Infection and Development of Target Spot of Flue-Cured Tobacco Caused by *Thanatephorus cucumeris*

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ABSTRACT

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The effects of misting frequency and temperature regime on basidiospore infection and lesion development of target spot of tobacco were determined. Three isolates of *Thanatephorus cucumeris* (*Rhizoctonia solani* AG-2-2) produced hymenia and basidiospores equally when exposed to moderate temperatures and extended periods of high relative humidity. Optimum temperatures for hymenium production, infection, and lesion development were 16–30, 20–26, and 20–30 C, respectively. Hymenium production was very minimal at temperatures above 30 C or at any temperature in the absence of misting. Lesion development was limited at high temperatures. Basidiospores germinated by the production of a single germ tube that terminated in an appressorium. Following direct penetration, a stroma was formed in the infected epidermal cell before further colonization of host tissue. Initial colonization resulted in the formation of a primary lesion 1–2 mm in diameter that remained distinct even if lesion expansion occurred. Lesions expanded by hyphal growth through the leaf tissue and by hyphae that grew out of stomata and across the leaf surface and then penetrated other stomata. The relationship between environmental conditions required for development of target spot and disease forecasting is discussed.

Target spot (Rhizoctonia leaf spot) of tobacco (*Nicotiana tabacum* L.), caused by *Thanatephorus cucumeris* (Frank) Donk (anamorph *Rhizoctonia solani* Kühn AG-2-2), was reported originally from Brazil in 1948 (2), and from Costa Rica in 1973 (17). In 1984, the disease was described for the first time in the United States on flue-cured tobacco in North Carolina (13). Severe losses as a result of leaf destruction occurred in several fields and numerous fields suffered minor losses. The widespread occurrence and severity of target spot was associated with periods of frequent rainfall and below-normal temperatures in June and July throughout the state. Between 1984 and 1988, the disease occurred each year in some parts of the flue-cured tobacco growing region of North Carolina and was also observed in Virginia (C. S. Johnson, *personal communication*). In 1989, the disease again was severe throughout the flue-cured tobacco region of North Carolina, causing an estimated loss of over $20 million (T. Melton, *personal communication*). In addition, it was observed in burley tobacco in North Carolina and on tobacco in South Carolina, Tennessee, and Kentucky. Since 1984, the occurrence of target spot has been associated with high moisture conditions. Target spot occurred on plants in the seed bed or greenhouse only after leaves were large enough to close the canopy and in the field once plants were large enough to have leaves that shaded the ground and the lower leaves of the plant. This study was conducted to determine the effects of temperature and misting (leaf wetness) regimes on inoculum production, leaf infection, and lesion development by *T. cucumeris* on flue-cured tobacco (14).

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MATERIALS AND METHODS

Plant growth conditions. Tobacco plants, NC 2326, were grown in standard phytotron soil mix (1/3 peat:perlite, 2/3 gravel [5]) for all tests. Three-week-old seedlings were transplanted into 7-cm-diameter Styrofoam cups and grown in the phytotron greenhouse at 26–22 C (day-night temperatures) for 3 wk. Seedlings were then transferred to growth chambers (3.66 × 2.44 m) maintained at the desired temperature regime with a 9-hr day at a light intensity of 670–735 $\mu E \cdot m^{-2} \cdot s^{-1}$ (430–480 hlux). A 1-hr dark-interruption period was used to simulate a long day length and prevent premature flowering of the tobacco. Plants were watered three times daily with approximately 100 ml of deionized water per plant at each watering. A drip irrigation system was used to prevent wetting of leaves. Plants were watered twice weekly with 100 ml of nutrient solution per plant (5).

Fungus isolates and inoculum production. Three isolates (Rs 1600, Rs 1602, and Rs 1609) of *T. cucumeris* were used. The isolates were obtained from large target spot lesions on field-grown flue-cured tobacco collected from three different counties in North Carolina. Stock cultures were maintained on V-8 juice agar slant tubes at room temperature.

Each fungus isolate was grown on twice-autoclaved rice grains (30 g of long-grain rice and 10 ml of deionized water) for 2 wk at 22–25 C. Soil was infested by placing 0.6 g of the colonized rice grains 1 cm below the soil surface and 3 cm from the plant stem in each Styrofoam cup. Cups and plant stems were observed daily for the presence of hymenia. The number of total cups with hymenia in each treatment was recorded daily for the first 2 wk of each 4-wk test.

