Characteristics of Hymenial Isolates of *Thanatephorus cucumeris* on Sugar Beets in Ohio

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ABSTRACT

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Hymenia of *Thanatephorus cucumeris* were first found on diseased petioles of crown and root rot diseased sugar beets and 10–14 days later in association with a leaf blade blight during both 1979 and 1980. Hymenial isolates belonged predominately in anastomosis group (AG) 2 (80 of 91 isolates). These isolates were highly virulent on older sugar beets. Isolates other than AG-2 were found only on diseased petioles during 1979 and included nine AG-4 and two binucleate *Rhizoctonia*-like isolates. The AG-4 isolates were highly virulent on sugar beet seedlings but only moderately to slightly virulent on older plants. The binucleate isolate tested was avirulent on older plants.

The first report of the occurrence of the perfect state of *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris* (Frank) Donk) on sugar beets (*Beta vulgaris* L.) was apparently by Kotila (5) in 1947, who found hymenia on the underside of leaves on sound tissue adjacent to foliage blight lesions. The earlier report by Schenck (13) of the presence of the basidial state of what she termed "*Hypochnus betae*" or "*Corticium vagum* B. and C. var. *betae*" on sugar beets contained no formal description of the fungus, and no disease symptoms were reported to be associated with the perfect state, according to Kotila (5). Moreover, the fungus was not pathogenic on sugar beets and potatoes in Schenck's (13) inoculation tests. Therefore, Kotila (5) considered the perfect state discovered by Schenck to be distinct from strains of *R. solani* causing foliage blight of sugar beets in the United States.

Kotila (5) also attributed foliage blight to strains of *R. solani* differing from the strains causing crown and root rot and dry rot canker of sugar beets. Although Kotila (5) reported that sugar beet foliage blight was observed in Michigan, Illinois, Wisconsin, Minnesota, and under irrigation in Colorado, it is unclear from his account whether the perfect state of *R. solani* was found in these locations also, as it was in Virginia and Maryland. He described two stages of foliage blight: a marginal leaf burn of young leaves and a blight of older, expanded leaves. In Ohio, no hymenia of *T. cucumeris* have been noted in association with the marginal leaf burn stage of foliage blight; rather, hymenia were found associated only with the blight of older leaves (2). This is similar to Kotila's findings. Thus, a report of occurrence of foliage blight does not necessarily imply the concomitant occurrence of the perfect state of *R. solani*.

In 1978, Naito and Sugimoto (6) reported that foliage blight of sugar beets in Hokkaido, Japan, was caused primarily by the anastomosis group (AG) 2 type 2 (AG 2-2) of *T. cucumeris*. Group AG 2-2 was designated by Ogoshi (8) as including isolates causing crown and root rot of sugar beets in Japan. Therefore, the predominant strains of *R. solani* causing foliage blight in Japan must differ from those originally described by Kotila (5) as being distinct from crown and root rot strains.

In Ohio, hymenia of *T. cucumeris* developed first on diseased petioles of sugar beets affected with crown and root rot, and 10–14 days later the leaf blade blight stage with associated hymenia was noted (2). This suggests that a buildup of basidiospores on diseased petioles was required to initiate the leaf blade blight. Recently, Naito and Sugimoto (7) reported similar observations in Japan, in that hymenia of *T. cucumeris* AG 2-2 were first found on petioles of sugar beets affected with crown rot, followed later by foliage blight after basidiospore discharge from the petiole hymenia. Kotila (5), however, reported that hymenia occurred "almost exclusively" on the underside of leaf blades adjacent to necrotic lesions and on petioles adjacent to blackened lesions. He did not mention any differences in time of appearance of hymenia on leaf petioles as compared with leaf blades (5).

The primary objective of this research was to characterize hymenial isolates collected from sugar beet petioles and leaf blades in Ohio by the numbers of nuclei in vegetative cells, by anastomosis groupings, and by pathogenicity assays on seedlings and older plants. The signs and symptoms associated with occurrence of the perfect state of *R. solani* on sugar beets in Ohio are also described. Some of this information was published in preliminary form (2,3).

