CHARACTERIZATION OF *Thanatephorus cucumeris* ISOLATES CAUSING WEB BLIGHT OF BEANS IN COSTA RICA[1] /

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Resumen

Setenta y un aislamientos de Thanatephorus cucumeris *fueron obtenidos del tejido de hojas de frijol infectadas en el campo y recolectadas en diferentes áreas de cultivo de Costa Rica, localizadas entre 50 y 1 200 m sobre el nivel del mar. Otros dos aislamientos fueron obtenidos a partir de lesiones sobre las hojas de las malezas* Sida acutifolia *y* Rhotboelia exaltata en un campo de *frijol cerca de Esparza. Con base en las características del micelio, a la condición multinuclear y a la estructura del septo (tipo doliporo), todos los aislamientos correspondieron típicamente a* Rhizoctonia solani, *el estado imperfecto de* T. cucumeris. *La rata de crecimiento linear, determinado sobre agar papa – dextrosa (PDA) a 25°C varió mucho entre los aislamientos, siendo dicha variación entre 10 y 29 mm en 24 horas. Todos los aislamientos produjeron esclerocios de color pardo a pardo oscuro, variando en diámetro desde 0.5 a 9.0 mm.*

Todos los aislamientos fueron patógenos hacia el tejido de hojas e hipocotilos de frijol (cultivar Mexico 27), siendo sus virulencias significativamente variables. Hubo una correlación positiva entre la rata de crecimiento de un aislamiento y su virulencia a hojas e hipocotilos de frijol. De los 73 aislamientos probados, 26 y 38 pertenecen a los grupos de anastomosis (AG) 1 y 2, respectivamente. Los nueve aislamientos restantes no hicieron anastomosis con ninguno de los cuatro AG de ensayos usados.

Introduction

In the humid lowlands of Latin America and the Caribbean, web blight (WB) is one of the most destructive diseases of beans (*Phaseolus vulgaris* L.) (3, 5, 6, 8, 10, 11, 19, 25). It causes a very rapid defoliation and sometimes even complete crop failure during the rainy season in the lowland tropics (8, 10, 25). Weber (24) was the first to demonstrate that WB of beans in Florida is caused by *Rhizoctonia solani* Kühn and its perfect state, *Thanatephorus cucumeris* (Frank) Donk. Both the perfect and imperfect states of this fungus often have been observed and reported occurring on beans in Costa Rica (5, 6, 7, 8, 11) and other bean growing areas in the tropics (3, 10, 19, 24, 25).

In nature, *T. cucumeris* exists in the form of many strains that differ in cultural appearance and pathogenicity. Such variability has been demonstrated to occur among isolates obtained from within one field or from different bean fields (9). It has been shown that *T. cucumeris* generally consists of 4 anastomosis groups (AG) that are characterized by distinctive morphological, physiological, and pathogenic traits, although overlapping may occur among the groups (1, 16, 20).

1 Received for publication on May 11, 1982.
Part of a Ph. D. dissertation presented by the senior author to the Graduate School of Cornell University.
We thank personnel of the Ministry of Agriculture and the University of Costa Rica for their help and use of their facilities.

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The WB isolates of *T. cucumeris* have a faster growth rate, produce larger number of sclerotia, and are more sensitive to CO_2 than the subterranean isolates of this fungus (4). Nevertheless, the aerial isolates were found capable of causing damping-off and both hypocotyl and root rot of beans. In contrast, the subterranean bean isolates of *T. cucumeris* with a fast growth rate were able to cause aerial infection under conditions of high humidity and temperature (9). Similar results have been reported with *T. cucumeris* isolates from cowpea (17) and soybean (23).

The objectives of this study were to characterize and to determine the range of variability among isolates of *T. cucumeris* causing WB of beans in Costa Rica.

Materials and methods

Isolations and identification of *T. cucumeris* from naturally infected leaf tissues. Leaf samples were collected from bean fields in the Central Plateau and the eastern and western regions of Costa Rica during the rainy season (August-December) of 1979. Small leaf pieces were obtained from the margin of expanding lesions, surface sterilized for 1-2 min in 0.05% NaOCl solution, and then plated on potato-dextrose agar (PDA) or the selective medium of Ko and Hora (14). After 24-48 hr of incubation at 24°C, hyphal tip transfers were made to 0.5% yeast-PDA (Y–PDA) slants from colonies showing the mycelial characteristics of the imperfect state, *R. solani* (2). Stock cultures were maintained by periodic transfer to Y–PDA slants at 20°C. All isolates were examined for the presence of the characteristic dolipore septal apparatus and the multinucleate condition of the hyphal tip cells (12, 13). The isolates were grown on 2% water agar plates at 25°C until the colony had almost covered the surface of the agar plates. Several drops of a wetting solution (1 ml each of Tween 20 and 85% lactic acid/L distilled water) were placed about 2-cm proximal to the advancing hyphal tips and spread thoroughly with a bent spatula. The wetted areas were then stained with a drop of 0.05% trypan blue in lactophenol. Pieces of agar (1 x 1 cm) containing the stained hyphae were thinly cut, placed on a slide, covered with a slip, and examined microscopically after 5-10 min.

