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Relative longevity of *Leptosphaeria maculans* and associated mycobiota on canola debris

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Survival of the blackleg pathogen (*Leptosphaeria maculans*) in canola (*Brassica napus* var. *oleifera*) stem debris was studied at three locations during two separate tests from August 1995 through October 1998. In combined isolation frequencies from both locations, *L. maculans* decreased over time from 85.0 % before burial in July to 15.4 % in September and 3.4 % in December. Eleven months after initiation of this test, *L. maculans* could not be isolated from the infected canola stem pieces. Isolation frequencies of *L. maculans* were similiar among the three soil depths over all sampling dates at both locations. All other fungi that were present in a preliminary assay also declined after burial. *Trichoderma* spp. were undetectable after initiation of the test and increased to 27.5 % on the last sampling date in July 1996. The increase of *Trichoderma* spp. corresponded with the decrease in isolation frequencies of *L. maculans* and other fungi in the stem pieces. The first test differed from previous studies, consequently a second test located near Griffin, Georgia, was initiated in 1996. In the second test, the relative longevity of *L. maculans* was determined on intact debris (two to three times greater in size) left on the soil surface after harvest in a field using either minimum or no-tillage. *Leptosphaeria maculans* was isolated from 26.4, 31.2, and 20.8 % of the pieces, respectively, for May 1997, November 1997, and October 1998. Pycnidia containing viable conidia were also identified on 43.7, 26.4, and 18.0 % from debris present in the D-V-8 plates even though no visible fungal colonies of *L. maculans* grew on the agar. The difference in relative longevity of *L. maculans* between the two tests was directly related to the condition and size of the canola debris. In the first test, the debris was sectioned into 10-cm pieces. These pieces were badly deteriorated and fragmented 5 months after burial. In contrast, the debris in the second test was still intact 36 months after being placed on the soil surface. This survival was because of increased debris size that resulted in reduced fragmentation from tillage. Thus, intact canola debris can serve as an inoculum source for at least three seasons or longer.

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[Longévité relative du *Leptosphaeria maculans* et du mycobiote associé sur des débris de canola]

La survie de l'agent de la jambe noire (*Leptosphaeria maculans*) dans des débris de canola (*Brassica napus* var. *oleifera*) a été étudiée à trois sites lors de deux tests séparés effectués entre août 1995 et octobre 1998. Selon les données d'isolement de tous les sites combinés, la fréquence d'isolement du *L. maculans* a diminué avec le temps de 85,0 % avant l'enfouissement en juillet, à 15,4 % en septembre et à 3,4 % en décembre. Onze mois après le début du test, le *L. maculans* n'était plus isolé de morceaux de tige de canola. Les fréquences d'isolement du *L. maculans* à trois profondeurs étaient semblables quels que soient la date et le lieu d'isolement. La présence de tous les autres champignons, présents selon une analyse préliminaire, a aussi diminué après l'enfouissement. La présence des *Trichoderma* spp. n'était pas détectable au début du test, mais elle était de 27,5 % lors du dernier échantillonnage de juillet 1996. L'accroissement de la présence des *Trichoderma* spp. coïncide avec la diminution des fréquences d'isolement du *L. maculans* et des autres champignons dans les morceaux de tige. Comme les résultats du premier test étaient différents de ceux d'études précédentes, un second test a débuté en 1996 près de Griffin, Georgie. Dans le second test, la longévité relative du *L. maculans* a été déterminée sur des débris intacts (deux à trois fois plus gros) laissés sur le sol après la récolte d'un champ avec travail minimum ou sans travail du sol. En mai 1997, novembre 1997 et octobre 1998, le *Leptosphaeria maculans* a été respectivement isolé de 26,4, 31,2 et 20,8 % des échantillons. Des pycnides contenant des conidies viables ont été aussi identifiées dans 43,7, 26,4 et 18,0 % des débris présents dans les boîtes de D-V-8, même si aucune colonie de *L. maculans* ne se développait sur l'agar. La différence de longévité relative du *L. maculans* entre les deux tests était directement reliée à la qualité et à la grosseur des débris de canola. Lors du premier test, les débris furent coupés en morceaux de 10 cm. Ces morceaux étaient très détériorés et fragmentés après 5 mois d'enfouissement. Par contre, dans le second test, les débris étaient encore intacts 36 mois après avoir été placés en surface du sol. Cette meilleure survie est attribuable à une plus forte dimension des débris, ce qui a réduit la fragmentation lors du travail du sol. Ainsi des débris de canola intacts peuvent servir de source d'inoculum pour au moins trois saisons.