Rotorod spore samplers were placed 25 cm above plant canopies to confirm the presence of basidiospores in the chamber atmospheres (mean air velocity 20 m/min). Air was sampled hourly for 2 days during peak production of hymenia to determine when basidiospores were present in the chambers.

There were 108 infested cups, 36 per isolate, and 36 uninfested cups per chamber in each experiment. The experimental design was a randomized complete block with replication over time. Treatments were assigned randomly to chambers before each run of the experiment. All experiments were repeated at least once. Data were analyzed by analysis of variance and subjected to the Waller-Duncan *k*-ratio *t* test for the level of significance where appropriate.

Misting and temperature regimes. The effects of misting regimes on inoculum production and disease development were determined at day-night temperatures of 28–22 C. This temperature regime was chosen because it was similar to temperatures present when severe disease was first observed in the field. Four misting regimes were established: 1) no mist, 2) mist three times per hour at night only (15-hr duration), 3) mist 12 times per hour at night only, and 4) mist 12 times per hour during the day and night. Mist duration was 12 sec in all treatments. Plants were misted with atomized (compressed-air driven), deionized water beginning immediately after the soil was

infested. Nozzles and air pressure in all chambers were calibrated before each run of the experiment to dispense an equal volume of water aerosol into each chamber. Relative humidities were determined for each misting regime with a Vaisala HMI 14 humidity sensor (Vaisala, Woburn, MA) in an aspirated system. Disease was measured as the percent of leaf area damage (leaf spots and loss of leaf tissue) on 10 randomly selected plants in each misting treatment.

Four temperature regimes (34–28, 30–24, 26–20, and 22–16 C, ± 1 C) were established in conjunction with the day and night misting regime. The continuous misting regime, 12 times per hour, was chosen because it was the most conducive regime for disease development. The effect of each temperature regime on formation of hymenia was determined by recording the total number of cups with hymenia as previously described. The effect of the temperature regime on infection was also determined. Twenty healthy plants were placed at 26–20 C in a chamber that contained severely diseased plants and had abundant basidiospore inoculum present (based on rotorod samples). After 24 hr, plants were removed and five randomly selected plants were placed at each of the four temperature regimes. Plants were observed after 5 and 10 days for the number of primary lesions (1–2-mm circular, water-soaked spots) that developed on each of five previously identified leaves at each temperature.

The rate of lesion expansion at the different temperature regimes was measured in a separate experiment on plants that had newly formed primary lesions 5 days after inoculation. Plants were inoculated as described above for the infection experiment. Twenty lesions (10 on each of two plants) at each temperature were identified at random and the diameter of each was measured daily for 14 days. The percentage of lesions that expanded and the maximum rate of lesion expansion was determined.

Histopathology. One-centimeter-diameter leaf disks were collected from leaf lesions at various stages of lesion development and placed immediately in glacial acetic acid:95% ethanol (1:1 v/v). After 24–48 hr, leaf disks were removed and placed in 70% lactic acid for an additional 24 hr to clear leaf tissue. Fungal tissue was stained by heating cleared leaf disks for 20 min in a solution of lactic acid:glycerin:water (2:2:1) containing 0.05% aniline blue. Leaf disks were mounted whole in lactophenol on glass slides for microscopic observation. Some leaf disks were fixed in Formalin-2-propiono-proponal immediately after removal from leaves. They were dehydrated in an isopropyl alcohol series, embedded in Paraplast + (Sherwood Medical Industries, St. Louis, MO), and sectioned on a rotary microtome at 12 μm. Sections were stained with Triarch's Quadruple Stain (Triarch Inc., Ripon, WI).

RESULTS

The three isolates of *T. cucumeris* used in this study produced hymenia and basidiospores abundantly. No differences were observed among isolates in production of hymenia under the various misting and temperature regimes, so data from the three isolates were combined. Hymenia were first observed 5 days after infesting soil and were abundant after 6 days. They were produced on the surface of the soil, on pots and irrigation equipment, on the stems of plants, and occasionally on the abaxial and adaxial side of leaves (Fig. 1). No hymenia developed in pots that were not infested with the pathogen. Production of basidiospores by each of the three isolates was not quantified, but microscopic observation of the hymenia of each isolate revealed abundant sporulation (Fig. 2). Basidiospores were present at all hourly sampling periods over the 2-day period, but the greatest numbers were detected at 0800 hours on each day.