MATERIALS AND METHODS

Diseased petioles from sugar beets with symptoms of Rhizoctonia crown and root rot and leaf blades affected with Rhizoctonia leaf blade blight bearing readily discernible hymenia were collected during the last half of August and the first week of September 1979 from sugar beet fields near Fremont, OH. Portions of hymenia from representative petioles and leaf blades were examined microscopically to confirm the presence of basidia and basidiospores. Isolations were made from this plant material, and 62 cultures were selected for further studies. All petiole and most leaf blade isolations were made by plating portions of hymenia on 2% water agar (WA). Nine leaf blade isolates were obtained from mass transfer of basidiospores as follows. Portions of leaves bearing hymenia were placed, hymenial side up, on sterile, moistened filter paper in petri dish tops and were held in place with a doubly bent wire holder. The tops were then placed over petri plate bottoms containing WA. After 24 hrs of incubation at 25 C and 100% relative humidity in darkness, spores deposited on the WA surface were located microscopically. Areas of dense spore concentrations were marked and transferred to potato-dextrose agar plates for germination and growth. All colonies were hyphal tipped and maintained on potato-dextrose agar test tube slants.

Two isolates, one originating from a hymenial fragment and one from a cluster of basidiospores, were tested for pathogenicity to foliage and for reproduction of leaf blight symptoms. Agar disks (6 mm diam) cut from the margins of actively growing cultures on potato dextrose-yeast extract agar (12) were placed on the upper surface of 6-wk-old

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sugar beet leaves. Four 15-cm pots, each containing a single USH 20 cultivar sugar beet, were used per isolate. Four additional plants served as uninoculated controls. The plants were then placed in a growth chamber (25 C, 12-hr days, ~20,000 lux) equipped with a centrifugal humidifier. The humidifier produced a fine mist and maintained a film of water on leaf surfaces. Plants were examined for foliage blight symptoms over a 2-wk period.

Numbers of nuclei in the vegetative cells of all isolates were ascertained using both rapid and Giemsa staining procedures (1). Anatomosis groupings were determined using a previously described technique (4) and known anastomosis tester isolates (courtesy of Neil Anderson, Department of Plant Pathology, University of Minnesota, St. Paul). Pathogenicity and virulence of all *T. cucumeris* isolates to sugar beet seedlings were tested by the modified inoculum layer method used previously by Herr and Roberts (4).

A completely randomized design with four replicates was used. Results were recorded as numbers of surviving healthy seedlings of 25 seeds planted per pot. Additionally, 30 selected cultures were tested for pathogenicity and virulence on older (6- to 7-wk-old) sugar beets using washed mycelium as inoculum (4). Each replicate consisted of seven single-plant pots; replicates were arranged in three randomized blocks in all tests. Disease ratings of harvested beet crowns and roots were assigned on a 0–5 scale, with 0 = healthy and 5 = dead. Sugar beet cultivar USH 20 was used for both seedling and older plant disease assays, and a greenhouse temperature of ~26 C, with ~8,500 lux supplemental lighting, was used throughout all assays.

During July, August, and September 1980, additional hymenial isolations were made from petioles and leaf blades by plating portions of hymenia on WA. Numbers of nuclei, determined by an aniline blue rapid-staining procedure (1), and anastomosis groupings of 29 isolates were ascertained.

RESULTS

Field observations. Hymenia (appressed, white to beige colored, superficial, membranous to felty fungal growths) of the perfect state of *R. solani* (*T. cucumeris*) were found on the undersides of diseased sugar beet leaf petioles (Fig. 1) in mid-August 1979. Generally, the hymenia were located on apparently sound petiole tissue adjacent to basal, blackened disease lesions; however, they were occasionally found on healthy young petioles within the rosette surrounded by infected older petioles on diseased plants. These hymenia were found only on petioles of diseased plants, never on lesion-free healthy sugar beets.

Within 2 wk of these observations (early September 1979), a Rhizoctonia foliage blight consisting of large, irregular, collapsed, water-soaked and blackened lesions on leaf blades became apparent. As the blight progressed, the lesions tended to dry out and disintegrate, and the blighted leaves appeared ragged or shredded (Fig. 2). In most instances, hymenia occurred on apparently healthy tissue adjacent to leaf blade lesions (Fig. 2). The basidial state was found again on petioles in 1980 in mid-July, 1 mo earlier than its discovery in 1979. By early August 1980, the leaf blade blight phase was clearly evident. Hymenia on diseased petioles and leaf blades were present in abundance into the first week of September. By the second week of September, fresh-appearing, active hymenia were rarely found on petioles or leaf blades, and spread of the leaf blight phase apparently was checked. This coincided with a similar cessation of leaf blight activity observed during the second week of September 1979.

