Rhizoctonia Crown Rot of Canola in Indiana

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ABSTRACT

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Dead and declining canola (winter rape) plants during the winter of 1990 had a severe crown (basal stem) rot with varying degrees of cortical necrosis caused by *Rhizoctonia solani* (cardinal temperatures of 4, 16–24, and 30 C). The fungus was compatible with *Rhizoctonia* anastomosis group 2 type 1. Aminopeptidase profiles were characteristic of *R. solani* but distinct from those of several other *Rhizoctonia* anastomosis groups profiled. Crown tissues were infected by early winter, but root tissues below the crown generally were not infected until midwinter and spring. The base of leaves frequently had a dark brown to black necrotic lesion that was associated with leaf death. Early-seeded plants, which grew extensively in the fall, were more severely infected than late-seeded plants, which were smaller; however, very small plants appeared to be the most susceptible and seldom survived through the winter. Infection of crown tissues appeared to be by direct penetration near the soil surface as well as through leaf scars. A late winter (January) and early spring (March) survey of canola fields in Indiana during 1990, 1991, and 1992 showed a plant kill of 0–100%, depending on the cultivar and seeding date. The winters and early springs of 1990, 1991, and 1992 were cool with abundant moisture. Crown rot was generally more prevalent in wet and poorly drained areas of fields but also was severe on early-seeded canola on sandy soils. This disease will be a limiting factor for production of canola in Indiana and adjacent states. Seeding date, nitrogen use, and cultivar selection may be important considerations in reducing severity of this disease.

Canola (*Brassica napus* L.), a form of winter annual rape that contains less than 2% erucic acid in the oil and less than 30 ppm of glucosinolates in the meal, has been cultivated in Indiana since 1985 as a result of consumer demand for low saturated fats and as an alternate crop to wheat (2). Approximately 5,000 acres were planted in Indiana in the fall of 1989 and 7,500 acres in the fall of 1990. Excellent stands in the fall were sometimes so severely thinned during the winter that the crop was abandoned in the spring. Although growers initially referred to this loss as winterkill, symptoms in midwinter were characteristic of damage caused by a cool season pathogen. Maceration of crown tissues, small developing necrotic cankers of cortical tissues, basal necrosis of leaves, and necrotic leaf scars (Fig. 1) (6) were similar to symptoms described for Rhizoctonia crown rot on beets (14) and damping-off, seedling blight, and brown girdling root rot of canola (4,7,8). This study was initiated to establish the etiology and severity of this disease in Indiana.

MATERIALS AND METHODS

Tissues from lesions on basal stems, leaf bases, and roots were surface-disinfected in 1.0% NaOCl for 1 min, plated on potato-dextrose agar (PDA), and incubated at room temperature in the laboratory. Cardinal temperatures for growth of the fungus were determined by incubating PDA cultures at 4, 8, 16, 24, 30, and 35 C. Diameter of growth was measured daily for 5 days, at which time 69–83% of the plate had been covered with growth at 16 and 24 C. Growth at each temperature was replicated five times.

The aminopeptidase profile was prepared by growing the fungus on cellulose membranes overlaying PDA for 7 days. The mycelium was then removed from the membrane, weighed, ground in a glass mortar and pestle in 0.05 M Tris-HCl buffer at pH 8.0, and diluted with Tris buffer to 10 mg/ml. β-Naphthylamide substrates (10^{-5} M) in Tris-HCl buffer at pH 8.0 were prepared from 10^{-3} M Jackson-Burdick methanol stock solutions (Table 1), and 1.9 ml of each was dispensed into separate 10 × 100 cm Pyrex tubes. The 23 substrates, a buffer blank, and a β-naphthylamide blank were assayed by inoculating each tube with 0.1 ml of the diluted inoculum of the fungus. Tubes were assayed for peptidase activity by recording fluorescence of the inoculated substrates after 6 and 24 hr of incubation at 22–23 C in an Aminco fluorophotometer with a Corning 7-60 narrow band pass primary filter and a Wratten 2a sharp cutoff secondary filter (13,15).