Relative humidities in the chambers varied with misting regime. In the absence of mist, relative humidities were generally in the range of 65–85%. Misting every 20 min at night resulted in humidities cycling between 95 and 100%, beginning approximately 1 hr after misting was initiated. Misting every 5 min at night gave humidities between 98 and 100%. Misting every 5 min during light periods resulted in humidities cycling between 85 and 100%. The extended periods of relative humidities at 100% resulted in perceptible leaf wetness in all misting regimes but was greatest in duration with continuous misting. Misting regime affected the production of hymenia and disease severity. Hymenia were observed more often in the misting treatments than in the no mist treatment (Table 1). The percent of leaf area damage increased as the duration of misting increased (Table 1).

Temperature regime significantly affected all pathogen and disease parameters measured. Optimum temperatures for hymenium production were between 20 and 30 C (Table 2); very little development occurred in the 34–28 C regime. Leaf infection was greatest at temperatures between 20 and 26 C (Table 2). Percent of primary lesions that expanded was greatest at 30–24 C (Table 3), while the rate of lesion expansion was greatest between 20 and 30 C (Table 3).

Histopathology. Basidiospores germinated on the leaf surface, usually with the production of a single germ tube, and an appressorium was formed before direct penetration of the epidermis (Fig. 3). After penetration, a stroma was formed in the infected epidermal cell (Figs. 4–6). Stroma varied in size and shape but always were formed in the infected cell before hyphae colonized adjacent cells. Colonization of host tissue

Table 1. Effect of misting regime on production of hymenia and on leaf area damage on flue-cured tobacco caused by *Thanatephorus cucumeris*

Misting regime[w]	Pots with hymenia (%)[x]	Leaf area damage (%)[y]
None	18 a[z]	1 a
Night (3 times per hour)	67 b	9 a
Night (12 times per hour)	82 b	20 b
Day + night (12 times per hour)	79 b	34 c

[w] Day-night temperature regime of 28–22 C.
[x] Each pot was infested by placing 0.6 g of rice grains colonized by *T. cucumeris* 1 cm below the soil surface. A total of 108 pots were infested per treatment. The experiment was run twice and data are means across both runs of the experiment.
[y] Percent of leaf area necrotic 21 days after infesting soil as the result of leaf infections by *T. cucumeris*. Mean damage from 10 randomly selected plants per treatment in each of two runs of the experiment.
[z] Values within a column followed by the same letter are not significantly different at $P = 0.05$.

Figs. 1–12. Stages in the development of target spot of tobacco. **(1)** Hymenium (hy) production on the stem and petioles of a young tobacco plant 7 days after infesting soil with *Thanatephorus cucumeris*; **(2)** mature and immature basidiospores (bs) of the pathogen; **(3)** germination of a basidiospore on the leaf surface showing a single germ tube and appressorium (ap); **(4)** direct penetration and initiation of stroma (str) in an epidermal cell; **(5)** well-developed stroma below penetration site (ps) in epidermal cell; **(6)** growth of hyphae from stroma; **(7)** primary lesion (approximately 2-mm in diameter) of target spot with stroma in center of lesion; **(8)** transverse section of leaf through primary lesion (pl) showing margin of lesion and hyphae (hp) in lesion; **(9)** extensive growth of hyphae in leaf tissue and emergence of hypha between guard cells (gc); **(10)** growth of hyphae across leaf surface and penetration of stomate, tr = trichome; **(11)** symptoms of target spot on leaf showing primary lesions (pl) and expanding lesions with concentric zones; and **(12)** hymenium (hy) production on leaf about 19 days after soil was infested.

