Etiology of Canola Blackleg in Kentucky and Seasonal Discharge Patterns of *Leptosphaeria maculans* Ascospores from Infested Canola Stubble

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ABSTRACT

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A limited survey was conducted to determine the pathogenicity groupings (PG) of *Leptosphaeria maculans* associated with canola (*Brassica napus* var. *oleifera*) blackleg in south central Kentucky. In addition, multiyear ascospore discharge patterns were determined for infested canola stubble from seven fields. Strains PG3 and PG4 of *L. maculans* were most commonly associated with blackleg in Kentucky (18.4 and 79% of the isolates tested, respectively). One weakly virulent PG1 isolate (2.6%) and no PG2 isolates were found. *L. maculans*-infested canola stubble discharged ascospores within 1 week of harvest in 1990 and 1991. Early discharge patterns were not determined in 1989. Stubble collected each year released significant quantities of ascospores in the fall and winter months following crop harvest. However, few ascospores were released from infested stubble after the first year following crop production. The implications of these data in blackleg management are discussed.

Canola-quality rapeseed (*Brassica napus* L. var. *oleifera* (Metzger) Sink.) is extensively produced in Canada and Europe as a source of edible oil and meal (12). Canola was introduced into Kentucky in 1987 in response to increasing demand in the United States for canola oil. The crop is being developed as a fall-seeded alternative to soft red winter wheat (*Triticum aestivum* L.). Like wheat, canola is usually doublecropped with no-till soybean (*Glycine max* L.) immediately following harvest.

Blackleg, caused by *Leptosphaeria maculans* (Desmaz.) Ces. & De Not. (anamorph *Phoma lingam* (Tode:Fr.) Desmaz.), is a serious concern in all countries where canola–rapeseed is extensively grown (7). Blackleg was first detected in the United States in 1989 when a localized (ca. 100 ha) epidemic developed in south central Kentucky (16). Preliminary studies indicated that the causal organism was a highly virulent strain (PG4) of *L. maculans*. This was the first report of this strain of *L. maculans* in the United States. It is unknown if other strains also occur in Kentucky. This information is important to canola breeders who are developing canola cultivars that are resistant or tolerant to blackleg.

The most important primary inoculum for blackleg epidemics is windblown ascospores of *L. maculans* originating from infested canola stubble (3,4,6,8,13). Stubble management, thus, is a critical component of blackleg management programs (2,5,7,8). Successful programs include deep tillage to bury infested canola stubble (2) and rotation of fields out of canola to allow time for stubble decomposition (5). Management of blackleg by tillage and crop rotation must be implemented on an area basis, because *L. maculans* ascospores are transported in air currents up to 5 km (8).

To effect blackleg management by tillage, stubble discharging ascospores must be buried before adjacent, newly seeded canola fields emerge (2,19). Studies from Australia (12), Canada (19), England (6), and France (1,4) indicate that virulent isolates of *L. maculans* discharge ascospores during the fall and early winter when fall-seeded canola–rapeseed is at peak susceptibility (i.e., plants are at or before the six-leaf stage [14]). Studies are needed to determine acceptable timing of tillage operations based on *L. maculans* ascospore discharge patterns in Kentucky.

The length of time for crop rotation depends upon stubble survival between canola crops and the capacity for *L. maculans* to survive in and discharge ascospores from canola stubble over time (5). In France, Alabouvette and Brunin (1) determined that *L. maculans* can survive in canola stubble for at least 5 years. They speculated that infested stubble would produce ascospores if brought to the soil surface by tillage. In western Canada, Petrie (18) found that planting canola in 3-year-old *L. maculans*-infested canola stubble resulted in serious blackleg infection. This contradicted earlier studies (17) where infested stubble was found to be an ineffective source of inoculum after 2 years. In southeast Australia, McGee (13) found that *L. maculans* ascospores are discharged for at least 3 years after the original crop was grown. He postulated, however, that under normal cultural practices, only first-year stubble is an important source of inoculum because very little crop residue survives in the field for more than 1 year. In contrast, Bokor et al. (3) found that it took 3 to 4 years for infested rapeseed residue to decompose in western Australia. Determining this aspect of blackleg epidemiology for Kentucky is critical to developing successful crop rotation programs involving canola.

This study was conducted to generate data needed to develop stubble-management programs for blackleg management in Kentucky. Specific objectives were to (i) determine the range of pathogenicity groupings (PGs) associated with canola blackleg, and (ii) determine annual patterns of ascospore discharge by *L. maculans* from infested stubble.