Characterization of the canola pathogen was initiated by first determining the number of nuclei in vegetative hyphal cells and the nature of the septal pore apparatus by a rapid aniline blue staining technique (5). Anastomosis grouping (AG) was ascertained by pairing the canola *R. solani* Kühn isolate, which resembled AG-2 isolates culturally, with known AG tester isolates, including two AG-2-1 testers (ATCC 62805 and one obtained from B. Nelson, North Dakota State University, Fargo) and three AG-2-2 testers (AG-2-2 IIIB from D. Bell and D. Sumner, Georgia Experiment Station, Griffin, and ATCC 66153 and AG-2-2 IV from L. J. Herr). A distilled water agar plate technique, modified from Ogoshi (9), was used for anastomosis tests. Isolates were paired approximately 3 cm apart on 9-cm-diameter plastic plates each containing 7 ml of water agar. After contact and intermingling of hyphae of the paired isolates, direct microscopic observations in the plates (×63–160) were made for hyphal fusions (anastomosis) between paired isolates, using the criteria of Ogoshi (10) for anastomosis.

A general survey of canola fields in Indiana was conducted to determine the frequency and severity of this disease.

RESULTS AND DISCUSSION

A multinucleate *R. solani*-like fungus was consistently isolated from cankers on upper roots and rotted crown tissues of canola in Indiana in February and early March of 1990. *Fusarium* spp. and several other common soilborne fungi isolated from necrotic tissues later in the spring were not pathogenic on canola. Isolates of *Fusarium* spp., *Pythium ultimum* Trow, and *Olpidium* sp. isolated from canola in the Canadian studies were only weakly virulent or nonpathogenic on canola (4). Healthy plants inoculated with a PDA culture of *R. solani* isolated from canola developed typical cortical and crown rot lesions, from which the fungus was reisolated. The staining results (5) confirmed the canola pathogen in Indiana to be *R. solani* with multinucleate vegetative hyphal cells and a prominent (doliform) septal pore. The isolate anastomosed readily with both AG-2-1 tester isolates but was not observed to anastomose with any of the three AG-2-2 testers or other anastomosis groups. Therefore, on the basis of anastomosis frequency within AG-2, the canola isolate belongs in anastomosis

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group 2 (AG-2), intraspecific group 1 (ISG-1), and is assigned to AG-2-1 (9,10). The aminopeptidase profile (Fig. 2) was characteristic of *R. solani* and distinctively different from those of *R. zeae* Voorhees and *R. cerealis* Van der Hoeven.

Typical cortical and crown rot lesions developed on plants inoculated with *R. solani* AG-2-1 at 10–15 C; the fungus was reisolated from the lesions. Symptoms on inoculated plants resembled those observed in the field (Fig. 1), and *R. solani* was consistently recovered from infected tissues. Cardinal temperatures for growth were 4, 16–24, and 30 (Table 2). Sclerotial production was profuse at 24 C and much less or absent at the other temperatures.

These observations are consistent with recent observations of brown girdling root rot of canola in Canada caused by *R. solani* AG-2-1 (4,7,8). Although the Canadian studies included several *R. solani* AG-4 isolates, they were less virulent than the AG-2-1 isolates that predominated. The Canadian isolates of *R. solani* AG-2-1 grew over a wide range of temperatures (0–32 C) and the growth range optimum was 24 C (8), which is similar to the optimum and temperature range of isolates from Indiana (Table 2). The optimum temperature for growth of the pathogen was above that commonly occurring in Indiana fields when disease was severe; however, the temperature range for fungal growth was broad enough to encompass field conditions. It also is possible that virulence of the pathogen and susceptibility of the canola are enhanced at temperatures lower than optimum for growth.

