Characterization of *Rhizoctonia solani* Isolates from Canola in West Central Alberta

S. F. HWANG, Plant Pathologist, Alberta Environmental Centre, P.O. Bag 4000, Vegreville, Alberta, Canada T0B 4L0; T. A. SWANSON, Deceased; and I. R. EVANS, Plant Pathologist, Alberta Agriculture, 7000 113 Street, Edmonton, Alberta, Canada T6H 5T6

ABSTRACT

Hwang, S. F., Swanson, T. A., and Evans, I. R. 1986. Characterization of *Rhizoctonia solani* isolates from canola in west central Alberta. Plant Disease 70:681-683.

From 69 isolates of *Rhizoctonia solani* recovered from diseased plants of canola in the west central region of Alberta, two anastomosis groups, AG-2 (50 isolates) and AG-4 (10 isolates), were found on the basis of pairing with known anastomosis group tester cultures. Isolates in AG-2 were more pathogenic to *Brassica napus* than those in AG-4.

Canada is the world's leading exporter of rapeseed or canola (*Brassica napus* L. and *B. campetris* L.). The crop is a source of edible oil and seedcakes used as livestock feed. None of the presently cultivated rapeseed or canola genotypes has good resistance to *R. solani* (1,5,6,8). *R. solani* is an important soilborne plant pathogen that may induce seed rot, preemergence and postemergence damping-off, stem canker, root rot, crown and bud rot, storage rot, or aerial blight on a wide diversity of crop species (3,4). On canola, symptoms consist of preemergence and postemergence seedling blight (wirestem). Stem lesions or cankers develop at the stem base. The hard, brown, sunken lesions may girdle the stem and eventually kill the plant (13).

Parmeter et al (10) recognized four distinct anastomosis groups of *Rhizoctonia solani* Kühn, the mycelial state of *Thanatephorus cucumeris* (Frank) Donk. In 1972, Ogoshi (9) described two additional groups, AG-5 and AG-6, in Japan. Sherwood (12) reported that each group had distinctive morphological, pathological, and physiological characteristics with a few overlapping isolates. According to current concepts, each anastomosis group is regarded as an independent and genetically isolated, noninterbreeding population (2,7).

This study was initiated to group the *R. solani* isolates from diseased canola plants according to their anastomosis reactions and cultural characteristics and to investigate the virulence of the different anastomosis groups on canola.

Accepted for publication 8 January 1986.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

©1986 The American Phytopathological Society

MATERIALS AND METHODS

In the summer of 1984, isolates of *R. solani* were obtained from five fields in the extensive canola-growing region of west central Alberta (Morinville-Westlock area) from mature canola plants with typical symptoms of basal stem or foot rot. Tissues were cut into sections about 0.5 cm long, washed for 1 hr in running tap water, and blotted dry. Each section was dipped for 30 sec in a solution of 50% ethanol with 1% sodium hypochlorite, flamed, and placed on acidified potato-dextrose agar (PDA) medium (2% Difco PDA and 0.05% lactic acid). When colonies of *Rhizoctonia* species developed, small blocks of the medium were transferred from each colony to slants of laboratory-prepared PDA. Sixty-nine isolates of *R. solani* were used in this experiment.

Anastomosis was determined by the method of Parmeter and Whitney (11). Each isolate was paired with a representative of each of the four available anastomosis group tester isolates on 2% distilled water agar in 9-cm petri dishes. Isolate pairs were plated 2–4 cm apart in each dish. The dishes were incubated at room temperature for 2–5 days, depending on the growth rate of the cultures. After contact and a slight overlapping of hyphae, a portion (1–2 cm^2) in the contact area was removed and mounted on a microscope slide. The slides were stained with 0.5% aniline blue in dilute lactophenol and examined for hyphal anastomosis (14,15).

Cultural characteristics of isolates were determined on 4-wk-old cultures grown on 20 ml of PDA in 9-cm-diameter petri dishes incubated at 22 ± 2 C. All cultures were initiated from 4-mm-diameter inoculum plugs from the margins of actively expanding 3- to 4-day-old cultures also grown on PDA at 22 ± 2 C. Descriptions of cultures included color, texture, growth rate, and zonation of the mycelia and color, distribution, size, amount, and shape of the sclerotia if present (12).

The canola seeds (*B. napus*) were surface-sterilized by soaking in 0.6% sodium hypochlorite for 5 min. After they were rinsed many times in water, they were planted in vermiculite and watered with half-strength Hoagland's solution once a week in a controlled-temperature greenhouse during July and August 1985. Mycelial inoculum for direct inoculation was prepared by culturing the test isolate on PDA for 5 days. Agar plugs (4 × 4 mm) were taken from the margins of cultures grown at a room temperature of 24 C. Six-week-old canola seedlings maintained at 25 C were inoculated by gently removing the vermiculite from one side of the canola stem, placing the inoculum plug in direct contact with the base of the stem, and covering the inoculum with vermiculite. A sterile, uncolonized PDA plug placed near the seedling stem served as a control. Ten plants were used for each isolate, with equal numbers of controls. Lesions were measured 7 days after inoculation.

