Characterization of Isolates of *Rhizoctonia solani* from Lima Beans Grown in New York State

Helene R. Dillard

Assistant professor, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva 14456.
I gratefully acknowledge the cooperation of E. Hamilton and R. F. Becker and of Comstock Foods. Appreciation is extended to G. C. Adams, Jr., for helpful suggestions, E. E. Butler for providing anastomosis group tester isolates of *R. solani*, and G. Wallis for technical assistance.

Accepted for publication 17 November 1986 (submitted for electronic processing).

ABSTRACT

Dillard, H. R. 1987. Characterization of isolates of *Rhizoctonia solani* from lima beans grown in New York State. Phytopathology 77:748-751.

In 1984, a pod rot was observed on lima beans grown in western New York State. Reddish brown, sunken lesions frequently developed on pods in contact with the soil surface. The seeds within the affected pods were discolored tan to reddish brown. *Rhizoctonia solani* was isolated from discolored seeds and pods. Inoculation of intact lima bean pods or detached seeds with sclerotial masses or with potato-dextrose agar disks colonized for 3 days by the isolates of *R. solani* produced lesions similar to those observed in the field. Hyphal tip cells of all isolates were multinucleate, had dolipore septa, and belonged to anastomosis groups 1 and 5. The sexual stage (*Thanatephorus cucumeris*) of anastomosis group 1 (microsclerotial-type) isolates was induced in the laboratory using a nutrient stepdown technique. In greenhouse studies, all isolates were pathogenic to lima bean hypocotyls and produced web blight symptoms at high relative humidities.

About 263 ha of small-seeded or baby lima beans (*Phaseolus lunatus* L.) were grown for processing in New York State in 1984. The crop has become a popular processing vegetable because of the high yields achieved in the western part of the state. *Rhizoctonia solani* Kühn commonly causes root and hypocotyl rot of lima beans, dry beans, and snap beans (10,26). In 1984, an unusual pod and seed rot of lima beans occurred in western New York (8). Stem lesions on mature plants were also associated with the disease. The disease was most common on the lower parts of the plant, and especially on pods in contact with the soil surface. *R. solani* was isolated consistently on artificial media from diseased tissue.

A similar disease occurred in 1904, when Hedgcock (14) reported brown, sunken lesions caused by *R. solani* on bean pods and noted that the fungus penetrated the pod lesions and colonized and discolored the seeds. Barrus (7) later observed that a large percentage of bean pods in contact with the ground became infected with *R. solani*. Subsequent reports have shown that *R. solani* and its teleomorph (*Thanatephorus cucumeris* (Frank) Donk) can cause aerial blights when weather conditions are warm and moist (1,9,11,27). Basidiospores, sclerotia, and hyphae in organic debris have been reported as possible inoculum sources for aerial blights (11,27).

The objectives of this report are to describe a pod rot of lima beans in New York State, to identify and characterize the fungus associated with the disease, and to demonstrate pathogenicity of the fungus to lima beans.

MATERIALS AND METHODS

Field samples of lima bean stems, petioles, pods, and seeds with characteristic symptoms were washed under running tap water for 1–2 hr. Small pieces of infected tissue were surface-disinfested in 0.5% NaOCl for 1 min and placed on Difco potato-dextrose agar (PDA) and Difco Bacto-Agar supplemented with streptomycin sulfate and chloramphenicol (100 μg/ml each). Cultures of 11 isolates of *R. solani* were transferred to PDA slants for 1 wk at about 25 C and later stored at 5 C. Inoculation of intact lima bean pods or detached seeds with sclerotial masses or PDA disks colonized for 3 days by the isolates of *R. solani* produced lesions similar to those observed in the field. Hyphal tips of the isolates were stained with 4',6-diamidino-2-phenylindole according to the procedure of Toda et al (24) for examination of the nuclear condition and the characteristic dolipore septum (21,22,25).