MATERIALS AND METHODS

Collection of *L. maculans* isolates and pathogenicity group (PG) determinations. During 1989 to 1991, canola production fields in Logan, Simpson, Todd, and Daviess Counties were surveyed during late May to early June for plants showing typical blackleg stem cankers (5). These fields represented the bulk of the canola hectarage produced in the state during this period. The number of fields available to survey in these counties was very limited due to the small number of farms involved in canola production. Of 37 fields examined, 13 were found to have plants with blackleg symptoms. Of these 13 fields, nine were in Logan County, two were in Simpson County, and two were in Todd County. Twenty plants with blackleg stem cankers were collected at random from each field. Collected, diseased stems were stored at 4°C until further use.

Isolates of *L. maculans* were obtained by surface-sterilizing (3 to 4 min in 10% sodium hypochlorite followed by serial rinsing in sterilized distilled water) a 2- to 3-mm piece of tissue from the borders of stem cankers and plating the tissue on V8 juice agar amended with Rose Bengal (40 µg/ml) and streptomycin sulfate (100

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µg/ml). After 3 days at room temperature, single hyphal tips of developing fungi were transferred to standard V8 juice agar and stored at 23 to 24°C under continuous fluorescent light (Sylvania Cool White bulbs) for 7 to 10 days.

Thirty-eight isolates obtained from the 13 fields (1 to 3 isolates per field) were selected for PG determination. PGs were determined using the *B. napus* differential cotyledon inoculation procedure described by Mengistu et al. (15). Seed of the three differential *B. napus* cultivars (Westar, Quinta, and Glacier) was provided by C. Hill, Ameri-Can Pedigreed Seed Company, Leesburg, Georgia. All tests included a known PG4 isolate from Australia for comparison. The differential cultivars and known PG4 isolate were replicated three times; the average disease reaction was used for PG determination.

Ascospore discharge studies. In 1989 to 1991, stubble from recently harvested canola crops was collected (June 13, June 22, May 31, respectively) for use in ascospore discharge studies. Samples were collected from two fields in 1989 and 1991, and three fields in 1990. All fields were in Logan County, Kentucky. Random PG testing indicated that each field was infested with *L. maculans* PG4. Each year, approximately 250 stems exhibiting girdling blackleg cankers were collected from each field. Following collection, diseased stems were trimmed to 15- to 20-cm lengths and loosely packed into 3-liter wire baskets. Three replicate baskets were prepared from each field each year. Baskets with stubble were stored outside in a semiprotected area, which was shaded from midday and late-afternoon sun, but were otherwise exposed to ambient temperatures and rainfall.

Ascospore discharge from stubble was monitored at 3- to 5-week intervals for 35, 34, and 22 months postharvest in 1989, 1990, and 1991, respectively. Ascospore monitoring for stubble collected in June 1989 did not begin until mid-December due to the time needed to develop ascospore monitoring methodologies. In contrast, stubble collected in 1990 and 1991 was monitored for ascospore discharge within 1 week of stubble collection.

Ascospore discharge was monitored using a modification of a procedure described by McGee (13) and McGee and Petrie (14). Instead of using "ascospore liberation tunnels," preconditioned stubble (i.e., stubble subjected to 100% relative humidity for 24 h [room temperature] followed by wetting with sterile distilled water) was placed inside 3.78-liter plastic jugs with the bottoms removed. The jugs with stubble were then connected to Kramer-Collins 7-day drum spore samplers (G-R Electric Mfg. Co., Manhattan, Kansas). The rotating drums of the samplers were fitted with double-sided tape (19 mm wide). The drum samplers were then placed under a vacuum by connecting the sampler to a 115 volt, 60 Hz vacuum pump pulling 30 liters/min. The pump and sampler were run for 4 h, during which time ascospores discharged from stubble were deposited on the double-sided tape. Spores were then counted by transferring the exposed tape to a 2.5 × 7.5 cm glass microscope slide and observing spores with a compound microscope.