INTRODUCTION

Canola (*Brassica napus* L. var. *oleifera* (Metzer) Sink.) is currently the world's third leading oil-seed crop after soybean and cotton (Woodruff and Hudson 1996). Commercial production in Georgia began in 1989 and approximately 6500 ha were planted in the southeastern United States in 1997. Current production includes primarily transgenic cultivars of laurate canola grown under contract with Calgene, LLC. The oil from these cultivars is excellent for use in production of confectionary coatings, detergents, and other specialty products. Current cultivars of high laurate canola grown in Georgia are highly susceptible to blackleg caused by the fungus, *Leptosphaeria maculans* (Desm.) Ces. & De not.

The control strategy for blackleg has depended heavily on moving production to avoid areas where severe disease occurred previously (Woodruff and Hudson 1996). Information on the survival of the pathogen in infested fields in the southeastern United States is

important since most recommended practices are based on studies or observations from other production regions (Hershman 1992; Hershman and Perkins 1995).

Leptosphaeria maculans is common worldwide and attacks numerous species of Cruciferae, including canola. Both virulent and weakly aggressive strains of *L. maculans* have been confirmed by researchers in the United States (Hershman 1992). In 1989, a virulent strain of *L. maculans* was responsible for 75 to 90 % yield losses of susceptible varieties in several canola production fields in southeast Kentucky. In southwest Georgia, significant damage to the canola crop first occurred during the 1993-1994 season (Baird 1996; Baird *et al.* 1996).

In Canada, *L. maculans* reportedly survives on canola debris for at least 5 yr after initial establishment in a field (Kharbanda and Tewari 1996). Therefore, a rotation where canola is planted every 4 to 5 yr is recommended if a field is infested with *L. maculans* (Curt Hill, Calgene LLC, personal communication). In western Washington, *L. maculans* was reported to overwinter on canola residues and on related cruciferous seed crops in western Washington (Gabrielson *et al.* 1978). In Kentucky, ascospores of *L. maculans* could be collected in a field up to 3 yr (Hershman and Perkins 1995). It is uncertain if primary survival of *L. maculans* is on canola debris or if the pathogen commonly occurs on alternative hosts in Georgia.

Certain fungal pathogens such as *Rhizoctonia solani* AG-4, have been reported to survive saprophytically in plant debris by utilizing cellulose as a carbon source; however, their longevity in debris varies depending upon pathogen and host (Daniels 1963; Garren 1966; Garrett 1970). Chung *et al.* (1988) observed that a hardwood-bark medium that contained high levels of cellulose proportionally increased cellulase activity of *R. solani* AG-4 and disease severity. It is uncertain what percentage of canola debris contains cellulose and what those levels may have on survival of pathogenic fungi such as *L. maculans.*

Soil depth has previously been reported to influence microorganism activity and survival. Seedling disease and root rot of soybean increased when peanut shells infested with *R. solani* AG- 4 were buried at 5-10 cm compared to 0-5 and 10-13 cm deep (Bell and Sumner 1984). Recently, isolation frequencies of *R. solani* AG-4 were similar among the three soil depths, but isolation frequencies of *Trichoderma* spp. were greater at 7.6 and 25.4 cm depths (Baird *et al.* 1993). Naturally occurring species of *Trichoderma* present in the rhizosphere are believed to produce some control of certain soilborne pathogens (Papavizas 1985). It is uncertain if deep burial of canola debris would also increase levels of *Trichoderma* and result in reduced survival of *L. maculans.*