RESULTS

Anastomosis groups and cultural characteristics. Of the 69 isolates

cultured, no isolates of AG-1 and AG-3 were recovered in this study. AG-2T2 was the most prevalent group (28 isolates), followed by AG-2T1 (22 isolates) and AG-4 (10 isolates). Only nine of the isolates examined were not assignable to any of the four tester anastomosis groups. Because of the consistent branching and septation patterns of *R. solani*, the sources of anastomosing hyphae were easily observed. The AG-2 isolates showed greater variation in culture than the AG-4 group (Fig. 1). The mycelium of AG-2T1 was light brown to brown and showed concentric zones of appressed and aerial mycelium. Sclerotia were produced in varying sizes and numbers; they were distributed over the surfaces of the colonies in the zones that had aerial mycelia. AG-2T2 had sparse brown appressed mycelia. The AG-4 mycelium was almost white at first but later turned brownish gray. It had a leathery, appressed, or mealy texture. Very few tiny light brown sclerotia were embedded in the agar.

Pathogenicity tests. Sunken brown lesions were produced on canola stems inoculated with plugs of mycelium from PDA cultures of AG-2 and AG-4. (Figs. 2 and 3). Lengths of lesions formed by AG-2T2 and AG-2T1 isolates ranged from 44 to 4 mm and from 37 to 11 mm, respectively. No lesions, or very small lesions (8–11 mm), were produced on stems inoculated with an agar plug of mycelium of AG-4 isolates. Stems of uninoculated plants were lesion-free.

DISCUSSION

Even with the limited sample size, isolates included members of two anastomosis groups, and nine isolates did not anastomose with testers of the presently recognized anastomosis groups. Parmeter et al (10) indicated, however, that the failure to anastomose was not necessarily an indication that the isolates were unrelated. Rhizoctonia root rot of canola plants was most evident from isolates in AG-2. It is probable that the