Table 1. Pathogenicity groupings (PGs) for isolates of *Leptosphaeria maculans* associated with canola blackleg in Kentucky, 1989 to 1991

County of origin	No. of fields	No. of isolates	No. in each PG			
			1	2	3	4
Logan	9	26	0	0	2	24
Simpson	2	6	1	0	2	3
Todd	2	6	0	0	3	3
Total no.	13	38	1	0	7	29

Table 2. Seasonal discharge patterns[a] for ascospores of *Leptosphaeria maculans*[b] released from canola stubble collected in June 1989 from two fields in Logan County, Kentucky

Year	Month	Day	Average[c] no. of ascospores discharged	
			Field L89A	Field L89B
1989	Dec	15	1066±694	390±216
1990	Jan	12	1390±209	355±192
	Feb	23	1740±622	742±264
	Mar	22	436±223	632±325
	Apr	27	496±280	259±203
	May	30	10±6	15±10
	Jun	29	13±8	65±45
	Jul	26	13±7	24±20
	Aug	24	13±12	16±18
	Sep	30	3±3	60±38
	Nov	6	10±14	13±13
	Dec	6	5±4	212±54
1991	Jan	8	11±7	114±71
	Feb	8	5±7	7±2
	Mar	7	0±0	1±1
	Apr	5	0±0	3±5
	May	8	0±0	0±0
	Jun	6	0±0	0±0
	Jul	9	0±0	0±0
	Aug	9	0±0	0±0
	Sep	17	3±2	1±1
	Oct	10	0±0	4±4
	Nov	14	0±0	1±1
	Dec	4	0±0	0±0
1992	Jan	14	0±0	0±0
	Feb	10	0±0	3±3
	Mar	15	0±0	0±0
	Apr	11	0±0	0±0
	May	15	0±0	0±0
	Jun	12	0±0	0±0

[a] Ascospore release measured using Kramer-Collins 7-day rotating drum spore sampler (with 30-liter/min sampling volume for 4 h).
[b] Pathogenicity group 4.
[c] Average ± standard deviation for three replicates per field.

RESULTS

Pathogenicity group determinations. Of the 38 isolates evaluated, 37 (97%) were highly virulent on canola and were classified as either PG3 (18%) or PG4 (79%) (Table 1). One PG1 and no PG2 isolates were detected. Both PG3 and PG4 isolates were detected in each of the three counties sampled. Five fields sampled were infested with both PG3 and PG4 isolates. The lone PG1 isolate was from one of the two Simpson County fields; this same field also produced one PG3 and one PG4 isolate.

Ascospore discharge patterns. *Stubble collected 13 June 1989.* Stubble collected in June of 1989 was not monitored for ascospore discharge until December, 6 months postharvest. Both stubble sets had similar ascospore discharge patterns, although the stubble from field L89A generally discharged more spores than did the stubble from field L89B (Table 2). For both stubble sets, ascospore discharge among replications varied considerably.

Stubble from both fields had significant ascospore discharge during mid-December 1989 to late April 1990. Peak release from both stubble sets occurred during late February 1990 (8 months after harvest). Ascospore discharge from L89A stubble de-

clined precipitously from late February to late March, with few ascospores being discharged from late May 1990 through early February 1991 (10 to 19 months after harvest). L89A stubble produced no ascospores 20 to 35 months after harvest except for a very minor discharge on 17 September 1991 (26 months after harvest).

Stubble from field L89B discharged low levels of ascospores between late May and early November 1990 (11 to 16 months postharvest). These low levels were followed by a slight increase in discharge during early December 1990 and early January 1991 (17 and 18 months postharvest, respectively). L89B stubble produced no or very few ascospores 19 to 35 months after harvest.

Stubble collected 22 June 1990. All three stubble sets discharged ascospores 7 days after collection (Table 3). In addition, each produced an initial peak discharge in late July (1 month after harvest). Average ascospore numbers at this time were similar for the different stubble sets, but replicates within individual sets were highly variable. For all stubble sets, moderate ascospore discharge was detected from late August to early November 1990, followed by another peak in early December (5 months after harvest). At this time, there was significant variation in the numbers of ascospores discharged by stubble sets. Greatest discharge was from L90B stubble, followed by S90A and L90A stubble, respectively. This peak was followed by a rapid decline in discharge between early January 1991 and early March. For L90A and S90A stubble, ascospore numbers were extremely low between early April 1990 and mid-March 1991 (8 to 19 months after harvest. In contrast, L90B stubble had moderate discharge during mid-January and mid-February 1992 (18 and 19 months after harvest, respectively). No or very few ascospores were discharged from any stubble set 21 to 34 months after harvest.

Stubble collected 31 May 1991. Ascospore discharge patterns during the 23-month monitoring period were similar for both stubble sets (Table 4); numbers of ascospores released, however, were consistently higher for L91A stubble than for L91B stubble. For both stubble sets, ascospore discharge among replications varied considerably.