Growth rates of the isolates were determined at 19 and 26 C on PDA in 9-cm-diameter plastic petri dishes. Mycelial agar disks (4-mm diameter) from the margins of actively growing colonies were transferred to the center of PDA plates. Six replicate plates were used for each isolate and the experiment was repeated. Plates were incubated in the dark at 19 and 26 C, and colony diameter was recorded after 24 and 48 hr. Anastomosis grouping of the isolates was determined with the procedure of Parmeter et al (20). The AG testers, which were provided by E. E. Butler (Department of Plant Pathology, University of California, Davis), consisted of AG 1 (43, 245, 465), AG 2 (229), AG 2-1 (455), AG 2-2 (456, 460, 481), AG 3 (141), AG 4 (283), and AG 5 (441).

Induction of the perfect stage of the isolates was attempted with the nutrient stepdown technique of Adams et al (3). The following formulae of glucose nitrate agar (grams of $NaNO_3$ per gram of glucose per liter) were tested with all isolates: 6/15, 3/15, 0.25/15, and 0.25/20. A basidiospore suspension was obtained by inverting cultures containing hymenial layers over 5 ml of sterile water at room temperature for 12 hr to collect discharged basidiospores. The suspension, containing about 2,000 basidiospores per milliliter, was sprayed onto lima bean pods and leaves, and the inoculated tissues were then incubated in petri plates at 25 C. All isolates were tested for their ability to produce the perfect stage on intact lima bean plants. The plants (cultivar Early Thorogreen) were inoculated by placing pieces of colonized green beans on the hypocotyl of the lima bean plant. The plants were incubated in the greenhouse in a plastic chamber (25–30 C) where high relative humidity (near 100%) was maintained.

Pathogenicity of the 11 isolates of *R. solani* was evaluated on hypocotyl tissue of intact lima bean plants of Early Thorogreen. Four bean seeds were planted about 2 cm deep in 10-cm-square plastic pots filled 60% with pasteurized soil. After the seeds germinated, 150 cm^3 of soil artificially infested with one of the isolates of *R. solani* was added to the pots around the hypocotyl to a depth of about 5 cm. Infested soil was prepared by adding 150 agar plugs (4-mm diameter) from 3-day-old colonies of *R. solani* and incubating the mixture for 36 hr before using. Control plants received pasteurized soil only. Each pot with four seedlings was considered a replicate. All treatments were replicated five times, and the experiment was repeated twice. Plants were maintained in a greenhouse at 21–28 C for 3 wk, then removed from the soil,

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.

washed, and rated for disease severity on a scale where 0 = no apparent disease; 1 = one to three small, restricted lesions; 2 = 1–25%, 3 = 26–50%, and 4 = >50% discoloration and decay of hypocotyl tissue; and 5 = dead plant.

RESULTS

Disease caused by *R. solani* became apparent as the lima beans approached maturity. Symptoms on pods and stems in the field appeared as reddish brown, slightly sunken lesions. Infected seeds were discolored tan to reddish brown, without distinct lesion margins (Fig. 1). Infected pods and seeds remained firm unless secondary organisms invaded the tissue.

Eleven isolates of *R. solani* were obtained from characteristic lesions on lima bean stems, petioles, pods, and seeds (Table 1). Hyphal tip cells of all isolates were multinucleate, and characteristic dolipore septa of *R. solani* were observed. Six isolates anastomosed with Butler's 43 (AG 1, microsclerotial type) and were similar to the descriptions of Sherwood (22) for AG 1 microsclerotial isolates (Table 1). Four isolates anastomosed with Butler's 465 (AG 1), and one isolate anastomosed with Butler's 441 (AG 5).

Growth rates at 19 and 26 C varied among the 11 isolates of *R. solani* (Table 1). Because most of the AG 1 isolates reached a radial growth diameter greater than 90 mm before 48 hr at 26 C, only data recorded after 24 hr of incubation are presented in Table 1. At 19 C, the AG 1 large-sclerotial isolates generally grew faster than the AG 5 and the AG 1 microsclerotial-type isolates. At 24 C, half of the AG 1 microsclerotial-type isolates showed the highest growth rate, and the AG 5 isolate grew significantly slower than all other isolates.