Occurrence of Cercospora leaf blight (*Cercospora beticola* Sacc.) at high disease intensity levels in many sugar beet fields in September 1980 made further observations and foliage loss assessments impractical. The leaf blade blight and hymenia, in contrast to hymenia on petioles, were found on plants without crown and root rot symptoms as well as on those with crown and root rot. Distribution of foliage blight within fields appeared to be random in contrast to the distribution of crown and root rot,

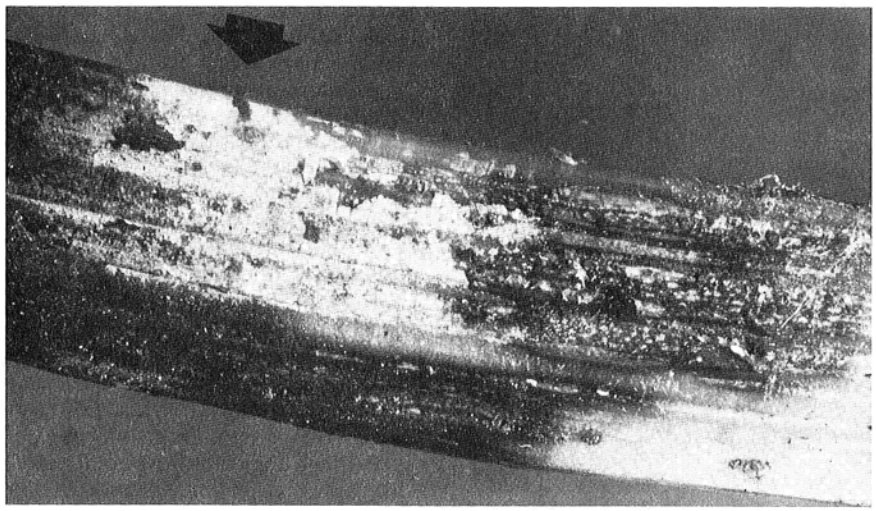

Fig. 1. Close-up view of hymenium on underside of diseased sugar beet petiole. Arrow points to hymenial layer on healthy tissue, surrounded by blackened disease lesion. A second hymenial layer can be seen at the bottom right.

Fig. 2. Underside of older sugar beet leaf exhibiting leaf blade lesion, with hymenium (arrow) on healthy tissue adjacent to lesion. Leaf tissues in older area of lesion have disintegrated, giving the basal portion of the leaf blade a shredded appearance.

which was definitely clumped (Herr, *unpublished*). Rainfall in the months hymenia were present was higher than normal in both 1979 and 1980; moreover, rainfall distribution was such that the sugar beet foliage was wet during much of these periods.

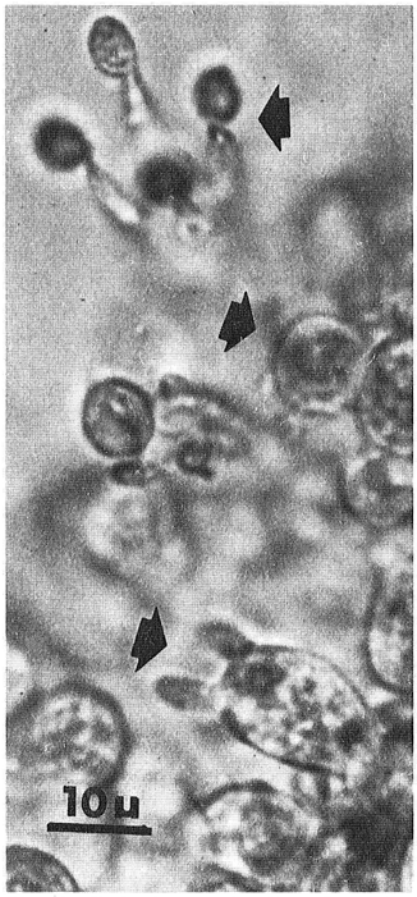

Fig. 3. Photomicrograph of portion of a hymenium depicting basidiospores borne on four sterigmata (upper arrow), partially developed sterigmata on young basidia (lower arrow), and new basidia without sterigmata (middle arrow).