Cultural variability and pathogenicity of WB isolates of *T. cucumeris*. Growth rate, sclerotial formation, zonation, and color of the isolates were determined as described previously (9). Four to 6 plates were used for each isolate per determination and were incubated at 25°C. Sclerotial formation and color of the colonies were identified according to Butler and Bracker (2) and Kuppers (15), respectively.

The bean cultivar Mexico 27 was used in the pathogenicity tests. Nontreated seeds were surface disinfested for 5 min in 0.25% NaOCl and rinsed several times in sterile distilled water. Pasteurized greenhouse-prepared soil mix (PS) (equal parts of top soil and sand) was used in this study. Test plants were watered daily and maintained in a greenhouse at 20°-24°C and relative humidity that varied between 60% -90%. Inoculum preparation, methods of inoculation, and incubation periods were essentially as reported earlier (9).

To test the pathogenicity of the isolates on hypocotyl tissues, 5 seeds were planted 2-cm deep in 20-cm clay pots, three-fifths filled with PS. Six to 8 days after planting, the plants were thinned to 3/pot and a layer about 4-cm thick of *T. cucumeris*-infested soil (14) (10%, v/v of inoculum source soil/PS) was added to the pots around the bean hypocotyls. Check plants were covered with PS that was treated identically as the *T. cucumeris*-infested soil. All treatments were replicated 5 times. After 14 days, plants were removed from the soil and their roots were washed and rated for disease severity. A rating of 0 to 5 was used with 0 indicating no apparent disease, whereas 5 referred to most severe disease (dead plants).

The pathogenicity of isolates of *T. cucumeris* was compared using the detached leaf method (9). Deteached leaves of 20-day-old plants were placed on a wire mesh about 3 cm above the bottom of plastic moist-chamber boxes (10x20x30 cm). High humidity was maintained by addition of water to the bottom of the boxes and by lining the sides with filter paper that extended to the water. The leaves were also sprayed thoroughly with sterile distilled water using an atomizer. The check treatments received noninfested PDA discs. Six leaves were used per box per isolate. Disease severity was recorded after 5 days' incubation at 25°C using a scale of 0 (no apparent lesion development) to 5 (100% of leaf surface is affected).

Anastomosis grouping. The 4 AG testers used in this study were obtained from Dr. E. E. Butler, Departament of Plant Pathology, University of California, Davis, CA 95616. Stock cultures of these isolates were maintained by periodical transfers to Y-PDA slants. Anastomosis between isolates was determined by using the method of Parmeter *et al.* (18). The anastomosis reactions recognized in this study (perfect fusion, imperfect fusion, and contact

fusion) were based on the definitions and descriptions previously reported in the literature (18). Isolates which did not anastomose with any set of the testers were paired in all possible combinations with other isolates. Pairings among isolates were made at least 2 different times with each of the testers.

Results

Identity of the WB isolates. The majority of the 81 isolates examined were recovered from lesions that appeared to be initiated either by sclerotial or mycelial form of inoculum (24). However, 3 isolates (R-70, R-72, and R-76) were obtained from lesions characteristic of basidiospore infection (7). All of the isolates possessed the mycelial characteristics of *R. solani* (imperfect state of *T. cucumeris*) as described in the literature (2). Eight of the isolates were discarded as they failed to grow after several transfers to either PDA or Y-PDA slants. The remaining 73 isolates exhibited the presence of a dolipore septal apparatus and the cells of their young hyphal tips were multinucleate (12).

Cultural variability among the collected isolates *of T. cucumeris*. Growth Rate. Significant differences in growth rate were found among the 73 WB isolates of *T. cucumeris* obtained from several bean growing regions of Costa Rica (Table 1). Average colony linear growth of these isolates ranged from 10 to 29 mm per 24-hr incubation on PDA at 25°C. Generally, isolates of the fungus collected from the bean growing regions at elevations between 200 and 750 m (Esparza, 200 m; San Isidro, 650 m; and Turrúcares, 700 m) appear to have the highest growth rate. In contrast, isolates collected from bean fields at elevations of 50 m (Upala) and above 750 m (Central Plateau region) exhibited a relatively slower growth rate on PDA.

Zonation. Zonate formation is due to the periodical changes in mycelial growth and density

Table 1. Anastomosis groups, growth rate, and virulence to beans of web blight isolates of *Thanatephorus cucumeris* in Costa Rica.