The sexual stage (*T. cucumeris*) of all AG 1 microsclerotial-type isolates was induced by using the nutrient stepdown technique (Table 1). Basidiospores collected from hymenial layers formed in culture produced small, brown, restricted lesions on detached leaves and pods of Early Thorogreen. In addition, the microsclerotial-type isolates (except 322) formed hymenial layers on stems of intact lima bean plants located in a greenhouse moist chamber (25–30 C) with the relative humidity adjusted near 100%. At high relative humidity, all isolates produced characteristic web blight symptoms (27), and prominent spiderweb-like mycelium was observed on the leaves and stems (Fig. 2).

All of the 11 isolates of *R. solani* were pathogenic on hypocotyl tissue of intact lima beans (Table 1). Disease severity was greatest on plants inoculated with AG 1 large, sclerotial-type isolates and was mild on plants inoculated with AG 5 or AG 1 microsclerotial-type isolates.

DISCUSSION

Production of baby lima beans for processing is concentrated in the western part of New York State. The pod rot caused by *R. solani* is more prevalent in the Genesee River flats area, where the low-lying topography permits the silty soils and plants to remain moist well into the morning after a dew or fog. Despite the small sample size, 10 of the 11 isolates of *R. solani* obtained from this area belonged to AG 1. Of these, six were of the AG 1 microsclerotial type, which have previously been reported to cause web blights or aerial blights on many crops, including soybeans in Louisiana (5,19), lima beans and snap beans in Florida (27), and common beans in Costa Rica (9,11,12). In contrast, Galindo et al (10) examined 33 isolates of *R. solani* associated with snap bean hypocotyls and soils in New York and found only 4 that belonged to AG 1.

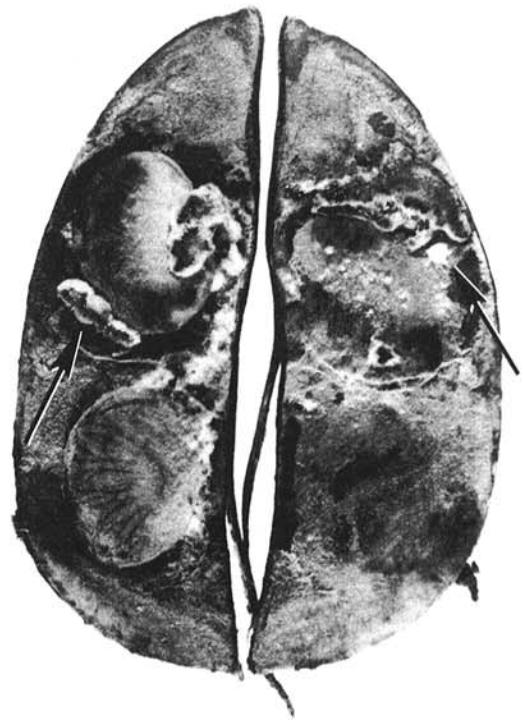

Fig. 1. Cross section of naturally infected pod and seeds with abundant mycelial growth and sclerotial production by *Rhizoctonia solani.*

TABLE 1. Characterization of isolates of *Rhizoctonia solani* recovered from lima beans