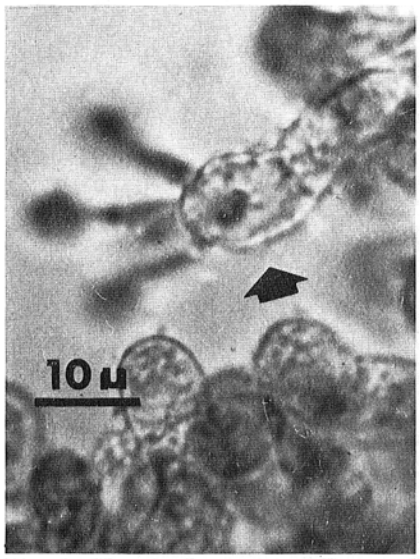

Fig. 4. Sharply focused basidium and supporting hyphae, showing the basidium to be only slightly wider than the supporting hyphae, as is characteristic of *Thanatephorus cucumeris*.

Morphology and inoculations. Portions of hymenia with basidia, sterigmata, and basidiospores are depicted in Figures 3 and 4. Basidia in various stages of development having none, young-developing, or developed sterigmata bearing basidiospores are evident in Figure 3. A clearly focused, mature basidium bearing sterigmata and basidiospores, slightly out of focus, is shown in Figure 4. Note that the basidium is less than twice the width of the supporting hyphae, which is characteristic of *T. cucumeris* (11,15). In growth chamber tests, typical foliage blight symptoms were produced on sugar beets by the two cultures tested (one from a hymenial fragment and one from a cluster of basidiospores). In some cases, incipient young hymenia were apparent surrounding the leaf blade lesions.

Anastomosis groupings. A total of 91 hymenial isolates was collected during the two seasons of 1979–1980. In 1979, hymenial isolates from petioles consisted of 24 AG-2 cultures, 9 AG-4 cultures (all multinucleate *T. cucumeris*), and 2 binucleate *Rhizoctonia*-like cultures (Table 1). In contrast to the petiole isolates, all leaf blade isolates, whether originating from hymenia or basidiospores, were multinucleate AG-2 cultures. Futhermore, all 29 isolates collected in 1980 both from petioles and leaf blades were AG-2 cultures (Table 1).

Pathogenicity and virulence. Results of the pathogenicity and virulence assays of *T. cucumeris* hymenial isolates on sugar beet seedlings are presented in Table 2 according to anastomosis groupings. There were 51 AG-2 cultures assayed and nine AG-4 cultures. With the exception of one moderately virulent isolate, the AG-4 isolates were highly virulent (0–1 surviving seedlings of 25 seedlings per pot). The LSD (0.05) for AG-4 isolates was 1.3 and the mean was 2.2 surviving healthy seedlings. The AG-2 cultures were considerably less virulent, with an LSD (0.05) of 4.5 and a mean of 10.8 surviving healthy seedlings. The isolates were organized into five virulence classes to simplify presentation of the results (Table 2). Only one isolate was slightly virulent (20–25 surviving seedlings). The median virulence category (10–15 surviving seedlings) contained the most isolates of the five virulence classes. The remaining AG-2 isolates were almost equally distributed in the three other classes (0–5, 5–11, and 15–20 surviving seedlings).

Relative virulence of the *Rhizoctonia* hymenial isolates to older plants was quite different from their virulence on seedling sugar beets (Table 3). The AG-2 isolates were much more virulent (average disease rating of 3.6) than AG-4 isolates (average disease rating of 0.6). The single binucleate isolate (not *R. solani*) tested was essentially avirulent (disease rating of 0.06).

DISCUSSION

The hymenial isolates from both petioles and leaf blades belonged predominately in AG-2 (80 of 91 isolates). Furthermore, these AG-2 isolates were highly virulent to older sugar beets. Thus,

Table 1. *Rhizoctonia* isolates arranged according to year, plant source, fungal structure, anastomosis group (AG), and numbers of nuclei in vegetative cells

Year	Plant source	Fungal structure	AG	Nuclei[a]	Number of isolates
1979	Petiole	Hymenium	2	MN	24
			4	MN	9
			...	BN	2
	Leaf blade	Hymenium	2	MN	18
		Basidiospores	2	MN	9
1980	Petiole	Hymenium	2	MN	13
	Leaf blade	Hymenium	2	MN	16

[a]MN = multinucleate; BN = binucleate, not *Thanatephorus cucumeris*.