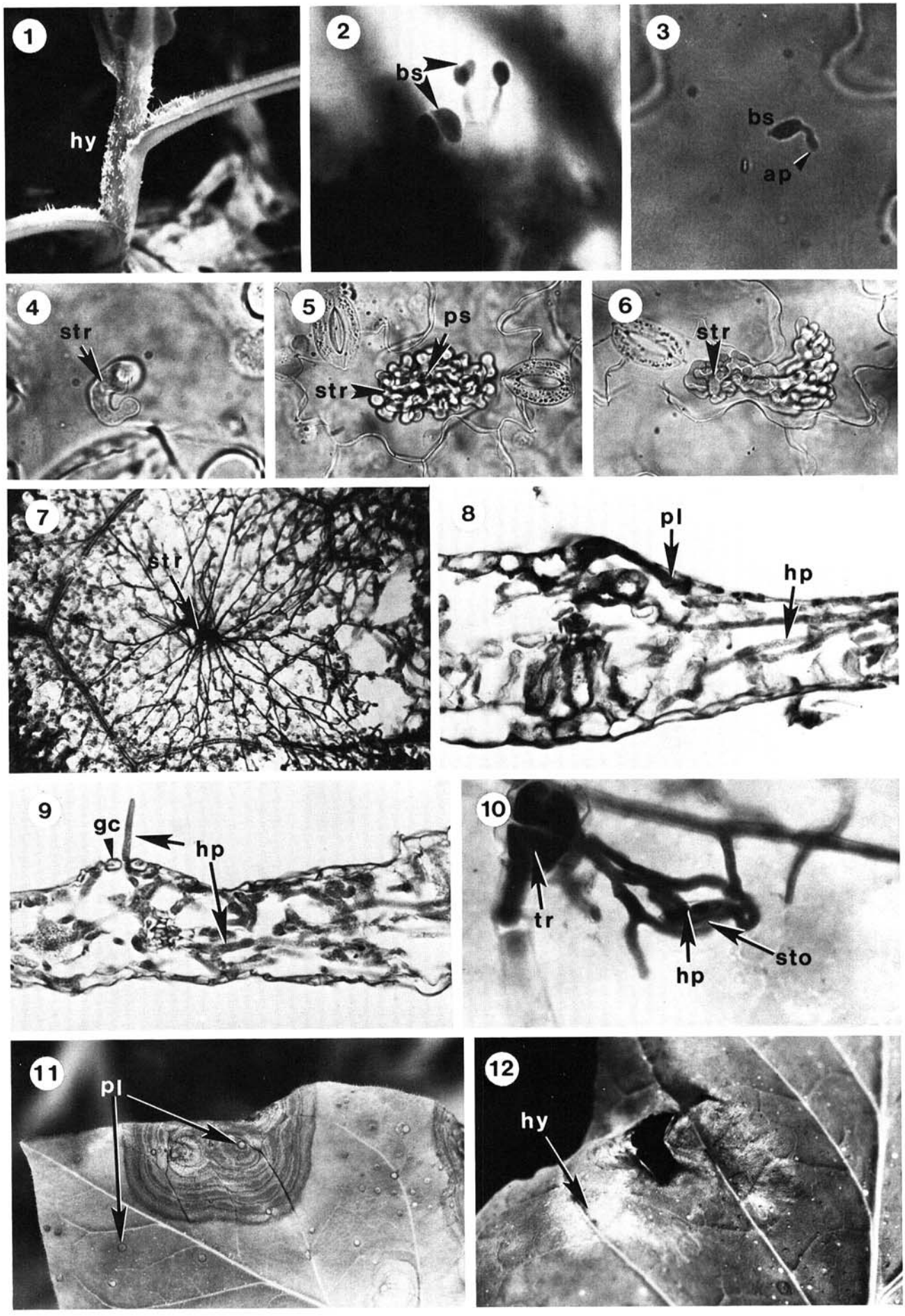
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Table 2. Effect of temperature on production of hymenia and infection of flue-cured tobacco by basidiospores of *Thanatephorus cucumeris*

Day-night temperature (C)	Pots with hymenia (%)[x]	Lesions per leaf (no.)[y]
22–16	69 b[z]	2 a
26–20	93 b	38 b
30–24	83 b	1 a
34–28	17 a	0 a

[x] Soil in each pot infested by placing 0.6 g of rice grains colonized by *T. cucumeris* 1 cm below the soil surface. A total of 108 pots per temperature regime were infested in each of two runs of the experiment. All temperature regimes received continuous (12 times per hour) mist.

[y] Plants were exposed to basidiospore inoculum for 24 hr before placement at the desired temperature regime. Value is the number of lesions that developed on each of five previously identified leaves (one leaf on each of five plants) at each temperature after 10 days. Plants were exposed to mist 12 times per hour during the experiment.

[z] Values within a column followed by the same letter are not significantly different at $P = 0.05$. Data were combined from both runs of the experiment for analysis.

Table 3. Effect of temperature on lesion development on leaves of flue-cured tobacco by *Thanatephorus cucumeris*

Day-night temperature (C)	Lesions expanding (%)[x]	Maximum increase per day (mm)[y]
22–16	10 a[z]	1.5
26–20	10 a	2.8
30–24	35 b	3.3
34–28	10 a	1.5

[x] Twenty primary lesions (10 on each of two plants) were identified on day 0 at each temperature regime and observed each day for 14 days.

[y] Lesion diameter measured daily for 14 days after primary lesion development. Data presented are maximum and not mean rates, so data were not analyzed.