Isolate no.[s]	Locality and altitude (m)	Growth rate (cm/24 hr)[t]	Virulence (0-5)[uv]		Anastomosis group[y]
			Hypocotyl[w]	Leaf[x]	
R–37	Turrúcares; 700	5.76	4.14	5.00	1–P
R–66	San Isidro; 650	5.72	4.14	5.00	1–P
R–49	Pasoagres; 200	5.70	3.60	5.00	1–P
R–53	Turrubares; 500	5.38	3.40	5.00	1–P
R–67	San Isidro; 650	5.36	4.14	4.83	1–P
R–25	Turrúcares; 700	5.20	3.67	5.00	1–I
R–80	Esparza; 200	5.18	4.07	5.00	1–I
R–55	San Mateo; 400	5.16	3.87	5.00	1–P
R–68	San Isidro; 650	4.98	4.27	4.83	1–P
R–75	Esparza; 200	4.94	4.07	5.00	1–P
R–26	Turrúcares; 700	4.86	4.07	5.00	1–P
R–14	Turrúcares; 750	4.74	3.80	5.00	1–I
R–21	Turrúcares; 700	4.64	2.95	4.00	1–I
R–44	Turrúcares; 750	4.36	3.80	5.00	1–I
R–73	Esparza; 200	4.36	3.87	4.83	1–P
R–76	Esparza; 200	4.24	3.40	5.00	1–P
R–69	Esparza; 200	4.22	3.47	5.00	1–P
R–74	Esparza; 200	4.22	3.54	5.00	1–P
R–72	Esparza; 200	4.18	3.80	4.83	1–P
R–24	Turrúcares; 700	4.08	3.14	5.00	1–P
R–65	San Isidro; 650	3.78	3.87	4.33	1–I
R–45	Turrúcares; 700	3.52	3.07	4.00	1–P
R–70	Esparza; 200	3.38	3.47	4.67	1–P
R–52	Orotina; 500	3.28	2.67	3.67	2–P
R–78	Esparza; 200	3.28	2.73	3.67	1–P
R–63	Fabio Baudrit; 850	3.02	3.87	3.83	2–P
R–71	Esparza; 200	3.02	2.47	3.33	1–P
R–60	San José; 1 000	2.90	2.67	3.50	1–C
R–4	Palmares; 900	2.76	1.27	3.66	2–P

Table 1 (Cont.)

R–23	Dulce nombre; 850	2.74	2.67	3.83	–[z]
R–51	Riogrande; 800	2.72	2.07	3.50	2–I
R–56	Dulcenombre; 850	2.70	3.20	4.00	1–P
R–11	Palmares; 1 100	2.64	1.20	3.33	2–I
R–2	Palmares; 950	2.58	2.14	3.33	2–I
R–34	Upala; 50	2.58	2.20	3.33	2–I
R–62	Fabio Baudrit; 850	2.58	2.07	3.33	2–P
R–7	Naranjo; 1 000	2.56	1.47	3.00	2–C
R–64	Fabio Baudrit; 850	2.56	2.40	3.33	2–C
R–29	Upala; 50	2.52	1.73	3.00	2–C
R–53	Villa Bonita; 900	2.52	1.20	3.00	–
R–61	Fabio Baudrit; 850	2.52	2.47	3.33	2–C
R–22	Dulcenombre; 850	2.48	1.00	3.00	2–I
R–59	San José; 1 000	2.48	2.07	2.83	–
R–20	Santa Eulalia; 900	2.44	2.20	2.50	2–C
R–8	Palmares; 900	2.42	1.47	3.00	–
R–19	Santa Eulalia; 900	2.42	2.14	2.50	–
R–10	Palmares; 900	2.40	1.00	3.00	2–C
R–28	Upala; 50	2.34	1.00	2.33	2–P
R–57	San José; 1 000	2.34	2.60	3.50	2–P
R–58	San José; 1 000	2.34	1.74	2.67	2–C
R–17	Santa Eulalia; 900	2.32	1.00	1.83	2–C
R–27	Dulcenombre; 850	2.32	1.20	2.50	2–C
R–46	Monserrat; 900	2.32	1.20	2.83	2–P
R–54	Santa Eulalia; 900	2.30	2.60	2.33	2–C
R–32	Upala; 50	2.30	2.00	2.16	2–P
R–3	Palmares; 1 000	2.22	0.74	2.33	–
R–6	Palmares; 1 100	2.18	0.74	1.67	2–C
R–15	Palmares; 900	2.16	1.20	1.83	–
R–30	Upala; 50	2.16	1.00	2.00	–
R–9	Palmares; 900	2.12	0.54	2.00	2–C
R–12	Palmares; 900	2.12	1.00	1.33	2–C
R–40	Ciruelas; 800	2.12	0.60	1.83	–
R–41	Ciruelas; 800	2.08	0.60	2.00	2–I
R–33	Upala; 50	2.08	1.47	1.16	2–I
R–36	Upala; 50	2.08	1.26	1.00	2–P
R–42	Villa Bonita; 900	2.08	0.54	1.83	2–C
R–31	Upala; 50	2.06	1.26	2.33	2–C
R–47	Dulcenombre; 850	2.06	1.00	1.83	2–C
R–50	Estanquillo; 1 200	2.06	0.60	1.00	2–I
R–1	Palmares; 900	2.04	1.60	1.66	2–P
R–16	San Ramón; 1 100	2.04	1.20	1.50	2–C
R–39	Ciruelas; 800	2.04	0.47	1.33	2–P
R–48	Dulcenombre; 850	2.04	1.00	1.33	2–I
	LSD: 0.05	0.019	0.030	0.021	

[s] Refers to locality or regions in Costa Rica where the isolates were collected.