Isolate	Tissue[u] type	Disease[v] severity	AG[w]	Growth rate (mm/day)[x] 19 C	Growth rate (mm/day)[x] 26 C	Sporulation after growth on glucose nitrate agar[y] 6 g $NaNO_3$, 15 g glucose	3 g $NaNO_3$, 15 g glucose	0.25 g $NaNO_3$, 15 g glucose	0.25 g $NaNO_3$, 20 g glucose
348	Pod	3.8 a[z]	1	27 fg	38 c	−	−	−	−
325	Stem	3.7 a	1	31 bc	41 b	−	−	−	−
326	Bean	3.2 ab	1	33 a	43 a	−	−	−	−
335	Pod	3.1 bc	1	32 ab	39 c	−	−	−	−
322	Stem	2.9 bc	1 (m)	29 de	38 c	+	+	−	−
332	Pod	2.8 bc	1 (m)	28 ef	43 a	+	+	+	+
330	Pod	2.6 bcd	1 (m)	26 gh	35 d	+	+	−	−
323	Petiole	2.5 cd	1 (m)	25 h	43 a	+	+	+	+
324	Pod	2.0 de	1 (m)	28 ef	38 c	+	+	+	+
327	Bean	1.8 e	1 (m)	30 cd	43 a	+	+	−	−
349	Pod	2.1 de	5	26 gh	30 e	−	−	−	−

[u] Type of baby lima bean tissue (cultivar Early Thorogreen) from which original isolation was made.
[v] Pathogenicity to intact baby lima bean hypocotyl tissue of Early Thorogreen in greenhouse studies using artificially infested soil.
[w] Anastomosis group; 1 (m) = anastomosis group 1, microsclerotial type.
[x] Growth on potato-dextrose agar; values represent average of six replicate plates per isolate at each temperature.
[y] Sporulation on salts thiamine agar after growth on different formulations of glucose nitrate agar.
[z] Values represent disease severity ratings on scale where 0 = no apparent disease; 1 = one to three small, restricted lesions; 2 = 1–25%, 3 = 26–50%, and 4 = >50% discoloration and decay of hypocotyl tissue; and 5 = dead plant. Means in column followed by same letter do not differ significantly (Waller-Duncan's Exact Bayesian *K*-ratio LSD, $P = 0.05$).

In western New York, the disease caused by *R. solani* was most prevalent on the lower parts of the lima bean plants, and especially on pods in contact with the soil surface. The AG 1 microsclerotial types isolated in this study readily produced basidia and basidospores in culture and hymenial layers frequently formed on lima bean stems of intact plants in the presence of high humidity in greenhouse chambers. Despite ideal moist conditions in the Genesee River flats area where lima beans are grown, hymenial layers were not found on plants in the field, and leaf lesions characteristic of basidiospore infection (9,23) were not observed. Thus, sclerotia and mycelium of *R. solani* in rain-splashed and cultivator-disseminated soil are thought to be the inoculum source for infection of lima beans in New York State. These results are in agreement with Weber (27) and Galindo et al (11), who proposed that sclerotia and mycelium either free in soil or in the form of colonized debris were the main sources of inoculum in Costa Rica. Furthermore, Galindo et al (11) showed that rice husks used as mulching material served as a physical barrier that reduced splashing of soil and debris onto bean tissues and lowered web blight severity.

At high relative humidity (near 100%) in greenhouse studies, all isolates produced characteristic web blight symptoms of equal severity on leaves and stems. Differences in disease severity were evident in pathogenicity tests using infested soil to inoculate hypocotyl tissue of intact lima bean plants. Disease severity was greatest on plants growing in soil infested with AG 1 large, sclerotial-type isolates. This observation is in agreement with those of Galindo et al (10), who found that AG 1 isolates were highly virulent to both bean hypocotyls and leaves. Growth rates of the AG 1 isolates used in this study were also in good agreement with previous descriptions (6,17).

