Rhizoctonia Foliar Blight of Cabbage in New York State

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ABSTRACT

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Late in the 1983 season, small, irregular, brown to black lesions developed on cabbage heads grown in western New York. These symptoms were observed initially on cabbage cultivars Superdane and Greenwinter, which are grown primarily for fresh market. *Rhizoctonia solani* anastomosis group 1 was isolated from these lesions. Cultures of these isolates were dark brown, produced numerous small sclerotia, and had an optimum temperature of about 28 C for mycelial growth. Growth rates at 28 C averaged 2.1 mm/hr. Attempts to induce the sexual state of this fungus (*Thanatephorus cucumeris*) under laboratory conditions were unsuccessful. Inoculation of intact cabbage heads or detached leaf segments with a mycelial suspension, sclerotial masses, artificially infested soil, or 3-day-old potato-dextrose mycelial agar disks of *R. solani* produced lesions similar to those observed in the field. Similar isolates of *R. solani* were recovered from inoculated plants. Cabbage foliar blight isolates of *R. solani* were also pathogenic to snap bean hypocotyls and leaves under high moisture conditions.

New York State is a major producer of cabbage (*Brassica oleracea* var. *capitata* L.). Cabbage grown for fresh market and processing was valued at $33 million in 1978 (21). *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) is known to cause several diseases such as damping-off, wire stem, root rot, bottom rot, and head rot in cabbage and other crucifers (3,6,20,24). Generally, sclerotia and mycelial fragments of *R. solani* free in soil or associated with organic debris are considered the main source of inoculum for these diseases. Initial infection sites are reported to be root, stem, or lower leaf tissues in contact with the soil surface. In their studies on bottom rot and head rots of cabbage, Weber (25) and Wellman (27) reported the occurrence of the perfect state of the fungus on soil surfaces and on cabbage stem and leaf tissues, suggesting the possible involvement of basidiospores as a primary source of inoculum. Weber (25) failed to demonstrate infection of cabbage heads directly from basidiospore suspensions but showed that mycelial fragments obtained from basidiospore cultures were effective inoculum.

During September 1983, necrotic lesions were observed on the tops of cabbage heads in several plantings in western New York. Isolations on artificial media yielded *R. solani*. This suggested that airborne inoculum of *R. solani* was responsible for this mode of infection. The weather in central and western New York in late June to early August was unusually dry (1.3 mm of rain in July) and relatively hot. Soil temperature 10 cm deep under a sod cover averaged 20–25 C during July. Average precipitation and temperatures for this region prevailed during the rest of the growing season (Climatological Reference Station 3031840, National Weather Service, Geneva, NY). These conditions may have played a major role in the incidence of this foliar blight.

R. solani and its teleomorph are known to cause severe aerial blight diseases under warm, moist conditions on a variety of crop plants including bean (*Phaseolus vulgaris* L.), sugar beet (*Beta vulgaris* L.), soybean (*Glycine max* (L.) Merr.), cotton (*Gossypium hirsutum* L.), tobacco (*Nicotiana tabacum* L.), and many others (3,7,10,12,14,16,23). Basidiospores, sclerotia, and/or hyphae in organic debris have been reported as the possible inoculum sources for the diseases.

The objectives of this report are to report and describe a foliar blight of cabbage in New York State, to identify and characterize the pathogen, to demonstrate pathogenicity of the fungus to attached and detached cabbage leaves, and to compare its pathogenicity to an anastomosis group (AG) 2 isolate of *R. solani* on bean leaf and hypocotyl tissues.