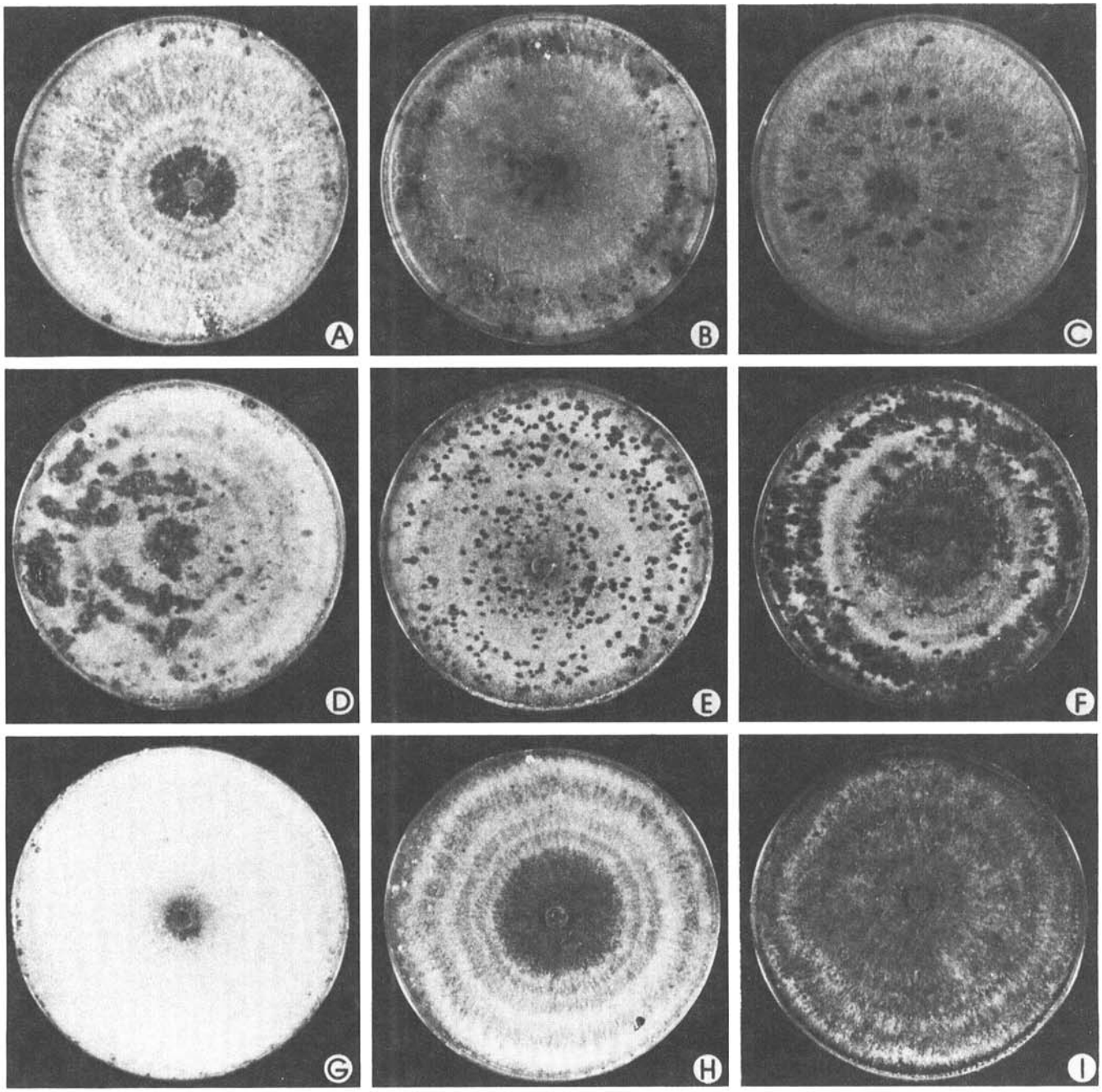


Fig. 1. Variation in the cultural characteristics of *Rhizoctonia solani* isolates of **(A–C)** AG-2T1, **(D–F)** AG-2T2, and **(G–I)** AG-4 grown for 4 wk on potato-dextrose agar.

isolates of this group are parasitically more active on canola plants than isolates of AG-4 when they are present together in a naturally infested field. The different disease syndromes on canola seedlings and adult plants may also result from different components of the pathogen population in the soil. Further research of this aspect needs to be done.

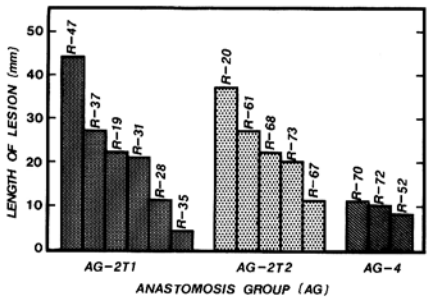
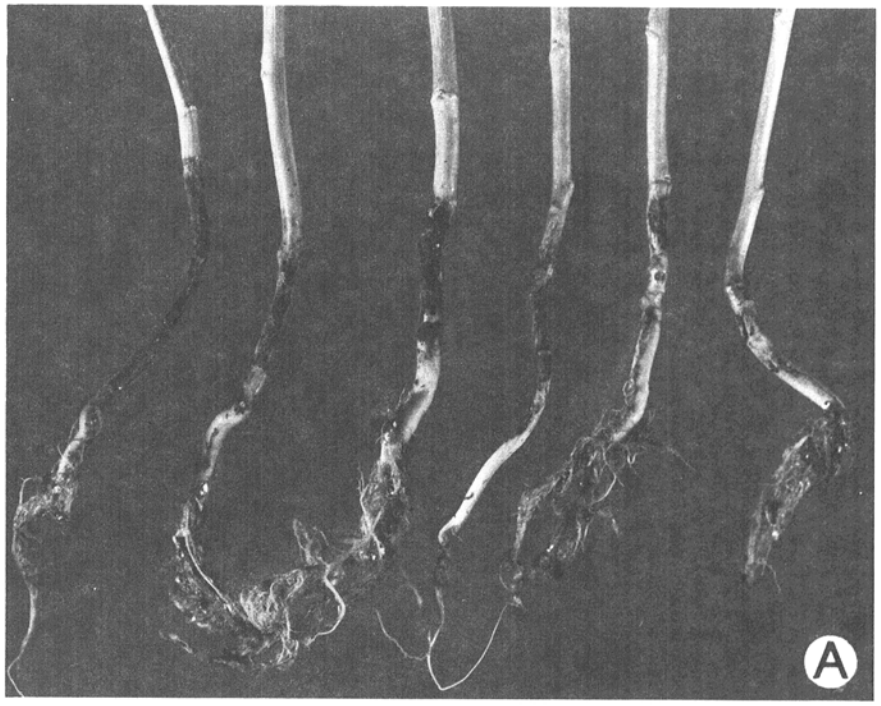


Fig. 2. Lengths of lesions on canola stems 1 wk after inoculation with *Rhizoctonia solani* isolates of AG-2T1, AG-2T2, and AG-4.