Both stubble sets were actively releasing ascospores in early June, 4 days after crop harvest and stubble collection. Between mid-July and mid-August, spore release from both L91A and L91B stubble stopped entirely. The second of three peak ascospore discharge periods for both stubble sets occurred in mid-September (3 months after harvest). Numbers of ascospores released from both stubble sets then slowly declined during October through early December (4 to 6 months after harvest). In mid-January, 1992 L91A produced a third peak in ascospore discharge (7 months after harvest). L91B stubble did not perform similarly, and spore release continued to decline. The third peak observed for L91B stubble, which was significantly lower than the first two peaks, occurred in mid-February 1992 (8 months after harvest). During this same period, ascospore discharge from L91A stubble declined precipitously. Discharge from both stubble sets decreased during mid-March (9 months after harvest). L91B stubble discharged no or very few ascospores between mid-April 1992 and late May 1993 (10 to 22 months after harvest). L91A stubble performed similarly except for a minor ascospore discharge in mid-January and mid-March 1993 (18 and 20 months after harvest, respectively).

DISCUSSION

PG4 and, to a lesser extent, PG3 strains of *L. maculans* are the primary cause of canola blackleg in Kentucky. Our findings support an earlier report by Mengistu et al. (16) that *L. maculans* PG4 is associated with blackleg in Kentucky. It is unknown if PG3 and PG4 strains are endemic to Kentucky or if they were introduced with imported seed.

The PG2 strain is the most common cause of blackleg epidemics in Canada (15) and North Dakota (11). This strain of *L. maculans* was not detected in our study. Knowledge of the PGs involved in blackleg epidemics will be useful to breeders attempting to develop canola cultivars that have durable and effective blackleg resistance. For example, canola cultivars developed to resist *L. maculans* PG2 will likely not be effective in areas where PG3 and/or PG4 strains predominate (C. B. Hill, *personal communication*). Thus, studies evaluating cultivar resistance useful in Kentucky will need to be conducted in areas where *L. maculans* PG3 and PG4 occur or where it is acceptable to infest fields with those strains.

Stubble from five canola fields infested with *L. maculans* PG4 discharged ascospores within 1 week of harvest (Tables 3 and 4). This finding is important because it suggests that volunteer canola emerging after a diseased crop is harvested could be

Table 3. Seasonal discharge patterns[a] for ascospores of *Leptosphaeria maculans*[b] released from canola stubble collected in June 1990 from one field in Simpson County and two fields in Logan County, Kentucky

			Average[c] no. of ascospores discharged		
Year	**Month**	**Day**	**Field S90A**	**Field L90A**	**Field L90B**
1990	Jun	29	314±171	852±613	1173±586
	Jul	26	1822±1456	2283±1674	2220±1733
	Aug	24	247±62	85±75	260±164
	Sep	30	651±144	211±222	551±257
	Nov	6	90±70	151±101	157±151
	Dec	6	1973±414	1064±517	5186±1694
1991	Jan	8	1459±1324	559±372	3797±2984
	Feb	8	141±144	142±107	360±267
	Mar	7	227±293	11±10	49±60
	Apr	5	2±3	4±4	11±14
	May	8	0±0	0±0	3±4
	Jun	6	0±0	0±0	0±0
	Jul	9	0±0	0±0	0±0
	Aug	9	0±0	0±0	1±1
	Sep	13	12±6	8±2	1±1
	Oct	10	14±9	5±7	3±2
	Nov	14	1±1	8±1	2±2
	Dec	6	2±1	18±20	44±33
1992	Jan	16	8±8	11±16	531±395
	Feb	12	3±2	7±8	338±200
	Mar	12	3±2	5±3	48±60
	Apr	18	1±1	0±0	6±1
	May	22	0±0	0±0	1±1
	Jun	18	0±0	0±0	0±0
	Jul	24	0±0	0±0	0±0
	Aug	20	0±0	0±0	0±0
	Sep	18	0±0	0±0	0±0
	Oct	26	0±0	0±0	0±0
	Nov	18	0±0	0±0	0±0
	Dec	16	0±0	0±0	0±0
1993	Jan	13	0±0	1±1	2±1
	Feb	9	0±0	0±0	0±0
	Mar	4	0±0	0±0	1±1
	Apr	6	0±0	0±0	0±0
	May	20	0±0	0±0	0±0

[a] Ascospore release measured using Kramer-Collins 7-day rotating drum spore sampler (with 30-liter/min sampling volume for 4 h).
[b] Pathogenicity group 4.
[c] Average ± standard deviation for three replicates per field.

infected by *L. maculans* and serve as a source of inoculum for canola seeded in adjacent fields in the fall.