MATERIALS AND METHODS

Isolation and characterization of the pathogen (*R. solani*). Cabbage leaf segments with the characteristic brown to black necrotic lesions were first washed under running tap water for 15–60 min, then half of the segments were surface-sterilized for 1–2 min in 0.5% NaOCl solution. Washed and surface-sterilized pieces (2–5 mm^2) of cabbage leaf tissues with lesions were placed on acidified potato-dextrose agar (APDA), water agar with 100 μg/ml each of streptomycin sulfate and chloramphenicol, or a modified Ko and Hora medium (15). After 1–4 days of incubation at laboratory temperature (20–25 C), hyphal-tip transfers were made to APDA plates from colonies showing characteristics of *R. solani*. Stock cultures were maintained by periodic transfer to APDA slants for 1 wk at about 25 C. Cultures were stored at 5 C. All isolates were examined for the characteristic dolipore septum and the multinucleate condition of the hyphal-tip cells using 0.05% trypan blue in lactophenol according to the procedure of Burpee et al (4).

Anastomosis grouping of the cabbage-*Rhizoctonia* isolates was determined according to the procedure of Parmeter et al (18). The AG testers were provided by E. E. Butler (Department of Plant Pathology, University of California, Davis) and L. J. Herr (Department of Plant Pathology, Ohio Agricultural and Development Center, Wooster) and

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included isolates of the four common groups (AG 1, AG 2, AG 3, and AG 4) and AG 5. Growth rate of *R. solani* from cabbage was determined as colony diameter or colony dry weight on APDA or potato-dextrose broth (PDB) (Difco Laboratories, Detroit, MI) plates, respectively. Mycelial agar disks (6 mm in diameter) were transferred from the margin of a 2-day-old colony of *R. solani* to the center of APDA or PDB plates (each with 15 ml of medium). Five plates of each medium were incubated in the dark at each of the desired temperatures. Colony diameter was recorded daily, whereas colony weight was determined after 48 hr. Mycelial mats were each placed in an aluminum tray of known weight, dried overnight at 80 C, and weighed.

Pathogenicity of cabbage-*Rhizoctonia* isolates to cabbage and beans. Inoculum of *R. solani* used in pathogenicity tests consisted of sclerotial masses, hyphal fragment suspension, or infested pasteurized soil. Sclerotial masses were obtained from 2-wk-old APDA cultures. The hyphal fragment suspension was obtained by blending 2- to 7-day-old mycelial mats from APDA or PDB plates in sterile distilled water for 30–60 sec. The soil inoculum of *R. solani* was prepared according to the procedure of Ko and Hora (11) and used at a rate of 2% (inoculum source:soil mixture, v/v). Cabbage leaf segments were washed in running tap water, surface-sterilized for 5 min in 0.5% NaOCl, and placed on wire mesh resting about 2–3 cm above the bottom of a covered plastic box (27 × 20 × 11 cm). High relative humidity was maintained by adding water and lining the sides of the box with paper towels. Inoculated and check leaf segments were sprayed with sterile distilled water as needed to maintain a film of water on the leaf surface. Whole cabbage heads were inoculated with a hyphal suspension (6-day-old mat blended with 30 ml of water, 5 ml/head) or *R. solani*-infested soil (20 cm^3/head), placed in fiberglass lugs,