The biodegradation rate of plant debris is dependent on several environmental factors, including temperature and available moisture which can directly influence microorganism activity. In Canada, for example, cooler temperatures and shorter growing seasons reduce microorganism activity as well as the rate of biodegradation. In warmer soils, such as occur in the southeastern United States, the potential microorganismal activity might be accelerated to result in increased nutrient depletion and biodegradation of plant debris. The biodegradation rate of canola debris by microorganisms in warmer climates such as Georgia may be critical to determine rotation length for canola, particularly if *L. maculans* is present. The purpose of this investigation was to determine the relative longevity of *L. maculans* and associated mycobiota in canola debris buried at three soil depths. In addition, the effects of canola debris size on the relative longevity of *L. maculans* were evaluated.

MATERIALS AND METHODS

In June 1995, canola debris which consisted of 30- to 50-cm-long stem pieces were collected from a blackleg nursery trial, containing over 300 varieties or breeding lines, located in Griffin (lat. 33°40' N, long. 85°40' W), Georgia, in

the first test. The collected debris was stored in polypropylene mesh bags suspended above the ground and exposed to external conditions for 35 d prior to initiation of the study.

In July 1995, canola stem debris containing natural infections of *L. maculans* were separated from disease-free tissue. Diseased stems were identified based on visible tissue discoloration and the presence of pycnidia of the anamorph, *Phoma lingam* (Tode ex. Schw.) Desm. The infested stem tissues were sectioned into 10-cm-long pieces and 20 of these were placed into 30-cm^2 polypropylene (mesh size 1 mm^2) packets and sealed with nylon thread. A total of 288 packets were prepared and 144 were buried at each of two sites.

Plots were established at two locations which had been planted with canola the previous season and had no prior history of *L. maculans*. A total of 48 plots, were established near Blakely (lat. 31°50' N, long. 85°0' W) (Site 1), Georgia, in a Tift loamy sand (fine-loamy, siliceous, thermic Plinthic Kandiudults; pH 6.2) on 1 August 1995 and Rome (lat. 34°30' N, long. 85°10' W) (Site 2), Georgia, in a Dyke clay loam (clayey, kaolinitic, mesic, Typic Rhodudults; pH 6.2) on 5 August 1995. At both sites, plots were subdivided into four replicate blocks of 12 microplots, with a spacing of 1.5 m center to center. Within each microplot, the packets with canola stem debris were buried at three soil depths: 0, 7.6, and 25.4 cm. Flags were used to mark each microplot during the investigation. Plot areas at each location were left undisturbed during the study period.

In the second test, canola debris was collected from a blackleg nursery site at Griffin. The debris, which consisted primarily of lower stem pieces 30- to 50-cm-long left standing after harvest, was collected in June 1996 and stored as in the first test. On 10 October 1996, a field site approximately 100 m x 30 m and centered midway between sites 1 and 2 of the first test was planted to canola. After planting, the debris, previously collected, was randomly scattered throughout the plot area and left undisturbed on the soil surface. The test site consisted of two soil types: 1) Lloyd clay loam (fine, kaolinitic, thermic Rhodic Kanhapludults; pH 6.2) and 2) Davidson loam (fine-loamy, mixed, superactive mesic Aeric Calciaquolls; pH 6.2). On 5 May 1997, just prior to canola harvest, 72 stem pieces were randomly collected from the test area, placed into paper bags, and returned to the laboratory for processing. To prevent contamination of the remaining debris during harvest, approximately 500 stem pieces were re-collected prior to harvest. Seven days after harvest and removed debris, was scattered over the surface of an approximately 20 m x 20 m area of the test site and left undisturbed for the remainder of the study. Random samples of 72 stems each were collected from this area on three sampling dates and processed in the laboratory.