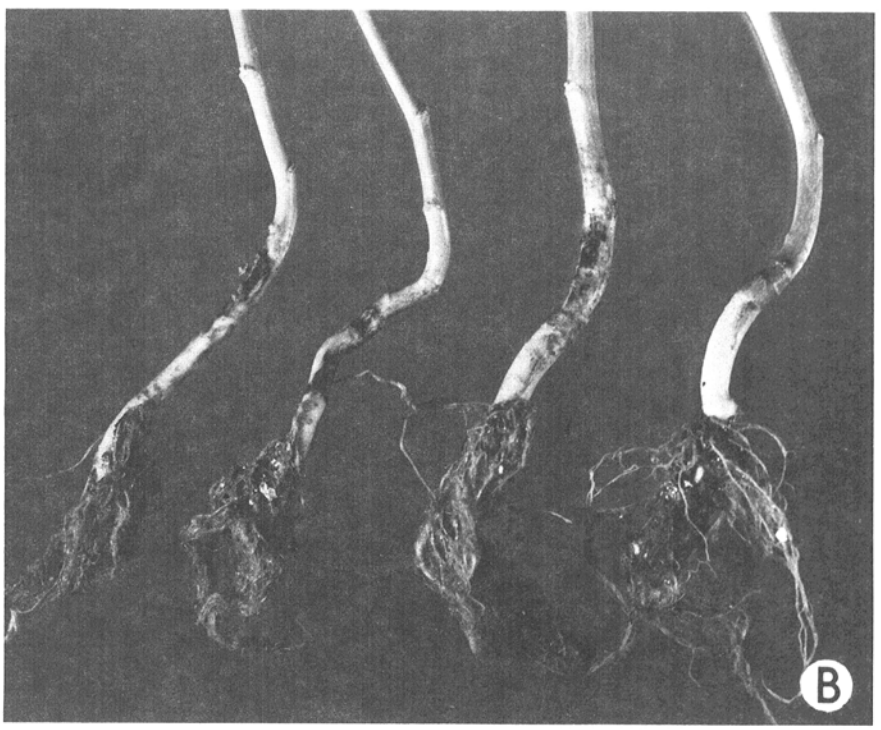


Fig. 3. Lesions on canola stems 1 wk after infection by mycelium-plug inoculum of the representative isolates of two anastomosis groups: **(A)** AG-2T1 and **(B)** AG-4.

ACKNOWLEDGMENTS

Thanks to P. R. Verma for providing the tester isolates and C. Louie and M. Herbert for their excellent technical assistance.

LITERATURE CITED

1. Acharya, S. N., Verma, P. R., Dueck, J., and Downey, R. K. 1984. Screening rapeseed/canola for resistance to damping-off and seedling root rot caused by *Rhizoctonia solani*. Can. J. Plant Pathol. 6:325-328.
2. Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. Annu. Rev. Phytopathol. 20:329-347.
3. Baker, F. K. 1970. Types of *Rhizoctonia* diseases and their occurrence. Pages 125-148 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
4. Baker, R., and Martinson, C. A. 1970. Epidemiology of diseases caused by *Rhizoctonia solani*. Pages 172-188 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
5. Berkenkamp, B., and Degenhardt, K. 1974. Diseases of rapeseed in central and northern Alberta in 1972. Can. Plant Dis. Surv. 54:35-36.
6. Berkenkamp, B., and Vaartnou, H. 1972. Fungi associated with rape root rot in Alberta. Can. J. Plant Sci. 52:973-976.
7. Bolkan, H. A., and Ribeiro, W. R. C. 1985. Anastomosis groups and pathogenicity of *Rhizoctonia solani* isolates from Brazil. Plant Dis. 69:599-601.
8. Ellis, P. J. 1983. Diseases of rapeseed in the Peace River region of Alberta. M.S. thesis. Simon Fraser University, Burnaby, BC, Canada. 89 pp.
9. Ogoshi, A. 1972. Some characters of hyphal anastomosis groups in *Rhizoctonia solani* Kühn. Annu. Phytopathol. Soc. Jpn. 38:123-129.
10. Parmeter, J. R., Jr., Sherwood, R. T., and Platt, W. D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 59:1270-1278.
11. Parmeter, J. R., Jr., and Whitney, H. S. 1970. Taxonomy and nomenclature of the imperfect state. Pages 7-19 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
12. Sherwood, R. T. 1969. Morphology and physiology in four anastomosis groups of *Thanatephorus cucumeris*. Phytopathology 59:1924-1929.
13. Sippell, D. W., Davison, J. G. N., and Sadasivaiah, R. R. 1985. *Rhizoctonia* root rot of rapeseed in the Peace River region of Alberta. Can. J. Plant Pathol. 7:184-186.
14. Tu, C. C., and Kimbrough, J. W. 1973. A rapid staining technique for *Rhizoctonia solani* and related fungi. Mycologia 65:941-944.
15. Tu, C. C., and Kimbrough, J. W. 1975. Morphology, development, and cytochemistry of the hyphae and sclerotia of species in the *Rhizoctonia* complex. Can. J. Bot. 53:2282-2296.