[z] Values within a column followed by the same letter are not significantly different at $P = 0.05$. Data were combined across runs of the experiment for analysis.

was slowed or halted after hyphae colonized an area 1–2 mm in diameter. This area became water soaked, and a primary lesion formed (Figs. 7 and 8). Many of the primary lesions failed to expand but usually remained distinct even when the lesion continued to expand. Large, irregularly shaped lesions characteristic of the disease in the field formed when hyphae grew out from the primary lesion. In some cases, hyphae grew out from the primary lesion through the leaf mesophyll and enlarged the necrotic area around the primary lesion (Fig. 9). In other cases, hyphae exited the leaf tissue through stomata (Fig. 9), grew onto and across the leaf surface, penetrated the leaf through other stomata, and initiated secondary lesions (Fig. 10). A distinctive pattern of concentric rings often developed within lesions as they enlarged, and hymenial formation was common on leaves in advanced stages of lesion development (Figs. 11 and 12).

T. cucumeris was isolated consistently from all primary and secondary lesions. Recovery was lower from old primary lesions that had failed to enlarge. The pathogen often failed to grow from primary lesions that were greater than 2 wk old, i.e. at the end of the experiments conducted in this study.

DISCUSSION

The environmental conditions conducive to the development of target spot of tobacco are similar to the conditions for the development of foliar blights caused by *T. cucumeris* on other hosts (6,8,11,16). Extended periods of high relative humidity and leaf wetness, in conjunction with moderate ambient temperatures, resulted in abundant production of hymenia and basidiospores of *T. cucumeris* and infection of tobacco leaves. Relative humidities above 98% are required for basidiospore production, germination, and leaf infection (8,10,16). The minimum duration of high relative humidity and leaf wetness required for infection by basidiospores was not determined in this study. Also, such information is lacking for other hosts infected by basidiospores of *T. cucumeris*. Studies in progress will examine the effects of the duration of postinfection periods of high humidity and leaf wetness on lesion development.

Hymenia of *T. cucumeris* developed at temperatures between 16 and 30 C. This temperature regime is within the mean high and low temperatures for the flue-cured tobacco region of North Carolina during the first half of the growing season, when the disease is most severe. During years that are cooler and wetter than average, the disease has continued to develop until temperatures were higher than 32 C for several consecutive days. Growth of the pathogen in host tissues was greatest between 20 and 30 C, which is similar to optimum temperatures for growth in vitro (13). The most sensitive stage in disease development to temperature apparently is infection, with greatest infection efficiency occurring between 20 and 26 C. Very high levels of infection also occurred at 28–22 C in the experiments with various misting regimes. However, in those experiments, plants were exposed to a high concentration of basidiospore inoculum over a 3-wk period, so infection efficiency may have been somewhat lower than at 26–20 C but was masked by the high inoculum level. We did not attempt to quantify inoculum at each temperature regime, but it's possible that hymenia that form at high and low temperatures produce fewer basidiospores that are of poorer quality than those at moderate temperatures.

The source of primary or overwintering inoculum for target spot isolates of *T. cucumeris* has not been determined. In the field, primary inoculum of stem rot or sore shin isolates of *T. cucumeris* is hyphae that arise from sclerotia or colonized organic matter. These hyphae give rise to infection cushions or lobate appressoria that penetrate host tissues directly or through stomata (3,4). Infection cushions also are formed following basidiospore germination on cotton (9) and tomato (7), while appressoria are formed before direct penetration of sugar beet leaves (11). On tobacco, basidiospores germinated, produced appressoria, and penetrated the epidermis directly. These observations are in agreement with observations of the penetration of sugar beet leaves by isolates of *T. cucumeris* (10,11).

The function of the stroma, formed in the infected epidermal cell, is not known, but it may be similar to an infection cushion except that it is formed inside host tissue. Stroma varied in size and shape but were always formed. Initial colonization resulted in development of small, circular, water-soaked lesions, approximately 1–2 mm in diameter. Similar symptoms have been described on bean (6), jute (16), and sugar beet (10) following infection by basidiospores. On tobacco, less than half of these primary lesions continued to develop under the most favorable environmental conditions tested, even though the fungus remained viable in host tissue up to several weeks after primary lesion formation. Primary lesions remained distinct even when the lesion continued to expand and is considered a diagnostic feature of this disease. These observations are again in agreement with stages in lesion development of the foliar blight of sugar beet (10,11).