Laboratory analyses

Prior to the canola stems being placed into the packets, 60 stems (10 cm long) were randomly selected to determine the natural mycobiota using the isolation methods discussed below. During each monthly sampling, four replicate microplots from both locations, each containing three packets of stem debris, were assayed for survival of *L. maculans* from each location. The 12 packets (four replicate plots) were sent by overnight mail to the laboratory at Tifton, Georgia, for analysis. Within 72 h after removal from the fields, the packets of debris were thoroughly rinsed in running tap water for 1 h to remove excess soil and plant material. Ten stem pieces were then randomly selected from each packet and four 1-cm sections were excised at 1.5-2.5, 3.5-4.5, 5.5-6.5, and 7.5-8.5 cm from the end of each stem. The 1-cm pieces were placed into a sodium hypochlorite solution w/v 0.524 % for 3.0 min. After 5 mo, the canola stems became progressively more decomposed and surface disinfection time was reduced to 1.0 min. The samples were then plated on D-V-8 medium in petri plates amended with 5 $\mu g\ mL^{-1}$ of chlortetracycline and 5 $\mu g\ mL^{-1}$ of tergitol NPX (Erwin *et al.* 1987). The cultures were incubated at room temperature up to 7 d and all mycelial colonies were transferred to

potato-dextrose agar (PDA) for identification (Baird *et al.* 1991). Both microscopic and macroscopic cultural characteristics were used to identify the fungi. Isolates of *Rhizoctonia* were assigned to anastomosis groups by pairing the isolates with "Testers" and observing hyphal fusion as described previously (Parmeter *et al.* 1969). Single spores of cultures initially identified as *Fusarium* spp. were transferred to carnation leaf agar and identified using the classification system of Nelson *et al.* (1983). Keys for general identification of the fungi were those developed by Ellis (1971), Sutton (1980), and Barnett and Hunter (1986).

In the second test, canola stem debris was returned to the laboratory on 5 May, 15 November 1997, and 8 October 1998 from the plot located at Griffin, Georgia. For all three sampling dates, 72 stem pieces were randomly selected and from each piece, a 4-cm section was excised and then subdivided into 2-cm pieces. One of the two 2-cm pieces was surface disinfested with sodium hypochlorite for 3 min and the other was disinfested for 1 min. All debris sections were placed in plastic petri plates (100 mm x 15 mm) containing D-V-8 medium. The timing of the sodium hypochlorite treatment was used to determine if the length of sterilization influenced the results from the first test. Identification of the fungi followed the methods previously described. Additional data was obtained from the 2-cm pieces by counting the sections containing pycnidia and conidia of the anamorphic stage of *L. maculans* when no visible cultures formed on the plates.

Statistical Analysis

All data was analyzed employing the Proc ANOVA (SAS 1989) to establish the correct error terms using a split-split plot design where location was the main plot and month of sample was the first split within the test. The second split included replicates and sampling depth. The reduced model included linear and quadratic effects to determine trend in percent isolation across months. Linear and quadratic analyses were used to determine the isolation frequency trend among the three sampling depths using Proc GLM.

RESULTS

Ambient temperatures varied between the two locations in the first test. Soil temperatures were below 15.6°C for 99 d in northwest Georgia (Site 2) and 21 d in the southwest trial at site 1 (Hoogeboom, unpublished data), but *L. maculans* could not be isolated from debris at either location on the last two sampling dates. Average air temperatures were 17.1°C at site 1 and 14.1°C at site 2 and the average soil temperatures were 21.4°C at site 1 and 16.6°C at site 2. Even though there were 3.3°C differences for average air and 4.8°C for soil temperatures between site 1 and site 2, visible tissue degradation and fungal activity were similar. Precipitation differences at the two sites (163.7 mm at site 1 versus 131.7 mm at site 2) had minimal impact on mean isolation frequencies.

In the preliminary assay conducted prior to the first test, 10 species of fungi were identified from the 60 stem pieces in July (Table 1). Of the 10 species, *Leptosphaeria maculans* was isolated from 85 % of the stem pieces, *Alternaria* spp. from 90 %, and *Fusarium* spp. from 26.6 %. *Nigrospora sphaerica* (Sacc.) E. Mason was the only other species isolated from the debris before burial that was also identified during later sampling periods.