All stubble evaluated discharged ascospores during the fall and early winter months following crop harvest (Tables 2 to 4). This is the time when canola seeded in mid-September is most susceptible to infection by *L. maculans* (14). Others have reported similar results for fall-seeded canola–rapeseed (1,4,6,12,19).

The determination that ascospores are being discharged in the fall indicates that infested canola stubble must be buried prior to planting doublecrop soybeans in Kentucky (i.e., late May to mid-June). Delaying tillage operations until doublecrop soybeans are harvested (i.e., October to November) would result in the exposure of canola seedlings to ascospores of *L. maculans*. Waiting to bury stubble until the following spring (in preparation for planting corn [*Zea mays* L.]) would be even less acceptable than fall tillage. This practice, which is common in Kentucky, would expose canola seedlings to *L. maculans* inoculum during the fall and winter months.

The practice of burying infested canola stubble prior to planting doublecrop soybeans is not likely to be widely implemented by producers. In nearly all cases, doublecrop soybean is planted using no-tillage methods (9). This is done to conserve soil moisture, reduce labor and equipment costs, and achieve the earliest possible soybean planting date. Plowing canola stubble prior to planting doublecrop soybean would negate these benefits and thus be unacceptable to most producers.

It is likely, therefore, that resistance by producers to plowing infested canola stubble following canola harvest will increase future problems with blackleg. This, in turn, may slow or halt the development of canola as a viable crop in Kentucky. A similar situation occurred in Australia in the 1970s (5).

All stubble sets evaluated had minor ascospore discharge periods in the spring (February to late April) following the year the crop was produced. Spore release at this time, however, would be of little practical importance to the canola crop because of the advanced stage of the crop that time of year (4,14). Five of seven stubble sets discharged very low levels of ascospores 15 to 19 months after harvest (September to January). Two stubble sets discharged significant numbers of ascospores during this time. Discharge at this time could contribute to blackleg epidemics since newly seeded canola fields would be at the stage where they are most susceptible to blackleg. Almost no ascospores were discharged during this critical period in the third fall–winter following crop production.

Our findings support previous studies (1,3,10,13,14,18) that *L. maculans* is capable of surviving in and discharging ascospores from infested canola stubble for more than a year. Except for two stubble sets that discharged moderate numbers of ascospores 17 to 19 months after harvest, ascospore discharge was minimal from stubble after 10 months. Our observations are that under field conditions very little canola stubble survives by the twelfth month (D. E. Hershman, *unpublished*). Thus, it is unlikely that stubble would contribute significant inoculum beyond the first year following crop production. McGee (13) came to a similar conclusion for studies conducted in Victoria, Australia.

Our findings indicate that a break of 15 months between canola crops may be sufficient to significantly reduce the threat of blackleg. This length of time allows for the production of doublecrop soybean (following canola) and corn before again planting canola. Best results would be obtained by allowing two growing seasons between canola crops (26 months).

Although natural ascospore discharge under field conditions was not measured in this study, McGee (13) showed that laboratory-induced ascospore discharge from stubble closely approximates natural discharge. Thus, the results presented here should be useful for developing blackleg management strategies in Kentucky.

Table 4. Seasonal discharge patterns[a] of *Leptosphaeria maculans*[b] ascospores released from canola stubble collected in late May 1991 from two fields in Logan County, Kentucky

Year	Month	Day	Average[c] no. of ascospores discharged	
			Field L91A	Field L91B
1991	Jun	4	1502±589	989±586
	Jul	24	0±0	0±0
	Aug	8	1±1	0±0
	Sep	12	606±294	1463±758
	Oct	11	323±125	670±327
	Nov	12	191±33	584±381
	Dec	6	165±32	195±36
1992	Jan	15	1628±856	75±42
	Feb	14	610±314	227±114
	Mar	12	119±102	68±14
	Apr	16	0±0	25±24
	May	19	6±6	0±0
	Jun	17	5±4	0±0
	Jul	21	0±0	0±0
	Aug	18	0±0	0±0
	Sep	17	0±0	0±0
	Oct	27	0±0	1±1
	Dec	16	0±0	1±1
1993	Jan	13	33±25	4±2
	Feb	10	0±0	9±6
	Mar	14	111±106	3±5
	Apr	6	1±1	0±0
	May	21	0±0	0±0

[a] Ascospore release measured using Kramer-Collins 7-day rotating drum spore sampler (with 30-liter/min sampling volume for 4 h).
[b] Pathogenicity group 4.
[c] Average ± standard deviation for three replicates per field.

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