The tobacco and sugar beet pathogens are in anastomosis group 2, type 2 (AG-2-2). Even though AG-2-2 isolates of *T. cucumeris* are generally considered to cause root rots (1,12,15), foliar blights of other plants also are caused by isolates from this anastomosis group (10,12). Tobacco isolates of *T. cucumeris* that cause target spot also cause damping off, root rot, and sore shin (stem rot) of tobacco. However, most sore shin isolates of *T. cucumeris* from tobacco are not AG-2-2 and do not cause leaf spot of tobacco (H. D. Shew, *unpublished data*). Other natural hosts for the isolates of *T. cucumeris* that attack tobacco currently are not known. Basidiospores of the isolates failed to infect other field and vegetable crops commonly grown in North Carolina (H. D. Shew, *unpublished*). Isolates of AG-2-2 are important pathogens of corn in the southern United States (15), and corn is a primary rota-

tion crop with tobacco in North Carolina. Corn may thus be serving as another host of target spot isolates.

The relatively narrow range of environmental conditions that are favorable to the initiation and development of target spot of tobacco should make it possible to predict when the disease will cause the most damage to the flue-cured tobacco crop. Moderate temperature and extended periods of frequent rainfall and high relative humidities that serve to maintain leaf wetness and high soil moisture satisfy the requirements of the fungus to produce inoculum and infect tobacco leaves. Conversely, periods of seasonally high temperature will prevent inoculum production and infection of host tissue. This information should be helpful in developing fungicide strategies should the disease become an even more severe problem in future years.

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LITERATURE CITED

1. Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. Annu. Rev. Phytopathol. 20:329-347.
2. Costa, A. S. 1948. Mancha aureolada e requeima do fumo causades por *Corticium solani*. Biologico 14:113-114.
3. Dodman, R. L., Barker, K. R., and Walker, J. C. 1968. A detailed study of the different modes of penetration by *Rhizoctonia solani*. Phytopathology 58:1271-1276.
4. Dodman, R. L., and Flentje, N. T. 1970. The mechanism and physiology of plant penetration by *Rhizoctonia solani*. Pages 149-160 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. Univ. Calif. Press, Berkeley.
5. Downs, R. J., and Bonaminio, V. P. 1976. Phytotron procedural manual. N. C. Agric. Exp. Stn. Tech. Bull. 244 37 pp.
6. Echandi, E. 1965. Basidiospore infection by *Pellicularia filamentosa* (= *Corticium microscterotia*), the incitant of web blight of common bean. Phytopathology 55:698-699.
7. Gonzalez, L. C., and Owen, J. H. 1963. Soil rot of tomato caused by *Rhizoctonia solani*. Phytopathology 53:82-85.
8. Kotila, J. E. 1947. Rhizoctonia foliage blight of sugar beets. J. Agric. Res. 74:289-314.
9. Luke, W. J., Pinckard, J. A., and Wang, S. C. 1974. Basidiospore infection of cotton bolls by *Thanatephorus cucumeris*. Phytopathology 64:107-111.
10. Naito, S. 1984. Studies on foliage blight of sugar beets. Res. Bull. Hokkaido Natl. Agric. Exp. Stn. 139:145-188.
11. Naito, S., and Sugimoto, T. 1978. Basidiospore infection and lesion development on sugar beet leaves by *Thanatephorus cucumeris*. Ann. Phytopathol. Soc. Jpn. 44:426-431.
12. Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and interspecific groups of *Rhizoctonia solani* Kühn. Annu. Rev. Phytopathol. 25:125-143.
13. Shew, H. D., and Main, C. E. 1985. Rhizoctonia leaf spot of flue-cured tobacco in North Carolina. Plant Dis. 69:901-903.
14. Shew, H. D., and Main, C. E. 1986. Effect of temperature and misting regime on development of Rhizoctonia leaf spot of tobacco. (Abstr.) Phytopathology 76:1119-1120.
15. Sumner, D. R., and Minton, N. A. 1989. Crop losses in corn induced by *Rhizoctonia solani* AG-2-2 and nematodes. Phytopathology 79:934-941.
16. Tu, C. C., Cheng, Y. H., and Schenck, N. C. 1977. Leaf spot caused by basidiospores of *Thanatephorus cucumeris* on jute, and survival of single basidiospore isolates in soil. Plant Dis. Rep. 61:80-84.
17. Vargas, E. 1973. Infection by basidiospores of *Thanatephorus cucumeris* causing a foliar disease in tobacco. Turrialba 23:357-359.