Leptosphaeria maculans and *Alternaria* spp., which had the two greatest mean isolation frequencies just prior to initiation of the first test in July (Table 1), were isolated at greatly reduced frequencies following burial in July 1995. Mean isolation frequencies of *L. maculans* showed a decreasing linear trend with time, with an increase during the last 3 mo of the investigation that resulted in a quadratic response (Table 1). The mean isolation frequency of *L. maculans* was 15.4 % in September, 8.0 % in October, and decreased to 2.8 % in November 1995. As of June 1996, *L. maculans* could no longer be isolated at either test site. *Alternaria* spp., a

Table 1. Mean isolation frequencies of *Leptosphaeria maculans* and other fungi from canola debris at various sampling dates after burial at three depths and at two locations

	Sampling date													
Taxa	July	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	L[a]	Q
Leptosphaeria maculans	85.0[b]	15.4[c]	8.0	2.8	3.4	2.5	4.4	0.6	2.6	0.3	0	0	**	**
Alternaria spp.	90.0	0.5	3.0	3.6	1.8	1.8	2.6	3.4	0.9	0.9	9.3	2.0	**	NS
Total *Fusarium*[d] spp.	26.6	2.5	19.3	13.0	8.6	18.9	16.2	14.7	17.9	17.1	13.1	14.0	**	NS
F. solani	10.0	1.4	7.7	6.2	2.6	5.5	2.0	0.9	6.0	0.6	1.2	1.0	**	NS
F. sambucinum	5.0	0.7	1.3	0.9	0.2	1.9	1.2	3.1	1.8	3.6	1.7	1.5	NS	**
F. oxysporum	3.3	1.2	4.0	3.2	1.4	3.4	4.4	0	3.6	2.9	2.3	4.7	NS	**
F. equisiti	0	1.8	4.6	0.8	3.1	4.8	8.0	9.9	4.3	8.0	4.2	3.8	NS	**
Nigrospora sphaerica	1.2	0.3	2.2	2.9	1.4	3.3	2.3	0	2.0	2.7	1.4	0.3	**	**
Rhizoctonia solani AG-4	0	2.8	0.9	3.0	0.3	0	0	0	0	0	0	0	NS	NS
Pythium spp.	0	1.4	5.7	0.4	2.8	1.0	0.3	1.7	1.9	2.5	1.1	0.8	NS	NS
Trichoderma spp.	0	9.1	18.8	26.4	11.6	17.7	24.9	21.5	23.6	18.1	19.1	27.5	NS	NS
Total fungi	81.1	42.0	73.0	68.0	38.9	62.3	67.1	51.5	61.7	54.8	63.0	63.9	NS	**

[a] L refers to a linear response and Q a quadratic response of the mean isolation frequencies; ** significant linear or quadratic responses; NS non significant.

[b] Percent isolation frequencies of fungi from the preliminary assay in July 1995 from 60 canola stem pieces (four 1-cm pieces per stem) grown on V-8 medium just prior to initiation of the study.

[c] Numbers for September 1995 through July 1996 sampling dates are the mean isolation frequencies of the fungi from 480 (1-cm) pieces per sampling date over two locations.

[d] *Fusarium equisiti, F. nivale, F. oxysporum, F. sambucinum, F. solani,* and *Fusarium* spp.

common saprophyte on numerous hosts, were isolated at a level of 0.5 % in September from both locations and the mean isolation frequencies for *Alternaria* spp. ranged between 2.0 and 9.3 % for the remainder of the study. Isolation frequencies showed a linear decline with sampling date and depth for *Alternaria* spp. (Tables 1 and 2). As soil depth increased, isolation frequencies decreased (Table 2). As with *L. maculans*, mean isolation frequencies of *Alternaria* spp. decreased over time, except in June 1996 when the percentage was at 9.3 % compared to 0.9 % for May and 2.0 % for July.