Most AG 5 isolates have been recovered from soils in Japan, where this group was defined in 1972 (15,17,18). Because of the relatively recent discovery of AG 5, there are few reports concerning the pathogenicity of the isolates in this group (4). Kuninaga et al (15) reported that 8.5% of the isolates obtained from noncultivated soils in Hokkaido were members of AG 5. In 1978, Abe and Tsuboki (2) reported that 3% of the isolates obtained from lesions on potato stems and sclerotia on potato tubers in Hokkaido were members of AG 5. Research conducted in the United States has shown the involvement of AG 5 isolates from tall and red fescue in brown patch and damping-off diseases (16). Bandy et al (6) found that 18% of the isolates of *R. solani* collected in a survey of potato fields in Maine were members of AG 5 and were capable of infecting potato stems. In contrast, Grisham and Anderson (13) isolated two nonpathogenic AG 5 cultures from carrots. In this study, the AG 5 isolate obtained from lima bean pod tissue was weakly virulent to lima bean hypocotyls, causing small restricted lesions and colonizing 1–25% of the hypocotyl tissue. Crop rotations in the lima bean growing area of western New York do not (or rarely) include crops with a previous history of disease caused by AG 5 members, such as carrots, potatoes, or turfgrass species, but normally include peas, sweet corn, field corn, and winter wheat.

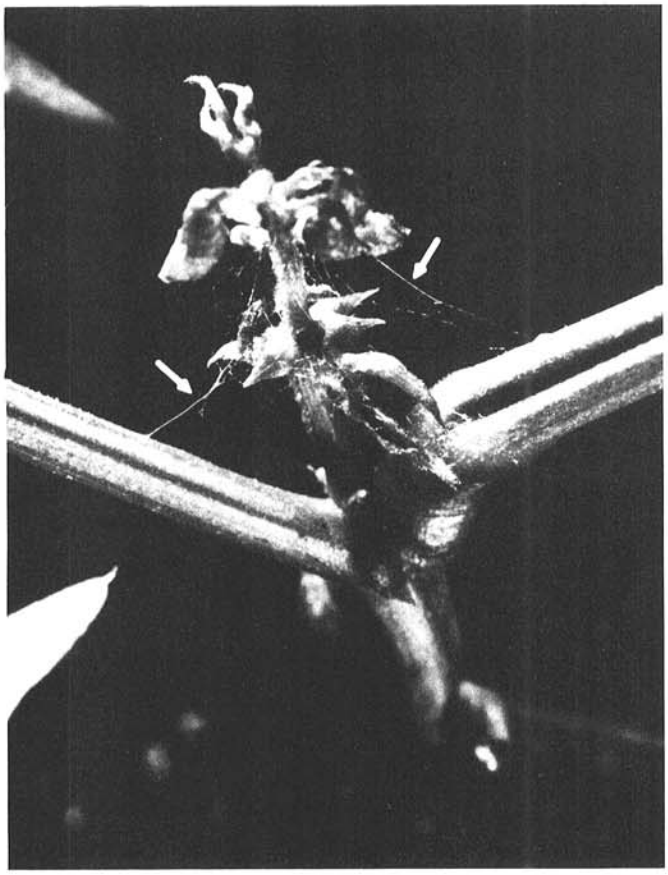

Fig. 2. Characteristic web blight signs caused by mycelium of *Rhizoctonia solani* (arrows).