[t-u] Each number is an average of 6 and 5 replicates, respectively.

[v] Disease severity rating was recorded using a scale of 0 to 5, with 0 referring to no apparent disease symptoms and 5 indicating 100% of inoculated tissues were infected.

[w-x] Disease rating was determined 14 days and 5 days after inoculation, respectively.

[y] Anastomosis grouping was according to the designation given by Parmeter *et al.* (18). P, I, and C refer to perfect, imperfect, and contact fusion, respectively.

[z] These isolates failed to anastomose with any of the tester isolates or any of the other isolates used in this study

resulting in the production of a sparse and a dense aerial mycelial mat regions (21). All the isolates examined in this study failed to exhibit zonation. However, concentric rings composed of the aggregation of sclerotia were observed in cultures of isolates R-6, R-7, R-9, R-31, R-32, R-33, R-41, R-57, R-58, R-63, and R-69 that were incubated in the dark, but none was found under artificial illumination.

Sclerotial Formation. All the isolates of *T. cucumeris* included in this study produced dark brown sclerotia. However, these sclerotia varied in size and distribution on the surface of the agar plates. Small sclerotia (2-4 mm diam) that were uniformly distributed on the surface of the colony were produced by 47 isolates which exhibited low to intermediate growth rate (Figure 1c). Within this group, 2 isolates (R-11 and R-15) showed aggregates of sclerotia in the center of the plate, whereas 10 others produced aggregates of sclerotia in the form of concentric rings. The latter were produced when the isolates were incubated in darkness but not under continuous artificial illumination. Isolates of *T. cucumeris* with fast growth rates produced sclerotia that were evenly distributed on the agar plates and were either few and large (7-9 mm diam; isolates R-24, R-26, R-37, R-66, R-67, R-68, and R-69) (Figure 1a) or numerous and very small (0.5-1.0 mm diam; R-49, R-71, R-72, R-73, R-74, R-75, and R-76) (Figure 1b).

Color of Vegetative Hyphae. The color of the mycelium of all isolates studied was typically brown. The brown color of most of these isolates is characterized as N90 A60 M60 and N99 A50 M50 for the mycelium and sclerotia, respectively (15). Few isolates had a mycelial and sclerotial color of dark brown which was designated as N99 A50 M50. The latter isolates had a fast growth rate and produced small sclerotia (0.5-1.0 mm diam).

Virulence on hypocotyls and leaves. Significant differences were found in the relative virulence of the 73 isolates of *T. cucumeris* to bean hypocotyls and leaves (Table 1). Virulence of the isolates was found to be related to their relative growth rates. The isolates with the highest growth rate were the most virulent, whereas those with the slowest growth rate exhibited the least virulence.

The highly virulent isolates of *T. cucumeris* caused a rapid and severe necrosis on leaves and branches of bean seedlings. Lesions first became visible after 36 hr of incubation and the foliage of inoculated plants was completely affected within 5 days. Small sclerotia (0.5-1.0 mm diam) were produced on infected tissues 5 days after inoculations. The least virulent isolates incited restricted necrotic lesions that did not coalesce nor result in infection of