In the preliminary assay, *Fusarium* spp. were isolated from 26.6 % of the 60 stem pieces (Table 1). After burial, the mean isolation frequency declined to 2.5 % in September and varied between 8.6 and 19.3 % throughout the remainder of the study. A decreasing linear trend over dates was observed for *Fusarium* spp. *Fusarium solani* (Mart.) Sacc. and *Fusarium equisiti* (Corda) Sacc. were the most commonly isolated *Fusarium* spp. during the study (Table 1). Individual species of *Fusarium,* except *F. solani,* had significant quadratic trends over the sampling dates during this investigation. In contrast, only *F. solani* showed a linear decreasing trend over the sampling dates (Table 2).

Nigrospora sphaerica, a common soil saprophyte, was isolated from debris throughout the study in the first test, but the isolation frequencies did not have a consistent trend. Mean isolation frequencies were significantly linear over sampling dates. A quadratic trend was also observed among soil depths for this fungal species with the 7.6 cm depth having the lowest percentage. Mean isolation frequency of *N. sphaerica* increased from 0.3 % in September to 2.9 % in November 1996, but remained 3.3 % or less for the remaining sampling dates (Table 1). Furthermore, isolation frequencies for *N. sphaerica* were significantly greater from site 2 than from site 1 (Table 2).

Table 2. Mean isolation frequencies of *Leptosphaeria maculans* and other fungi from canola debris buried at three soil depths at two locations

	Soil depth (cm)[a]					Location	
Taxa	0	7.6	25.4	L[b]	Q	1	2
Leptosphaeria maculans	3.6	3.4	4.0	NS	NS	3.0	4.3
Alternaria spp.	3.0	1.8	2.3	**	NS	2.1	2.6
Total *Fusarium* spp.	14.8	12.8	16.2	**	**	13.8	15.4
F. solani	3.5	3.0	3.1	NS	NS	2.3*	4.1*
F. sambucinum	2.2	1.1	1.6	**	**	1.5	1.8
F. oxysporum	3.1	2.4	3.1	NS	**	2.7*	3.0*
F. equisiti	4.1	4.1	6.2	**	**	5.2	4.5
Nigrospora sphaerica	4.7	0.9	0.9	**	**	1.4*	2.9*
Rhizoctonia solani AG-4	0.6	0.7	0.6	NS	NS	1.1	0.2
Pythium spp.	2.1	1.9	1.4	NS	NS	2.0	1.5
Trichoderma spp.	15.6	22.4	21.5	**	**	22.2	17.5
Total fungi	59.3	56.7	60.2	NS	NS	60.6	56.9

[a] Mean percent isolation frequencies of fungi isolated from 160 (1-cm) canola debris pieces per soil depth and among locations.

[b] L refers to linear response and Q a quadratic response of the mean isolation frequencies; ** significantly linear or quadratic responses; NS non significant.

During the preliminary sampling, *Trichoderma* spp. were not isolated from the canola debris (Table 1). However, *Trichoderma* spp. quickly colonized the canola stem tissues following burial in the soil and by September the mean isolation frequency was 9.1 %. Mean isolation frequencies for *Trichoderma* spp. did not show either linear or quadratic responses over sampling dates (Table 1). A greater percentage of stem pieces contained *Trichoderma* spp. from 7.6 and 25.4 cm depths than from the 0 cm soil depth, but *Trichoderma* spp. were isolated at similar percentages from both locations (Table 2). Significant linear and quadratic responses occurred over soil depths for *Trichoderma* spp. (Table 2). Over 90 % of the isolates of *Trichoderma* spp. were tentatively identified as *Trichoderma harzianum* Rifai and the remainder as *Trichoderma hamatum* (Bonord.) Bainier, but further studies are necessary to confirm their identifications.