Although *R. solani* AG-2 type 1 is a common pathogen on *Brassica* spp. (10), *R. solani* AG-2-1 also is reported to be highly pathogenic on cowpea and moderately virulent on snap and lima bean (11). It also has been isolated from visibly sound peanut seed, snap bean, rye, sorghum, and corn (12). The widespread distribution of this pathogen on canola in Indiana soils indicates it is probably an indigenous organism that has persisted on wild weed hosts or as an epiphyte in the traditional corn and soybean rotations for this area. Mustard and a few possible alternate weedy hosts are common during the late summer following wheat harvest until fall tillage but are very limited during the growing season. *Rhizoctonia* is reported to be seedborne (1), and AG-2-1 may have been introduced to Indiana on infested canola seed. However, this possibility appears unlikely to explain the rapid dispersion and incidence of canola crown rot observed in Indiana. It is more likely that this pathogen survives as a saprophyte on plant residues or on symptomless hosts (3,12).

Overall losses of canola in Indiana during the relatively mild, wet winters of 1990 and 1991 are estimated at 8–12% on the basis of stand reduction; 5–8% of the fields were damaged enough that the canola was abandoned and an alternate crop planted. Observations in January 1992 indicated that the disease was much more severe than in 1990 and 1991, and by late March, it was apparent that no canola would survive the optimum conditions for disease development that occurred in 1992. Early seeding in the fall or other conditions, such as high nitrogen fertility, that result in extensive fall growth appear to predispose plants

Fig. 1. Crown and cortical rot symptoms caused by *Rhizoctonia solani* AG-2-1 on canola.

Table 1. β-Naphthylamide substrates for aminopeptidase profiling of *Rhizoctonia solani* AG-2-1

No.	Substrate[a]	Abbreviation
1	Alanine	ALA
2	Arginine	ARG
3	Asparagine	ASP
4	Aspartic acid	ASPA
5	Benzoyl arginine	BARG
6	Cystine	CYS
7	Glutamic acid	GLU
8	Glycine	GLY
9	Histidine	HIS
10	Hydroxyproline	HPRO
11	Leucine	LEU
12	Isoleucine	ILEU
13	Lysine	LYS
14	Methionine	MET
15	Ornathine	ORN
16	Phenylalanine	PHE
17	Proline	PRO
18	Pyrrolidonyl	PYR
19	Serine	SER
20	Threonine	THR
21	Tryptophan	TRY
22	Tyrosine	TYR
23	Valine	VAL

[a] 5×10^{-5} M in 0.05 M Tris-HCl, pH 8.0.

Table 2. Growth rate of *Rhizoctonia solani* isolated from canola at various temperatures

Temperature (C)	Diameter[a] (mm) per day of growth			
	2	3	4	5
4	8	14	22	29
8	8	13	25	28
16	18	33	53	69
24	27	50	59	83
30	20	29	32	34
35	0	0.5	0.5	0.5

[a] Average diameter of five replicate plates per temperature.

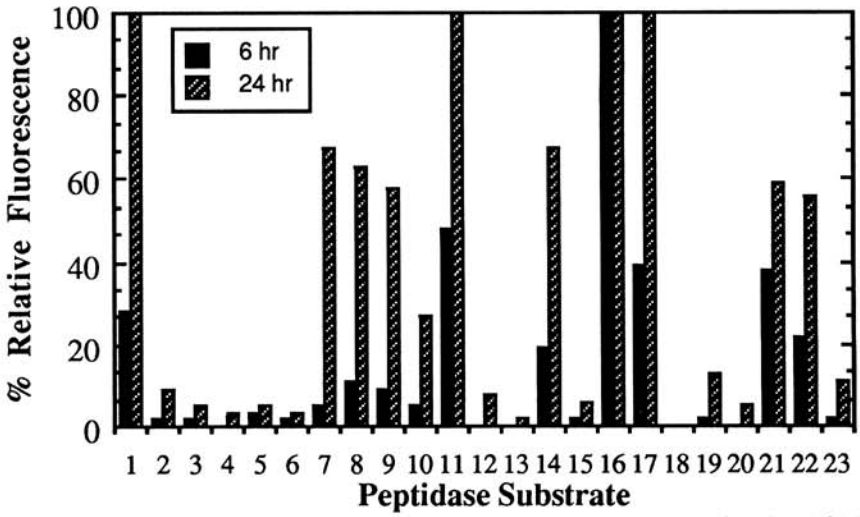

Fig. 2. Aminopeptidase profile of *Rhizoctonia solani* AG-2-1 from canola after 6 and 24 hr of incubation at room temperature. Peptidase substrates are listed in Table 1.