Table 2. Relative virulence of *Thanatephorus cucumeris* hymenial isolates to sugar beet seedlings

Anastomosis group	Class	Class intervals	No. of isolates per class	Percentage of total isolates
AG-2[a]	1	0–5[b]	10	20
	2	5–10	11	22
	3	10–15	19	37
	4	15–20	10	20
	5	20–25	1	0.2
AG-4[c]	1	0–1	8	89
	2	1–15	0	...
	3	15–20	1	11
	4	20–25	0	...

[a]Frequency distributions of 51 anastomosis group 2 (AG-2) isolates by specified class intervals.
[b]Number of surviving healthy seedlings of 25 in each class.
[c]Frequency distribution of nine AG-4 isolates by specified class intervals.

the isolates from Ohio were quite different from the foliage isolates originally described by Kotila (5) as being incapable of invading uninjured roots of half-mature or older sugar beets. The studies of Kotila (5) made in 1947 predate the use of anastomosis groupings in classifying *R. solani* isolates. However, his characterization of foliar isolates as (for the most part) highly virulent on sugar beet seedlings, weakly virulent on older sugar beets, possessing a wide host range, and having rapid growth rates appears definitely similar to recent descriptions of AG-4 characteristics (4,12,14). More directly, Parmeter et al (10) tested one sugar beet leaf isolate of Kotila's (apparently a foliage blight isolate) that was an AG-4. Ruppel (12) reported that sugar beet foliage isolates from Colorado were AG-4 and caused less severe root rot than did AG-2 isolates from sugar beet roots. Surprisingly, the anastomosis grouping composition of Ohio hymenial isolates appeared to be more similar to those reported by Naito and Sugimoto (6,7) in Japan (ie, predominately AG 2-2 by Ogoshi's system [8]) than to those previously reported in the United States (5,12).

All isolates other than those grouped in AG-2 were isolated from petioles during 1979. Included in this collection were nine AG-4 and two binucleate isolates that were not *R. solani*. The AG-4 isolates were mostly (eight of nine) highly virulent to sugar beet seedlings and only moderately to weakly virulent to older sugar beet plants. These results correspond well with previous reports (4,5,12). Isolation of the two binucleate isolates may have been simply a chance occurrence because both isolates were isolated from the same field on the same date. Hymenia occurring in the field are not sterile, and the binucleate isolates may have been present as contaminates of *T. cucumeris* hymenia. Alternatively, these isolates may have occurred as hymenia of their own perfect state (not *T. cucumeris*). However, in a previous study Herr and Roberts (4) reported that soil and weed binucleate isolates were essentially not pathogenic to sugar beet seedlings and older plants. The binucleate isolate tested in this study was also not pathogenic to older seedlings. These nonpathogenic, binucleate, *Rhizoctonia*-like fungi may be similar to the nonpathogenic "*Hypochnus*" basidial isolate reported by Schenck (13) in 1924. No basidia conforming to the description of the genus *Ceratobasidium,* which is the perfect state of most binucleate, *Rhizoctonia*-like fungi (9), were observed during microscopic examination of hymenia from any of the Ohio sugar beet fields.

Table 3. Relative virulence of *Rhizoctonia* hymenial isolates on 6- to 7-wk-old sugar beets

Anastomosis group (AG)	Nuclear condition[a]	Number of isolates tested	Disease rating[b]		
			High	Average	Low
AG-2	MN	20	4.4	3.6	2.4
AG-4	MN	9	1.7	0.6	0.1
	BN	1	0.1	0.06	0

[a]MN = multinucleate, *Thanatephorus cucumeris*; BN = binucleate, not *Thanatephorus cucumeris*.
[b]Disease rating scale: 0 = healthy, 5 = dead.