Two major genera of soilborne pathogens were identified during this investigation including *Pythium* spp. and *Rhizoctonia* spp. *Pythium* spp. were not observed in the preliminary tissue assay, but colonized the debris over all three soil depths by October. The mean isolation frequency for *Pythium* spp. was 5.7 % in October and was never greater than 2.8 % during any of the other sampling dates (Table 1). *Rhizoctonia solani* AG-4 was not isolated from the debris before burial in July 1995 but was present at 2.8 % in September. However, the fungus could not be detected after December 1996. The pathogen survived up to 3 mo on the debris at low isolation frequencies at all three soil depths (Table 1). In December, *R. solani* AG-4 could only be isolated from stem pieces collected at the 25.4 cm depth at site 1. Mean percent isolation frequencies of *R. solani* AG-4 did not show linear or quadratic trends over the three soil depths, but the pathogen was isolated most frequently from site 1 than site 2 (Table 2).

The canola stems left on the soil surface remained intact throughout the second test (Table 3) and *L. maculans* was isolated from 26.4 % of the stem

Table 3. Isolation frequencies of *Leptosphaeria maculans* and *Trichoderma* spp. from canola debris from the 1995-1996 crop scattered over the soil surface in October 1996

	Disinfection time					
	May 1997[ψ]		Nov. 1997		Oct. 1998	
	3 min	1 min	3 min	1 min	3 min	1 min
Leptosphaeria maculans isolated	27.8 a[ξ]	25.0 a	31.9 a	30.6 a	22.2 a	19.4 a
Average total	26.4		31.3		20.8	
Pycnidia with conidia observed	45.8 a[†]	41.6 a	27.8 a	25.0 a	13.8 a	19.4 a
Average total	43.7		26.4		16.6	
Trichoderma spp. isolated	0	0	5.5 a	6.9 a	15.3 a	18.1 a
Average total	0		6.2		16.7	

[ψ] For each sampling date 144 (2-cm) pieces were assayed on D-V-8 medium for the presence of *L. maculans*; 72 pieces were surface sterilized with sodium hypochlorite for 3 min and 72 for 1 min.

[ξ] Mean percent isolation frequencies compared between surface disinfection times; numbers followed by the same letter are not significantly different ($P \leq 0.05$) according to Waller-Duncan's K-ratio T test.

[†] Mean percent isolation frequencies based on the percent of 2-cm plant tissues containing pycnidia and conidia for the anamorph of *L. maculans* on plates without visible mycelial growth present.

sections 7 mo after the debris were scattered over the soil surface. Even when *L. maculans* was not isolated, visible pycnidia and conidia were observed on an additional 43.7 % of the 2-cm tissue pieces. *Trichoderma* spp. were not identified from the tissues on this sampling date. Six months later, in November 1997, *L. maculans* was isolated from 31.2 % of the stem pieces, pycnidia with conidia were identified from 27.8 % of the pieces and *Trichoderma* spp. were isolated from 6.2 %. In October 1998, *L. maculans* was isolated from 20.8 %, pycnidia with conidia were observed on 18.0 %, and *Trichoderma* spp. were isolated from 16.7 % of the stem pieces (Table 3). There was no significant difference between the 3- and 1-min disinfection times for the isolation rates of either *L. maculans* or *Trichoderma* spp. on any of the sampling dates.

DISCUSSION

In the first test, mean isolation frequencies of *L. maculans* declined rapidly after burial and the pathogen could no longer be cultured 11 mo after the 10-cm-long stem pieces were buried in the soil. During this same period, canola stem debris decomposed considerably. There were no differences in isolation frequencies of *L. maculans* buried at different depths or at the two locations. The rapid decline in survival of *L. maculans* in small pieces of canola debris might indicate a shorter acceptable rotation time between canola crops if all canola debris was destroyed through tillage and subsequent decomposition. However, some canola fields are planted with a summer crop using no-tillage or minimum-tillage operations that leave much of the canola stubble standing. Still other fields are not disturbed until time to plant a crop the following autumn or a spring crop nearly a year later. The predetermined size of the debris and use of polypropylene bags may have influenced the relative short longevity of *L. maculans*.