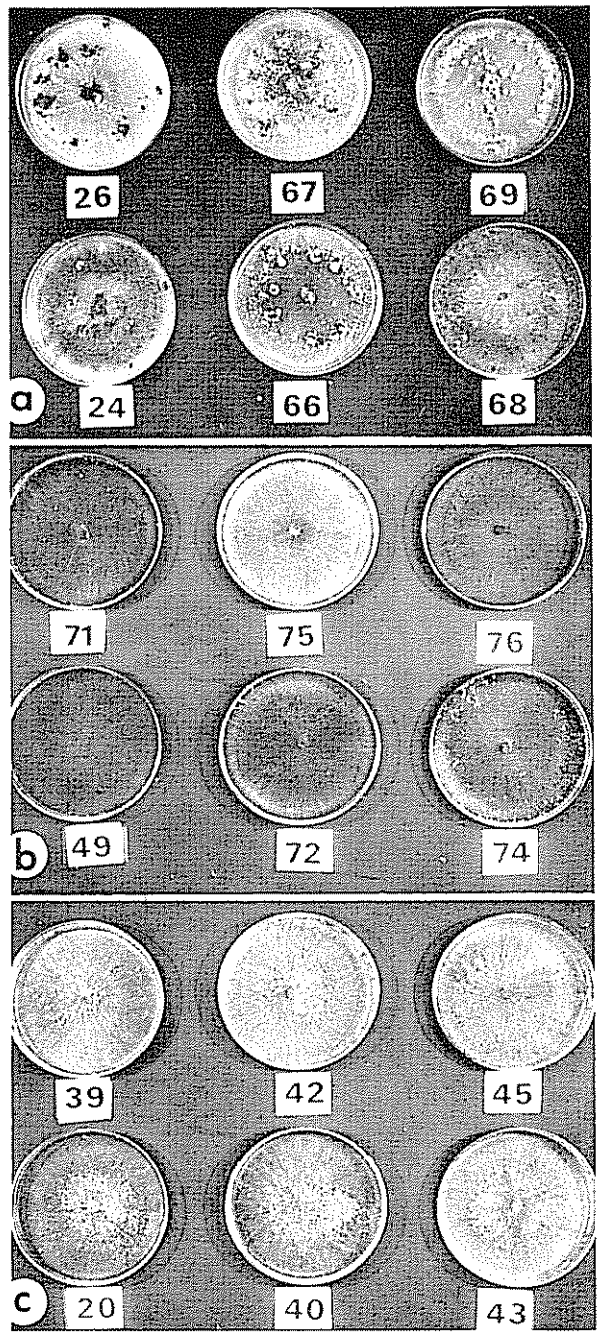

Fig. 1. Representative cultures of *Thanatephorus cucumeris* isolates causing web blight of beans in Costa Rica after 14 days' growth on potato-dextrose agar at 25°C with continuous light. a-b) Large (7-9 mm diam) and small (0.5-1.0 mm diam) sclerotial isolates of Anastomosis Group (AG) 1, respectively. c) Isolates of AG-2 showing sclerotia (2.0-4.0 mm diam) that often aggregate toward the center of the culture.

branches. Sclerotia were not visible around such restricted lesions over the duration of the test.

Sunken lesions were produced by the highly virulent isolates on hypocotyl tissues. Lesions were dark brown and sometimes coalesced resulting in girdling of the stem tissues at the soil line or slightly above. The soil surface of the pots infested with isolates of *T. cucumeris* often were covered by numerous small sclerotia (0.5-1.0 mm diam) which first became evident 3 days after inoculations. Brick-red sunken lesions were produced on hypocotyl tissues by the least virulent isolates. The most virulent isolates generally were collected from the bean growing regions of eastern, central, or western parts of Costa Rica with an altitude between 200 and 700 m (San Isidro de El General, Turrúcares, and Esparza).

Anastomosis grouping. Sixty-four out of the 73 WB isolates of *T. cucumeris* examined were assignable to the anastomosis groups designated by Parmeter *et al.* (18). Of these, 26 and 38 were found to belong to AG-1 and AG-2, respectively. None of the isolates were members of AG-3 or AG-4 (Table 1). Nine isolates failed to anastomose with the tester strains, among themselves, or with any of the web blight isolates belonging to AG-1 or AG-2. The 3 types of the anastomosis reaction observed in this study were similar to those reported in the literature (18). The "contact fusion" reaction was observed with 18 of the isolates in AG-2, the "imperfect reaction," with 7 isolates of AG-1 and 9 in AG-2, whereas the "perfect fusion" was observed with 19 isolates of AG-1 and 11 isolates in AG-2.

Isolates within AG-1 varied in color of mycelium and in size and number of sclerotia. Several isolates including R-66 exhibited a light brown and sparse mycelium with few large (6-9 mm diam) sclerotia that were dark brown in color. Other isolates including R-74 (Figure 1b) had an appressed and coarse mycelium with numerous small (0.5-1.0 mm diam) sclerotia. Sclerotia were imbedded in the agar, uniformly distributed on agar surface, and along with the mycelium were dark brown in color. Isolates of both groups had a fast growth rate and were highly virulent on hypocotyls and leaves.

Isolates within AG-2 showed only minor variation in color of mycelium and in size and number of sclerotia as compared to the isolates in AG-1 (Figure 1c). The mycelium was appressed to moderately aerial and brown in color. Sclerotia were 2-4 mm diam, often aggregated into compound sclerotia, and had a darker brown color than the mycelium. Isolates in AG-2 were characterized by slow to intermediate growth rates as well as low to moderate in virulence to bean hypocotyls and leaves.

Discussion

Only *T. cucumeris* was isolated from naturally infected bean leaves in Costa Rica exhibiting typical symptoms of WB. Identification of the collected isolates was based on the mycelial characteristics of the imperfect state, *R. solani* (2), possession of a prominent dolipore septal apparatus, and the multinucleate condition of the young hyphal tip cells. The last 2 features provide reliable diagnostic criteria in distinguishing *T. cucumeris* and its imperfect state from many Rhizoctonia-like fungi, which are similar in cultural appearance and mycelial morphology (12, 13). The binucleate Rhizoctonia-like fungi have been reported as causal agents of foliar blights on grasses and sugarbeets (13).