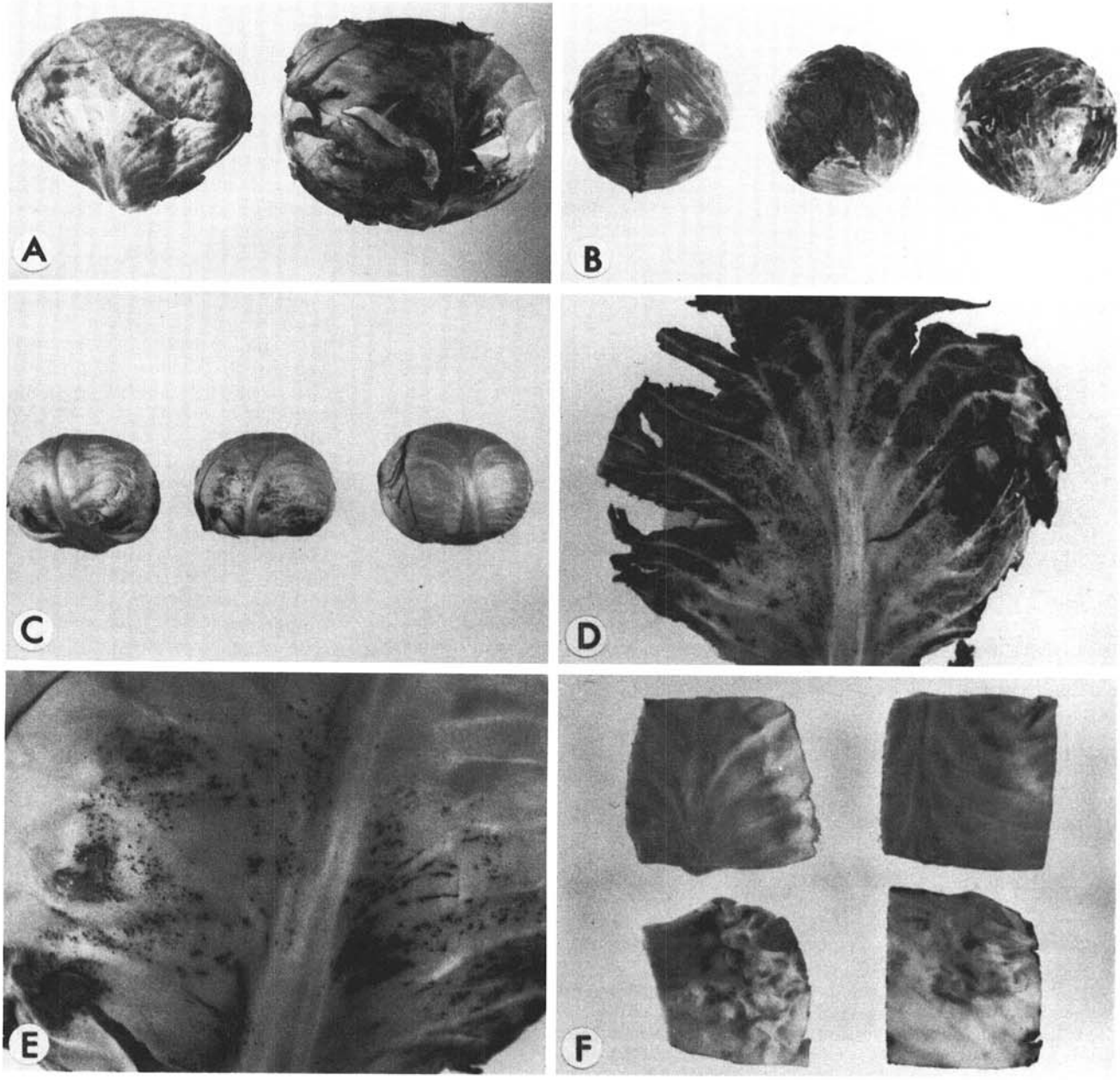

Fig. 1. Symptomatology of foliar blight of cabbage caused by *Rhizoctonia solani*: **(A)** Naturally infected heads of cabbage after several weeks of storage; **(B)** (left to right) uninoculated (water check) or artificially inoculated heads using the soil-potato or hyphal fragment suspension sources of inocula; **(C)** symptoms observed on cabbage heads after the outer eight to 10 leaves were removed from heads (left) artificially inoculated with hyphal fragment suspension, (center) inoculated with soil-potato source, and (right) not inoculated; **(D)** close-up of inoculated outer leaf showing typical small lesions and decay; **(E)** close-up illustrating shape, size, and distribution of lesions; and **(F)** detached leaf segments (beginning clockwise in upper left corner) that were not inoculated (lack of lesions) and inoculated with hyphal fragment suspension, mycelial agar disk, or sclerotial mass.

enclosed in large plastic bags, and incubated for 7–10 days.