Field observations indicated that the first appearance of hymenia (on petioles) was definitely associated with the occurrence of crown and root rot and that they were never found on lesion-free, healthy sugar beets. After 10–14 days, a foliage blight of leaf blades was found. The occurrence of leaf blade blight on beets not exhibiting crown and root rot symptoms and its random distribution in fields, as contrasted to the clumped distribution of crown and root rot, provided good evidence for involvement of aerial dissemination of basidiospores in the spread of older leaf blade blight. Naito and Sugimoto (7) demonstrated the role of basidiospores in the etiology of sugar beet foliage blight in Japan and found that aerial spore densities correlated well with disease severity.

In view of the extensive evidence of the extreme variability of single basidiospore isolates (adequately reviewed in Parmeter [9]), only mass isolates from portions of hymenia or clusters of basidiospores were used in this study. The similarities in pathogenicity and virulence characteristics of these hymenial isolates (ie, AG-2 isolates weakly to moderately virulent on seedlings, highly virulent on older plants; AG-4 isolates highly virulent on seedlings, weakly to moderately virulent on older plants) to those of the *R. solani* AG-2 and AG-4 soil isolates previously investigated by Herr and Roberts (4) were clearly evident, although the greater numbers of isolates examined in the latter study gave a somewhat wider range of isolate characteristics.

The occurrence of the perfect state of *T. cucumeris* and the older leaf blade blight stage in Ohio coincided with unusually wet conditions in 1979 and 1980, with above normal rainfall in July and August distributed such that the sugar beet foliage was wet much of these periods in both years. These circumstances appear to agree with Kotila's (5) observations that high incidence of sugar beet foliage blight and occurrence of the perfect state of *R. solani* required prolonged periods of high humidity. Naito and Sugimoto (7) also commented on the relation of hot, humid weather to the occurrence of the perfect state of *T. cucumeris* on sugar beets.

LITERATURE CITED

1. Herr, L. J. 1979. Practical nuclear staining procedures for *Rhizoctonia*-like fungi. Phytopathology 69:958-961.
2. Herr, L. J. 1980. New form of old disease found in Ohio sugarbeets. Ohio Rep. 65:19-21.
3. Herr, L. J. 1981. Basidial stage of *Rhizoctonia solani*, anastomosis groups 2 and 4, on sugarbeets in Ohio. (Abstr.) Phytopathology 71:224.
4. Herr, L. J., and Roberts, D. L. 1980. Characterization of *Rhizoctonia* populations obtained from sugarbeet fields with differing soil textures. Phytopathology 70:476-480.
5. Kotila, J. E. 1947. Rhizoctonia foliage blight of sugar beets. J. Agric. Res. 74:289-314.
6. Naito, S., and Sugimoto, T. 1978. Basidiospore infection and lesion development on sugar beet leaves by *Thanatephorus cucumeris* (Frank) Donk. Ann. Phytopathol. Soc. Jpn. 44:426-431. (In Japanese; English abstract)
7. Naito, S., and Sugimoto, T. 1980. Relationship between basidiospore dispersal of *Thanatephorus cucumeris* (Frank) Donk and development of foliage blight of sugar beets. Ann. Phytopathol. Soc. Jpn. 46:216-223. (In Japanese; English abstract)
8. Ogoshi, A. 1976. Studies on the grouping of *Rhizoctonia solani* Kühn with hyphal anastomosis and on the perfect stages of groups. Bull. Nat. Inst. Agric. Sci. Ser. C 30:1-63. (In Japanese; English summary)
9. Parmeter, J. R., Jr., ed. 1970. *Rhizoctonia solani*: Biology and Pathology. University of California Press, Berkeley. 255 pp.
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., Whitney, H. S., and Platt, W. D. 1967. Affinities of some *Rhizoctonia* species that resemble mycelium of *Thanatephorus cucumeris*. Phytopathology 57:218-223.
12. Ruppel, E. G. 1972. Correlation of cultural characters and source of isolates with pathogenicity of *Rhizoctonia solani* from sugar beet. Phytopathology 62:202-205.
13. Schenck, E. 1924. Über das auftreten einer Hypochnusart auf zuckerruse. Centrabl. f. Bakt. Abt. II 61:317-322. (As cited by Kotila [5])
14. Sherwood, R. T. 1969. Morphology and physiology in four anastomosis groups of *Thanatephorus cucumeris*. Phytopathology 59:1924-1929.
15. Talbot, P. H. B. 1970. Taxonomy and nomenclature of the perfect state. Pages 20-31 in: *Rhizoctonia solani:* Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.