to this Rhizoctonia crown rot, and late-seeded plants appear to be highly susceptible. There were some indications that the form as well as the rate and time of application of nitrogen may be important; however, additional studies are needed to confirm these relationships. Poor soil drainage and extensive spring rainfall also favor the disease. Differences in varietal susceptibility were apparent in the field, and screening cultivars for resistance or susceptibility to Rhizoctonia crown rot appears warranted.

Current recommendations to reduce disease losses and maximize yield potential of canola include: 1) seeding to provide a 30-day growth period before winter "dormancy" for plant establishment, 2) planting only on well-drained fields, 3) applying rates of fertilizer similar to those for wheat and splitting the nitrogen (20% fall, 80% spring), and 4) selecting winter-hardy cultivars (2). All of these recommendations are consistent with those practices identified as providing the greatest resistance to *Rhizoctonia.*

LITERATURE CITED

1. Baker, K. F. 1947. Seed transmission of *Rhizoctonia solani* in relation to control of seedling damping-off. Phytopathology 37:912-924.
2. Christmas, E. P. 1991. Canola—an alternative crop in Indiana. Agron. Guide AY-272. Purdue Univ. Coop. Ext. Ser.
3. Daniels, J. 1963. Saprophytic and parasitic activities of some isolates of *Corticium solani.* Trans. Br. Mycol. Soc. 46:485-502.
4. Gugel, R. K., Yitbarek, S. M., Verma, P. R., Morrall, R. A. A., and Sadasivaiah, R. S. 1987. Etiology of the Rhizoctonia root rot complex of canola in the Peace River region of Alberta. Can. J. Plant Pathol. 9:119-128.
5. Herr, L. J. 1979. Practical nuclear staining procedures for *Rhizoctonia*like fungi. Phytopathology 69:958-961.
6. Huber, D. M., Herr, L. J., Christmas, E. P. and Roseman, T. S. 1991. Crown rot, a serious disease of canola in the Midwest. (Abstr.) Phytopathology 81:1186.
7. Hwang, S. F., Swanson, T. A., and Evans, I. R. 1986. Characterization of *Rhizoctonia solani* isolates from canola in west central Alberta. Plant Dis. 70:681-683.
8. Kaminski, D. A., and Verma, P. R. 1985. Cultural characteristics, virulence, and *in vitro* temperature effect on mycelial growth of *Rhizoctonia* isolates from rapeseed. Can. J. Plant Pathol. 7:256-261.
9. Ogoshi, A. 1975. Grouping of *Rhizoctonia solani* Kuhn and their perfect stages. Rev. Plant Prot. Res. 8:93-103.
10. Ogoshi, A. 1985. Anastomosis and intraspecific groups of *Rhizoctonia solani* and binucleate *Rhizoctonia.* Fitopatol. Bras. 10:371-390.
11. Sumner, D. R. 1985. Virulence of anastomosis groups of *Rhizoctonia solani* and *Rhizoctonia*-like fungi on selected germ plasm of snap bean, lima bean, and cowpea. Plant Dis. 69:25-27.
12. Sumner, D. R. 1987. Efficacy of pencyuron against isolates representing different anastomosis groups of *Rhizoctonia solani* and *Rhizoctonia*-like binucleate fungi. Plant Dis. 71:515-518.
13. Sumner, D. R., Bell, D. E., and Huber, D. M. 1979. Pathology, host range, and ecology of a sterile basidiomycete causing root disease of corn. Plant Dis. Rep. 63:981-985.
14. Walker, J. C. 1952. Diseases of Vegetable Crops. McGraw-Hill, New York.
15. Warren, H. L., Jones, A. J., and Huber, D. M. 1977. Morphological and physiological differences between *Bipolaris maydis* races O & T. Mycologia 69:773-782.