GROWTH AND PATHOGENICITY OF SIX ISOLATES OF CORYNESPORA CASSIICOLA (BERK. & CURT.) WEI

by

James Cleveland Adams III

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APPROVED:

L. D. Moore, Chairman

M. K. Roane

J. A. Fox

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INTRODUCTION

In 1973, Fajola and Alasoadura (7) reported a new disease of tobacco in Nigeria they called Corynespora leaf spot, caused by Corynespora cassiicola. The new disease caused up to 15% yield reduction on tobacco farms in southern Nigeria. As described by Fajola and Alasoadura, symptoms of C. cassiicola on tobacco began with tiny chlorotic spots on the adaxial surfaces of older leaves. As lesion development progressed, fungal fructifications produced distinctly zonate "target spots," composed of concentric dark colored rings of conidia. Isolates of the fungus were found to grow best in culture at 30 C with a similar, but lower optimum for disease development in the field. Corynespora leaf spot was found to be a late-season tobacco pathogen in Nigeria and, as such, could be controlled by timely harvesting of the leaves.

During the summer of 1976, a fungus was isolated from soil in a field of flue-cured tobacco in Virginia where plants exhibited necrotic stem and leaf lesions. The fungus isolated from the tobacco field was subsequently identified as C. cassiicola. Because of the lack of knowledge surrounding the pathogenicity of C. cassiicola on tobacco, it was decided that the isolate from Virginia should be characterized as to its morphology and pathogenicity and compared to other isolates of the fungus from other crops.

Five isolates of C. cassiicola, along with the Virginia isolate (VI) were tested for pathogenicity on several crops including selected

cultivars of tobacco. The isolates were also evaluated for their relative growth rates and compared as to their colony characteristics and spore morphology.

There were four basic objectives to the characterization of the Virginia isolate of C. cassiicola. These were:

(1) To compare macro- and microscopic colony characteristics of the Virginia isolate with those of the other isolates.

(2) To compare growth of the Virginia isolate to that of the other isolates.

(3) To determine the pathogenicity of the Virginia isolate of C. cassiicola to several cultivars of tobacco and establish a host range for this isolate of the fungus.

(4) Having completed the above objectives, to evaluate the possible economic importance of the VI isolate of the fungus.

LITERATURE REVIEW

Corynespora cassiicola (Berk. & Curt.) Wei is a member of the order Moniliales, class Deuteromycetes. Synonyms listed by Wei (26) for *C. cassiicola* include *Helminthosporium cassiicola* Berk. & Curt. (1869), *Cercospora melonis* Cooke (1896), *Corynespora mazei* Gussow (1910), *C. melonis* (Cooke) Lindau (1910), *H. papayae* H. Syd. (1923), *Cercospora vignicola* Kawamura (1931), and *H. vignae* Olive, Bain & Lefebvre (1945). According to Wei (26) *C. cassiicola* was originally described in 1869 by Berkeley as *H. cassiicola*, after the fungus was collected in Cuba on *Cassia alata*. Thereafter, *C. cassiicola* was found on several different hosts in Europe and China during the latter part of the 19th century. Poor lines of communication during this time resulted in the many synonyms for the fungus. In 1950, Wei was able to examine collections of these fungi and determine that all were, in fact, *C. cassiicola*.

Corynespora cassiicola is the type species for the genus *Corynespora*, which is closely related to *Helminthosporium*. Species within the genus *Corynespora* are differentiated by size, shape, and color of the conidia, and the presence or absence of a stroma. *Corynespora cassiicola* is separated from the other species by absence of a stroma and production of a superficial mycelium.

The morphology of *C. cassiicola* varies among and within given isolates. The following is a summary of the Commonwealth Mycological Institute description given by Ellis (5). Colonies are colored gray

to brown and may appear hairy. The mycelium is generally immersed and does not form a stroma. The fungus produces erect conidiophores that are occasionally branched, straight or flexed to a small extent, subhyaline to dark brown, smooth, septate, 85-100 μ long, and 4-11 μ thick. The conidia of C. cassiicola may be solitary or connected in a series, variable in shape, subhyaline to greenish-black, 4-20 pseudoseptate, 400-500 μ long, 10-20 μ thick at the widest point, and 4-8 μ wide at the base of the structure. The hilum is usually thick and dark.