LITERATURE CITED

1. Abawi, G. S., and Martin, S. B. 1985. Rhizoctonia foliar blight of cabbage in New York State. Plant Dis. 69:158-161.
2. Abe, H., and Tsuboki, K. 1978. Anastomosis groups of isolates of *Rhizoctonia solani* Kühn from potatoes. Hokkaidoritsu Nogyo Shikenjo Shuho 40:61-70.
3. Adams, G. C., Jr., and Butler, E. E. 1983. Influence of nutrition on the formation of basidia and basidiospores in *Thanatephorus cucumeris*. Phytopathology 73:147-151.
4. Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. Annu. Rev. Phytopathol. 20:329-347.
5. Atkins, J. G., Jr., and Lewis, W. D. 1954. Rhizoctonia aerial blight of soybeans in Louisiana. Phytopathology 44:215-218.
6. Bandy, B. P., Zanzinger, D. H., and Tavantzis, S. M. 1984. Isolation of anastomosis group 5 of *Rhizoctonia solani* from potato field soils in Maine. Phytopathology 74:1220-1224.
7. Barrus, M. F. 1910. Rhizoctonia stem rot of beans. Science 31:796-797.
8. Dillard, H. R., and Wallis, G. 1986. Rhizoctonia pod and bean rot of baby lima beans in New York State. (Abstr.) Phytopathology 76:651.
9. Echandi, E. 1965. Basidiospore infection by *Pellicularia filamentosa* (*Corticium microsclerotia*), the incitant of web blight of common bean. Phytopathology 55:698-699.
10. Galindo, J. J., Abawi, G. S., and Thurston, H. D. 1982. Variability among isolates of *Rhizoctonia solani* associated with snap bean hypocotyls and soils in New York. Plant Dis. 66:390-394.
11. Galindo, J. J., Abawi, G. S., Thurston, H. D., and Gálvez, G. 1983. Source of inoculum and development of bean web blight in Costa Rica. Plant Dis. 67:1016-1021.
12. Galindo, J. J., Abawi, G. S., Thurston, H. D., and Gálvez, G. 1983. Effect of mulching on web blight of beans in Costa Rica. Phytopathology 73:610-615.
13. Grisham, M. P., and Anderson, N. A. 1983. Pathogenicity and host specificity of *Rhizoctonia solani* isolated from carrots. Phytopathology 73:1564-1569.
14. Hedgcock, G. G. 1904. A note on *Rhizoctonia*. Science (n. s.) 19:268.
15. Kuninaga, S., Yokosawa, R., and Ogoshi, A. 1978. Anastomosis grouping of *Rhizoctonia solani* Kühn isolated from non-cultivated soils. Nippon Shokubutsu Byori Gakkaiho 44:591-598.
16. Martin, S. B., and Lucas, L. T. 1984. Characterization and pathogenicity of *Rhizoctonia* spp. and binucleate *Rhizoctonia*-like fungi from turfgrasses in North Carolina. Phytopathology 74:170-175.
17. Ogoshi, A. 1972. Some characters of hyphal anastomosis groups in *Rhizoctonia solani* Kühn. Nippon Shokubutsu Byori Gakkaiho 38:123-129.
18. Ogoshi, A. 1976. Studies on the grouping of *Rhizoctonia solani* Kühn with hyphal anastomosis and on the perfect stages of groups. Nogyo Gijutsu Kenkyusho Hokoku C 30:1-63.
19. O'Neill, N. R., Rush, M. C., Horn, N. L., and Carver, R. B. 1977. Aerial blight of soybeans caused by *Rhizoctonia solani*. Plant Dis. Rep. 61:713-717.
20. Parmeter, J. R., Jr., Sherwood, R. T., and Platt, W. D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 59:1270-1278.
21. 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, ed. University of California Press, Berkeley. 255 pp.

22. Sherwood, R. T. 1969. Morphology and physiology in four anastomosis groups of *Thanatephorus cucumeris*. Phytopathology 59:1924-1929.
23. Shew, H. D., and Main, C. E. 1985. Rhizoctonia leaf spot of flue-cured tobacco in North Carolina. Plant Dis. 69:901-903.
24. Toda, T., Yamamoto, M., and Yanagida, M. 1981. Sequential alterations in the nuclear chromatin region during mitosis of the fission yeast *Schizosaccharomyces pombe*: Video fluorescence microscopy of synchronously growing wild-type and cold-sensitive cdc mutants by using a DNA-binding fluorescent probe. J. Cell Sci. 52:271-287.
25. Tu, C. C., Kimbrough, J. W., and Aldrich, H. C. 1977. Cytology and ultrastructure of *Thanatephorus cucumeris* and related taxa of the *Rhizoctonia* complex. Can. J. Bot. 55:2419-2436.
26. Warren, H. L., Helfrich, R. M., and Blount, V. L. 1972. Evaluation of lima bean varieties and selections for resistance to *Rhizoctonia solani*. Plant Dis. Rep. 56:268-270.
27. Weber, G. F. 1939. Web-blight, a disease of beans caused by *Corticium microsclerotia*. Phytopathology 29:559-575.