Pathogenicity of the cabbage-*R. solani* isolate was also evaluated on hypocotyl and leaf tissue of intact plants of the snap bean cultivar Bush Blue Lake 47 and compared with a bean isolate of *R. solani* (R-103). Five bean seeds were planted about 2 cm deep in 10-cm clay pots 60% filled with pasteurized soil. One week later, *Rhizoctonia*-infested soil was added to the pots around the bean hypocotyl to a depth of about 5 cm. Check plants received pasteurized soil only. Each pot with five seedlings was considered a replicate. All treatments were replicated eight times. Plants were maintained in a greenhouse at 21–28 C for 3 wk, then they were dug carefully, washed, and rated for disease severity on a scale of 0 (no apparent disease) to 6 (most severe disease, dead plant). Plants were dried for 48 hr at 95 C and weighed. Pathogenicity to bean leaves was determined by placing 6-mm mycelial agar disks, drops of hyphal suspension, or *R. solani*-infested soil on leaves of 3- to 5-wk-old plants growing in clay pots. The plants and pots were enclosed in plastic bags, placed on clay saucers for watering, and maintained in the greenhouse for 1 wk. Severity of infection was recorded on a scale of 0 (no visible symptoms) to 6 (complete infection of leaf surface).

RESULTS

Symptomatology of the disease. Initial symptoms of Rhizoctonia foliar blight were small (1 mm long), irregularly shaped lesions (Fig. 1D–F) that initially were light brown and elongated or circular. Lesions sometimes coalesced, forming short streaks (Fig. 1E). With time, the lesions became slightly larger and dark brown to black. These lesions remained firm and restricted, especially in the field. After storage in cold-temperature houses, however, soft rot often occurred on the outer leaves as a result of bacterial activity (Fig. 1A). In 1983, foliar blight lesions were observed at an unusually high frequency on heads of cabbage cultivars Superdane and Greenwinter. Symptoms intensified after a few weeks of storage (Fig. 1A). Inoculation of intact cabbage heads or leaf segments with an isolate of *Rhizoctonia* recovered from a foliar blight lesion caused symptoms similar to those observed on naturally infected cabbage heads in the field or storage houses. Similar symptoms were obtained when the source of inoculum was a mycelial fragment suspension, sclerotial masses, or *Rhizoctonia*-infested soil. Discrete lesions and soft rot of marginal tissues of outer leaves occurred on inoculated plants (Fig. 1B,D). Discrete lesions and dry rot developed on inside leaves (Fig. 1C,E) and leaf segments (Fig. 1F). When large masses of inoculum (clumps of infested soil, hyphal masses) were placed on the cabbage tissues, however, rather large, expanding lesions developed (Fig. 1B,E).

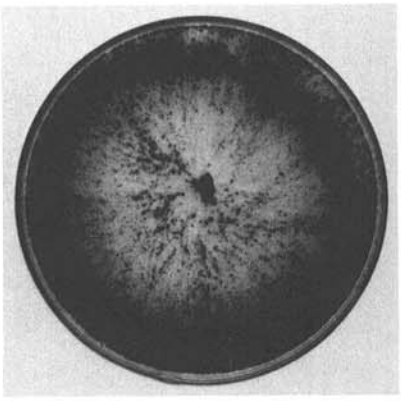

Fig. 2. Gross morphology of a 7-day-old culture of *Rhizoctonia solani* (R-169) growing on acidified potato-dextrose agar plate.

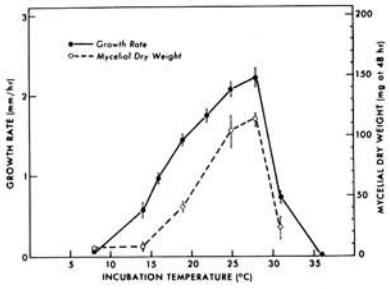

Fig. 3. Growth rate of a foliar blight of cabbage isolate of *Rhizoctonia solani* (R-169) as determined by colony diameter or dry mycelial weight.