Echandi (5, 6, 7, 8) and González (11) previously had reported on the occurrence of *T. cucumeris* and the severity of WB of beans in Costa Rica. Parmeter *et al.* (18) were successful in producing the perfect state under laboratory conditions using isolates of the fungus previously recovered from naturally infected bean leaves collected in Costa Rica. The basidial state of the fungus also has been reported occurring on beans (7) and tobacco (22) under field conditions in Costa Rica. The basidial state was also observed during the present study on bean tissues in fields in Esparza, San Isidro, and several areas in the Central Plateau. In this study, considerable cultural variability was demonstrated to exist among the WB isolates of *T. cucumeris* in Costa Rica. Cultural and morphological variability has been reported in the literature among isolates of *R. solani* that are associated with WB diseases on different crops and from different geographical regions (5, 23). Variability in cultural appearance also has been reported in the literature among the subterranean isolates of *R. solani* (9).

Rapid growing mycelium is characteristic of *T. cucumeris* isolates. Among the different habitats in which the fungus is found in nature (aerial, surface or subterranean), the aerial forms have been reported as having the fastest growth rates (4). However, several isolates of this fungus obtained from infected bean hypocotyls and roots previously were shown to have a comparable growth rate to the aerial WB isolates (9). The WB isolates examined in this study varied significantly in their growth rates which were also closely associated with their virulence to beans. The fastest growing isolates were distinguishable from the others by cultural appearance, higher virulence on

bean hypocotyls and leaves, and they were all in group AG-1.

All isolates of *T. cucumeris* obtained from WB lesions on beans were pathogenic to both hypocotyl and leaf tissues, although they varied considerably in their virulence (Table 1). Pathogenicity of the aerial isolates of *R. solani* to roots and hypocotyl tissues also have been reported on cowpeas (17), soybeans (23), and beans (9). In addition, *R. solani* isolates recovered from infected roots and hypocotyls have been shown to be pathogenic to the foliage of beans (9) and cowpeas (17). These results suggest that both foliar and root isolates of *T. cucumeris* have the capacity to infect the above-ground or subterranean bean tissues depending on the conditions prevailing in their habitats.

The sclerotia produced by the isolates of *T. cucumeris* in culture differed markedly from those formed on infected bean tissues. Sclerotia in culture were variable in shape and size (3-7 mm diam) and sometimes formed aggregates (1-2 cm diam), whereas those produced on infected tissue appeared round and very small (0.5-1.0 mm diam). This behavior also has been observed with *R. solani* isolates obtained from WB lesions on beans in Florida (24), but not from soybeans (23).

The majority of the WB isolates of *T. cucumeris* studied in this investigation were assignable to anastomosis groups AG-1 or AG-2. The characteristics of these isolates were in agreement with those given by Sherwood (20). Isolates of *T. cucumeris* in AG-1 and AG-2 have been previously isolated from naturally infected bean leaves in Costa Rica (18). Nine isolates obtained in this study failed to anastomose with any other isolates tested. The latter suggests that either anastomosis is rare among these isolates or that additional AG exist in nature as suggested by Parmeter *et al.* (18). Recently, field isolates from Japan have been placed in a new AG (1, 16).

The *T. cucumeris* isolates collected from the diverse geographical regions where beans are grown in Costa Rica varied greatly in cultural characteristics and virulence to bean tissues. Generally, the isolates obtained from Esparza, San Isidro de El General, and several from Turrúcares were highly virulent to beans. In contrast, isolates from Upala, San Ramón, Palmares, and some from Turrúcares were only weak to moderately virulent. Esparza, San Isidro, and Turrúcares are at altitudes of approximately 208, 650, and 700 m, respectively. WB is endemic in these areas and often occurs in epidemic proportion as a result of favorable environmental conditions for disease incidence and development during the rainy season. In addition, the basidial state often has been observed occurring on beans and other crops in these areas. Thus, there are more opportunities for continued variability of the fungus and the prevelance of more variants than in areas where disease incidence and severity are lower, such as Upala (elevation 50 m) and Estanquillo (elevation 1200 m).

Summary

Seventy-one isolates of *Thanatephorus cucumeris* were obtained from naturally infected bean leaf tissues collected from several bean growing areas of Costa Rica that ranged in elevation from 50 to 1200 m. Two other isolates were obtained from lesions on leaves of the weed species *Sida acutifolia* and *Rhotboelia exaltata* in a bean field near Esparza. Based on mycelial characteristics, multinucleate condition, and presence of the dolipore-type septal structures, all isolates were typically *R. solani* which is the imperfect state of *T. cucumeris*. Linear growth rate, as determined on PDA at 25°C, varied greatly among these isolates and ranged from 10 to 29 mm in 24 hr. All isolates produced brown to dark brown sclerotia which varied in size from 0.5 to 9.0 mm diam. All isolates were pathogenic to bean leaf and hypocotyl tissues (cultivar Mexico 27), but their virulence varied significantly. There was a positive correlation between growth rate of an isolate and its virulence to bean leaves and hypocotyls. Of the 73 isolates tested, 26 and 38 isolates belonged to anastomosis groups (AG) 1 and 2, respectively. The remaining 9 isolates failed to anastomose with any of the 4 AG testers used.

Literature cited

1. ADAMS, G. C., Jr., and BUTLER, E. Serological relationships among anastomosis groups of *Rhizoctonia solani*. Phytopathology 69:629-633. 1979.

