Poulenc
Sportak	prochloraz	Elanco
Tilt	propiconazole	Ciba-Geigy
Tersan	thirarn	DuPont
Vitavax	carbathiin	Uniroyal/Gustafson
--	urea	BDH
Herbicides		
Avadex BW	triallate	Monsanto
Roundup	glyphosate	Monsanto
Sencor	rnetribuzin	Chernagro
Treflan	trifluralin	DowElanco
Surfactants		
Agral 90	alkyl phenol ethylene oxide	Chiprnan
Triton X-100	t-octyl phenol ethoxylates	Rohm & Haas

Table 2. Results of treating 1-year-old blackleg-infectedcanola stubble with chemicals prior to the development of pseudothecia of *Leptosphaeria maculans* (1992).

Chemical	Concentration	Mean no. ascospores**	% reduction or increase
Water (control)	—	2178	—
Urea (g/L)	50.0	1154	-47.0
Funaicides (a a.i./L)			
Benomyl	1.0	21*	-99.1
	0.25	691•	-68.3
Carbathiin	10	449*	-79.4
Iprodione	1.0	3127	+43.6
Thiabendazole	1.0	15*	-99.3
Herbicides (a a.i./L)			
Glyphosate	10.0	0*	-100.0
Metribuzin	10.0	1976	-9.3
Triallate	10.0	908	-58.3
Trifluralin	10.0	4974*	+128.4
Surfactants (g product/L)			
Agral-90	50.0	191*	-91.2
Triton X-100	50.0	1024*	-53.0

• Significantly different from the control at P = **0.05,** according to **LSD** test.

** Values are means of four replications.

Table 3. Results of treating 1-year-old blackleg-infected canola stubble with chemicals prior to the development of pseudothecia of *Leptosphaeria maculans* (1993).

Chemical	Concentration (g a.i./L)	Mean no. ascospores**	% reduction
Water (control)	—	6718	—
Urea	50.0	457*	93.2
Funaicides			
Benornyl	1.0	0*	100.0
	0.5	94*	98.6
	0.1	1419*	78.9
Chlorothalonil	1.0	1299*	80.7
	0.5	1266*	81.1
	0.1	2275*	66.1
Imazalil	1.0	0*	100.0
	0.5	0*	100.0
	0.1	599*	91.1
Nuarirnol	0.1-1.0	0*	100.0
Prochloraz	1.0	0*	100.0
	0.5	11*	99.8
	0.1	322*	95.2
Propiconazole	1.0	0*	100.0
	0.5	1*	99.98
	0.1	65*	99.0
Thiabendazole	1.0	651*	90.3
	0.5	2413	64.1
	0.1	4375	34.9
Thirarn	1.0	731*	89.1
	0.5	1133*	83.1
	0.1	1878	72.0
Triadimenol	1.0	0*	100.0
	0.5	0*	100.0
	0.1	33*	99.5
Vinclozolin	1.0	430*	93.4
	0.5	792*	88.2
	0.1	1778	73.5
Surfactants	(g product/L)		
Agral-90	50.0	1200*	82.1
	25.0	3125	53.5
	5.0	2750	59.1

* Significantly different from the control at P=0.05, according to LSD test.
** Values are means of four replications.

Table 4. Results of treating 2-year-old blackleg-infected stubble with chemicals after the onset of production of fertile pseudothecia by *Leptosphaeria maculans* (1992).

Chemical and conc. (g a.i./L)	Original no. ascospores (mid-May)	No. ascospores (mid-July)	Potential no. of ascospores*	% of potential	Reduction in potential (%)
Water	1741 ± 742	12058 ± 3870	12058	100.0	0.0
Urea (50)	1892 ± 906	15 ± 23	13097	0.1	99.9
Benomyl (1)	1431 ± 860	421 ± 327	9910	4.3	95.7
Trifluralin (10)	3110 ± 485	5056 ± 2174	21537	23.5	76.5
Triton X-100 (50 g product/L)	3420 ± 2384	810 ± 884	23680	3.4	96.6

* Numbers based on 6.9-fold increase in the control from mid-May to mid-July.

Table 5. Results of treating one- and two-year-old blackleg-infected canola stubble with propiconazole (Tilt) in 1994.

Propiconazole conc. (g a.i./L)	Mean no. of ascospores**	% reduction in ascospore numbers
One-year-old stubble		
0.00	13500	—
0.01	6500*	51.9
0.10	2300*	83.0
1.00	0*	100.0
Two-year-old stubble		
0.00	343	—
0.01	31*	91.0
0.10	18*	94.8
0.50	0*	100.0
1.00	0*	100.0

* Significantly different from check at $p = 0.05$, according to LSD test.
** Values are means of four replications.