Isolation and characterization of the pathogen. The modified Ko and Hora medium and the water agar with antibiotics medium were equally effective in isolating *Rhizoctonia* from typical lesions without interference from contaminating organisms. All isolates obtained from naturally infected cabbage heads were similar and had the characteristic mycelium of *Rhizoctonia* spp. (5). The cabbage foliar blight isolates had a dark brown mycelium and produced an abundance of individual as well as masses of sclerotia (Fig. 2). They had a rapid growth rate as evidenced by colony diameter and weight (Fig. 3). Hyphal-tip cells of these isolates are multinucleate and had the characteristic dolipore septa, indicating that these isolates belong to *R. solani*. Results of pairing tests between cabbage foliar blight isolates and known AG testers clearly indicated affinity of these isolates with AG 1. Attempts to produce the perfect state of this fungus using the procedure of Adams and Butler (1) were not successful.

Pathogenicity of cabbage-*Rhizoctonia* isolates to cabbage and beans. The foliar blight isolates infected detached leaf segments as well as whole heads of cabbage, producing symptoms similar to those induced by natural infections (Fig. 1, Table 1). Attempts to reisolate the fungus from artificially inoculated cabbage segments were always successful. Mycelial agar disks, hyphal fragment suspensions, sclerotial masses, and artificially infested pasteurized soil were all effective as inocula in causing disease on detached leaf segments, but the severity ratings differed. In one test, the incidences of disease were 100, 93, 100, and 50%, respectively, when mycelial agar disks, hyphal fragment suspensions, infested soil, or sclerotial masses were used as inoculum sources. Results of one test are summarized in Table 1. Disease severity was greatest (rating 3.7) with the infested soil inoculum used in another test. All uninoculated segments remained free of lesions and rot. Likewise, inoculation of whole cabbage heads with a mycelial fragment suspension or infested soil (Fig. 1B) resulted in an equal amount of typical disease. Both inoculum sources resulted in 100% infection and a severity rating of 4 on the outermost layers. Lesions typical of Rhizoctonia foliar blight were observed on as many as

Table 1. Comparative virulence of inoculum sources of *Rhizoctonia solani* isolate R-169 on detached cabbage leaf segments

Inoculum form[v]	No. of leaf segments inoculated/no. infected[w]	Severity of infection[x]
None[y]	0/12	0.0 a[z]
Hyphal fragment suspension	12/12	1.3 b
Sclerotia	12/12	2.3 c
Mycelial agar disks	12/12	3.5 d

[v] Hyphal fragment suspension was prepared by blending 48-hr-old mycelial mats growing on potato-dextrose broth at 25 C in 30 ml of water in a Waring Blendor. Six-milliliter mycelial agar disks were obtained from 48-hr-old cultures growing on potato-dextrose agar (PDA) at 25 C. Sclerotial masses were obtained from a 2-wk-old culture of PDA.

[w] Segments about 5 cm^2 were cut from inner leaves of healthy cabbage heads of cultivar Greenwinter and placed in moist chamber boxes lined with damp paper towels.

[x] Disease severity was recorded on a scale of 0–4, where 0 = no apparent symptoms, 1 = 1–10, 2 = 11–25, 3 = 26–50, and 4 = more than 50 lesions per leaf segment.

[y] Sprayed with sterile distilled water.

[z] Means within a column followed by the same letter are not significantly ($P = 0.05$) different according to the Waller-Duncan multiple range test.

10–12 consecutive inside leaves in two tests (Fig. 1C). Uninoculated heads remained free of *Rhizoctonia* infection and an isolate morphologically similar to that used in the tests was reisolated from infected tissues of plants in both tests.

Pathogenicity of a cabbage foliar blight isolate (R-169) to snap bean was compared with a bean isolate of *R. solani* (R-103) belonging to AG 2 type 2. The cabbage isolate was highly virulent on bean tissues under the test conditions (Table 2). The two isolates were similar in their virulence to beans, although the bean isolate appeared slightly more virulent on hypocotyl tissues, and the cabbage isolate was more virulent on the leaf tissues in one test.