2. BUTLER, E. E., and BRACKER, C. Morphology and cytology of *Rhizoctonia solani*. Pages 32-44 in: J. R. Parmeter, Jr., ed. *Rhizoctonia solani*, Biology and Pathology. University California Press, Berkeley. 255 pp. 1970.

3. CIAT. Bean Production Program. Annual Reports. Centro Internacional de Agricultura Tropical, Cali, Colombia. 1973-1979.

4. DURBIN, R. D. Factors affecting the vertical distribution of *Rhizoctonia solani* with reference to CO_2 concentration. American Journal Botany 46:22-25. 1959.

5. ECHANDI, E. La chasparria de frijol (*Phaseolus vulgaris* L.) "Web blight" provocada por *Pellicularia filamentosa* (Pat.) Rogers (sinónimo *Corticium microsclerotia* (Matz Weber). Pp. 463 **In:** 5a, Reunión Latinoamericana de Fitotecnia, Buenos Aires, Argentina, 5-18 de Noviembre 1961. Actas, Buenos Aires, Inst. Tecnl. Agropec. (INTA), Vol. 2. 1962.

6. ECHANDI, E. Enfermedades del frijol (*Phaseolus vulgaris* L.) observadas en Nicaragua, El Salvador, Guatemala y Honduras en la segunda siembra del año 1964. Pp. 104-106 **In:** 11a Reunión Anual Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos Alimenticios. Panamá, 11-17 de marzo 1965. Informes, Guatemala, Librería Indígena. 1965.

7. ECHANDI, E. Basidiospore infection by *Pellicularia filamentosa* (= *Corticium microsclerotia*), the incitant of web blight of common bean. Phytopathology 55:698-699. 1965.

8. ECHANDI, E. Principales enfermedades del frijol observadas en diferentes zonas ecológicas de Costa Rica. Turrialba 16:359-363. 1966.

9. GALINDO, J. J., ABAWI, G. S., and THURSTON, H. D. Variability among isolates of *Rhizoctonia solani* associated with bean roots and soils in New York. Plant Dis. 66: (Accepted). 1982.

10. GALVEZ, G. E., GUZMAN, P., and CASTAÑO, M. Web blight. Pages 101-110 **in:** H. F. Schwartz and G. E. Gálvez, eds. Bean Production Problems: Disease, Insect, Soil and Climatic Constraints of *Phaseolus vulgaris*. Centro Internacional de Agricultura Tropical, Cali, Colombia. 1979. p. 424.

11. GONZALEZ, L. C. Informe de la evaluación de enfermedades hecha en los ensayos del plan nacional de frijoles. Pp. 9-11 **In:** F. Hernández, ed. Plan Nacional de Frijoles de Costa Rica en 1969. 15a Reunión del Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos Alimenticios San Salvador, C.A. 1969. Vol. 1. 1969.

12. HERR, L. J. Practical nuclear staining procedures for Rhizoctonia-like fungi. Phytopathology 69:958-961. 1979.

13. HERR, L. J., and ROBERTS, D. L. Characterization of *Rhizoctonia* populations obtained from sugarbeet fields with differing soil textures. Phytopathology 70:476-480. 1980.

14. KO, W., and HORA, F. K. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopatholoy 61:707-710. 1971.

15. KUPPERS, H. Atlas de Colores. Edit. Blume, Barcelona. 161 p. 1979.

16. OGOSHI, A., and UI, T. Specificity in vitamin requirement among anastomosis groups of *Rhizoctonia solani* Küehn. Ann. Phytopathol. Soc. Japan 45:47-53. 1979.

17. ONESIROSAN, P. T. Comparison of *Rhizoctonia solani* isolates from web blight and basal canker of cowpea and from soil. Plant and Soil 46:135-143. 1977.

18. PARMETER, J. R., Jr., SHERWOOD, R. T., and PLATT, W. D. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 59:1270-1278. 1969.

19. SAÑUDO, S. B., and BENAVIDES, J. E. Presencia de la mustia hilachosa del frijol en el Departamento de Nariño. Ascolfi informa (Colombia). Vol. 2. pp. 2-3. 1976.

20. SHERWOOD, R. T. Morphology and physiology in four anastomosis groups of *Thanatephorus cucumeris*. Phytopathology 59:1924-1929. 1969.

21. UI, T. Zonation in cultures of *Rhizoctonia solani* Kühn under continuous darkness. Trans. Mycology Society Japan 14:179-184. 1973.

22. VARGAS, E. Infección por basidiosporas de *Thanatephorus cucumeris*, causante de una enfermedad foliar en tabaco. Turrialba 23:357-359. 1975.

23. VERMA, H. S., and THAPLIYAL, P. N. Rhizoctonia aerial blight of soybean. Indian Phytopathology 29:389-391. 1976.

24. WEBER, G. F. Web-blight, a disease of beans caused by *Corticium microsclerotia*. Phytopathology 29:559-575. 1939.

25. ZAUMEYER, W. J., and THOMAS, H. R. A monographic study of bean diseases and methods for their control. USDA Agriculture Technical Bulletin 868. pp. 63-65. 1957.