The second test was initiated to determine if debris left relatively intact without soil tillage would enable *L. maculans* to survive for a longer period. In this study, stem pieces were much larger, ranged in size from 30- to 50-cm long, and were scattered over the soil surface. Total biomass of the debris was approximately two to three times greater and was slower to deteriorate than in the first test. *L. maculans* grew from 26.4 % of the pieces and pycnidia with conidia typical of the pathogen were found on another 43.7 % of the debris pieces in May 1997, 11 mo after harvest. Twenty-eight months after harvest, *L. maculans* continued to be isolated but the difference in longevity may be attributed to factors other than temperature and moisture, *e.g.*, the presence of weed hosts which may serve as an inoculum source.

The debris in the second test was used as an inoculum source in the blackleg nursery at Griffin. To enable establishment of the second test, the plant debris was removed from the soil in June, within a mo after harvest. Debris were kept under normal external environmental conditions, but away from soil contact until scattered over the soil surface in October, 4 mo later. At the first sampling date in May, the debris was intact with little observed deterioration. One reason for this may have been that the stem pieces were 30- to 50-cm long, and due to irregularities in the soil surface, only a small portion of each stem was in direct contact with the soil. In contrast, the entire length of 10-cm-long pieces used in the first test was in close contact with soil and the stems were extensively deteriorated, 11 mo after initiation of the study.

The differences in the longevity of *L. maculans* in the two tests was probably a result of differences in the extent of biological degradation of the canola debris. In the first test, the debris was handled in a manner that would simulate a typical tillage operation. The stem pieces degraded so rapidly, that after 5 mo, the surface sterilization time was reduced to prevent excessive disintegration of the sample pieces during the culturing process. The pieces were in direct contact with the soil and even those placed on the surface were soon

covered with debris or soil as a result of soil movement by wind and rain. In addition, the polypropylene mesh bags used in the first test enabled soil to collect around the enclosed debris that resulted in greater soil contact and biodegradation. In contrast, the debris in the second test ranged in size from 30 to 50 cm, which is normal for the stalks left standing after harvest and, unlike the first test, the debris was still intact 28 mo after canola harvest.

In the first test as the debris deteriorated, sterilization time using 10 % sodium hypochlorite was reduced from 3 to 1 min and this change may have influenced the recovery of *L. maculans* from the debris during the later sampling dates. Therefore, a comparison between the two sterilization times was conducted in the second test. During all three sampling dates in the second test, the isolation frequencies were similar when compared at 3 and 1 min using 10 % sodium hypochlorite.

Hershman and Perkins (1995) studied the ascospore release from debris in Kentucky and found that, while the numbers of ascospores were reduced over time, inoculum could still be detected at the time the crop would be planted three seasons later. In Georgia, if the stubble remains intact and is not buried, the pathogen could survive, at least, past the planting time three seasons later. It appears that tillage operations that reduce the size of stem pieces and bury them might hasten decomposition of debris and reduce the survival time of *L. maculans.*

Alternaria and *Fusarium* spp. present at high levels in the debris before burial, rapidly declined after burial and remained at low levels throughout the first test. *Trichoderma* spp. were not present during the July 1995 assay, but invaded the debris soon after burial. Isolation frequencies of *Trichoderma* spp. fluctuated throughout the study, but were highest on the final sampling date in both tests. Rapid establishment, growth and cellulose degradation by *Trichoderma* spp. may be partially responsible for reducing the survival of *L. maculans, Alternaria* spp., and *Fusarium* spp. over time on the canola stems. The increase in isolation frequencies of *Trichoderma* spp. corresponded with the decrease in isolation frequencies of these other fungi. Further studies are needed to determine if *Trichoderma* spp. were directly antagonistic or parasitic to *L. maculans* and other soilborne mycobiota.

Tillage soon after canola harvest may result in rapid decomposition of canola stems and this would provide the benefit of a more rapid decline in the inocula of *L. maculans.* The recommendation to bury the debris and the 4- to 5-yr rotation without canola in blackleg infested fields could be reduced to 3 yr for canola producers in Georgia.

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