Table 2. Pathogenicity of a cabbage foliar blight (R-169) and a bean hypocotyl rot (R-103) isolate of *Rhizoctonia solani* to snap bean cultivar Bush Blue Lake 47 under greenhouse conditions

	Hypocotyl tissues		Leaf tissues		
			Disease severity[w]		
			Test 1		
Isolate	Disease severity[w]	Dry wt (g/pot)	A[x]	B	Test 2[y]
None	0.0 a[z]	1.5 a	0.0 a	0.0 a	0.5 a
R-169	3.9 b	1.1 b	1.0 b	6.0 b	5.3 b
R-103	4.5 b	0.7 c	0.0 a	4.5 c	5.8 b

[w] Disease severity was recorded on a scale of 0–6, where 0 = no apparent disease, 1 = 1–3 restricted lesions, and 2 = 1–10, 3 = 11–25, 4 = 26–50, 5 = 51–75, and 6 = more than 75% of hypocotyl or leaf tissues colonized.

[x] A = infested soil inoculum at 2% strength and B = infested soil inoculum at 100% strength.

[y] Inoculum consisted of a 6-mm mycelial agar disk from a 2-day-old potato-dextrose agar culture of *R. solani*.

[z] Means within a column followed by the same letter are not significantly ($P = 0.05$) different according to the Waller-Duncan multiple range test.

DISCUSSION

All isolates of *R. solani* obtained from cabbage heads with foliar blight symptoms belonged to AG 1. Members of AG 1 of *R. solani* are known to cause foliar blight diseases in many crops (2,10,12–14,16,17,22). Galindo et al (9) reported the occurrence of AG 1 isolates of *R. solani* associated with snap bean hypocotyls and soils in New York. They also showed that these isolates were pathogenic to bean leaves under moist conditions. Cabbage is often planted as a rotation crop with bean in New York.

The natural primary source of inoculum of Rhizoctonia foliar blight of cabbage in New York is not known. The initiation of *Rhizoctonia* lesions on top of cabbage heads, which was observed for the first time in New York State, however, suggests that the inoculum is airborne. In laboratory and greenhouse tests, infested soil, mycelial agar disks, and sclerotial masses were the most effective sources of inocula. The perfect state of *R. solani* (*T. cucumeris*) was not observed on infected cabbage tissues or on the soils, and attempts to induce it on agar media were unsuccessful. Weber (25) and Wellman (27) reported the occurrence of the perfect state on cabbage tissues at later stages of disease development but failed to reproduce the disease symptoms directly by basidiospore inoculum. Basidiospores of *T. cucumeris* have been reported to function as a primary source of inoculum for inciting aerial blight diseases on a variety of crops (8,14,22,23). Sclerotia and mycelium, either free in soil or associated with organic debris, have also been suggested as sources of inoculum for foliar diseases incited by *R. solani* (10,16,26). Sclerotia and hyphal fragments in soil can become airborne by wind or rain-splashed soil. The latter could be the inoculum source functioning in New York. Rhizoctonia foliar blight of cabbage might have occurred often in New York previously but was possibly and incorrectly recognized as black speck (19), a nonparasitic disease of cabbage with symptoms somewhat similar to the initial symptoms of Rhizoctonia foliar blight.

Rhizoctonia foliar blight was observed on the cultivars Superdane and Greenwinter. It is not known if there is real variability in the reactions of cabbage cultivars to *R. solani* isolates of AG 1. It is possible that these storage cabbage cultivars were in the proper growth stage when the inoculum was plentiful in 1983. Symptoms of Rhizoctonia foliar blight were first observed in the field in early September 1983. Subsequently, infected heads were detected at different time intervals in storage houses. Likewise, it is not known if infection detected after storage was initiated in the field or whether it was the result of inoculum present in storage areas. Further research on the ecology of the pathogen and epidemiology of Rhizoctonia foliar blight of cabbage is needed.

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