Reseña de libros

PAUL, E.A. y J.N. LADD (eds.). Soil biochemistry. Vol. 5. M. Dekker Inc., New York 1981, 480 p.

Este es el primer volumen donde el Prof. McLaren, iniciador de la serie no figura entre los editores. Su muerte dejó un apreciable vacío entre los que practican la bioquímica de suelos. El volumen consiste de diez capítulos independientes que reflejan áreas con investigación activa en los últimos años y no tratados en volúmenes anteriores.

El primer capítulo se refiere a la química y las transformaciones de los compuestos naturales resistentes a una descomposición rápida. Este capítulo discute las evidencias de la existencia de compuestos orgánicos resistentes en suelos. Se discute en un segundo subcapítulo la formación y química de estos compuestos y se termina con conclusiones y una bibliografía de casi 300 referencias.

En el segundo capítulo se estudia el análisis "in situ" de los componentes de origen biológico del suelo. Hace tiempo se estima deseable realizar estudios con materiales no extraídos, sin que hasta el presente haya sido posible este tipo de estudios. El reciente desarrollo en microscopia electrónica y otras técnicas instrumentales hacen ahora posible estudios sin extracción, indicando el lugar preciso de la materia orgánica en la estructura del suelo. Se discute en el capítulo la localización e identificación, además de la cuantificación de estos derivados.

La combinación oxidativa de compuestos aromáticos por enzimas de microorganismos de suelos es el tópico del tercer capítulo. Se discute el tópico en cuatro subdivisiones dedicadas a las enzimas que promueven la combinación oxidativa que producidas por microorganismos del suelo y aparte del suelo, la diferenciación de las enzimas de combinación oxidativa producto de las reacciones de combinación oxidativa y finalmente los significativos biológicos y ecológicos de las reacciones de combinación oxidativa.

En el cuarto capítulo se estudia el control de las transformaciones de urea en suelos. Se discute aquí las propiedades de la ureasa en suelos, los efectos de los inhibidores de ureasa y los efectos de los inhibidores de la nitrificación sobre las transformaciones de urea en el suelo. Por ser la urea una forma de abono nitrogenado muy importante, es crítico el conocer sobre sus transformaciones en suelos; aquí se presenta un resumen tanto sobre los aspectos teóricos como los prácticos de sus cambios de forma.

En el quinto capítulo se presenta la información sobre la química y la distribución de los amino azúcares en suelos y organismos de suelos; se resume la información sobre la química de los amino azúcares, su distribución en organismos vivos, su determinación y las cantidades y formas de amino azúcares, su distribución en organismos vivos, su determinación y las cantidades y formas de amino azúcares en suelos. Se presenta el conocimiento actual sobre la transformación de estos compuestos en suelos.

El sexto capítulo se dedica al tópico de los derivados de petróleo en suelos. Se analiza su bioquímica, ecología y microbiología. Este capítulo, el segundo más largo del volumen, reune información de una serie de ciencias. Se discuten las sustancias de importancia en este campo, su comportamiento en suelos y sus efectos sobre el mismo. Se incluye también informa-

ción sobre la dinámica de dos reacciones y el comportamiento en sistemas acuáticos.

La fijación biológica del nitrógeno es el tópico del séptimo capítulo. Este tema, que ha recibido mucha atención recientemente, es cubierto con base a la literatura de la última década. Los principales aspectos discutidos son la fijación de N_2, los organismos involucrados, la bioquímica del proceso y sus relaciones con O_2. Se discuten también sistemas donde asociaciones entre microorganismos, raíces y plantas, especialmente tropicales, resultan en fijación de N_2.

La desnitrificación es el tópico del octavo capítulo. Se estudian aquí los organismos responsables y la fisiología y la biquímica del proceso. Se le da consideración a los métodos de medición del proceso y el sistema del suelo donde ocurre. Se considera la producción no biológica de formas volátiles de N_2.

El capítulo noveno se dedica a los metales pesados en la biología y bioquímica de suelos. Se analizan las formas principales de interacción entre estos elementos, esenciales o no, y los procesos biológicos o bioquímicos en suelos. Se considera más que todo los aspectos microbiológicos del problema, tratados con menos profundidad en otros trabajos de revisión.

En el décimo capítulo se estudia la biomasa microbiana en suelos, su medición y transformaciones. Se presenta su medición por microscopia directa, por medición de componentes específicos de la biomasa, la velocidad de respiración y otras técnicas. Se analiza la concordancia entre las diferentes técnicas.

Un breve índice final ayuda al lector a encontrar el material deseado en este volumen de alto nivel que presenta y resume mucho material de difícil acceso y de gran actualidad. El libro requiere conocimientos básicos en microbiología de suelos y buenas bases en bioquímica para su aprovechamiento y se le recomienda para los especialistas y estudiantes de posgrado en el campo.

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