The optimum temperature for growth of C. cassiicola on artificial media varies with the isolate. Seaman et al. (21) and Boosalis and Hamilton (4) reported a 25 C optimum for soybean (Glycine max) isolates as did Awoderu (2) for para rubber (Hevea brasiliensis), and Sobers (22) for azalea (Rhododendron obtusum) isolates. Fajola and Alasoadura (7) found a tobacco isolate from Nigeria grew best in culture at 30 C. All of the above authors found somewhat lower temperature optima for development of the fungus under natural conditions.

As a plant pathogen, C. cassiicola has elicited a diversity of opinions relating to its ability to incite disease. According to Wei (26), at the turn of the century Cercospora melonis (C. cassiicola) caused a severe epidemic on melon and cucumber in greenhouses in Europe. Corynespora cassiicola was considered at that time to be a limiting factor in greenhouse cucumber production in France, Holland, England, Denmark, and Germany. Various chemical treatments failed, and it was not until the introduction of resistant varieties that C. cassiicola could be controlled in greenhouse situations. Several workers during

this period, however, concluded that C. cassiicola was only a saprophyte (26). It was argued that damage inflicted by other pathogens was attributed to C. cassiicola because of the latter's abundant sporulation on the leaf surface. Onesirosan et al. (16) recently compared several isolates of C. cassiicola from Nigeria, the southern United States, and western Mexico, and concluded that C. cassiicola was merely a secondary invader of diseased or senescent tissue. At present, these ambiguities surrounding the pathogenicity of the organism have not been resolved.

Corynespora cassiicola has an extensive geographical distribution, but is most commonly found in tropical and subtropical areas. Ellis (5) and Ellis and Holliday (6) list the fungus as a pathogen on over 70 plant species, including Agave sisalana, Albizzia zygia, Aleurites montana, Ananas comosus, Bauhinia purpurea, Boehmeria nivea, Bridelia ferruginea, Capsicum frutescens, Carica papaya, Cassia spp., Citrulus vulgaris, Clerodendron paniculatum, Corchorus olitorius, Crotalaria juncea, C. sericea, Cucumis sativus, Cucurbita melo, C. pepo, Cyamopsis psoraloides, Dalbergia sp., Eucalyptus grandis, Gaillardia aristata, Glycine max, Gossypium spp., Hevea brasiliensis, Hibiscus esculentus, H. sabdariffa, Hydrangea macrophylla, Impatiens balsameria, Jacobinia sp., Lactuca sativa, Lagenaria siceraria, Lupinus spp., Lycopersicon esculentum, Manihot esculenta, Musa sp., Newbouldia laevis, Nicotiana tabacum, Norantea guianeensis, Petrea sp., Phaseolus aureus, P. lunatus, P. vulgaris, Piper betle, Plumeria acutifolia, Poinsettia sp., Psophocarpus tetragonolobus, Rhododendron

obtusum, Ricinus communis, Salacia sengalensis, Salvia leucantha, Sesamum indicum, S. orientale, Sida urens, Solanum melongena, Stachytarpheta angustifolia, Tacazzea apiculata, Tithonia speciosa, Tragia sp., Vigna sinensis, Vitex pubescens, Warpuria clandestina, Wedelia biflora and Xanthosoma sagittifolium. In addition to these, Fajola and Alasoadura (7) list Eugenia uniflora, Fleurya aestuans, and Gliricidia sepium as hosts of C. cassiicola in Nigeria.

Corynespora cassiicola causes a foliar disease on several plant species commonly known as "target spot." The disease derives its name from the circular lesions composed of concentric rings resulting from sporulation of C. cassiicola on the leaf surface. Luttrell (12) observed that under natural conditions, conidiophores arise from hyphal tissue that has penetrated epidermal cells of the suscept. Initially, lesions are seen as tiny purple or chlorotic pinpoint spots which gradually expand during disease development, and in the case of severe infection, may coalesce to give the plant a blighted appearance.

Stone and Jones (24), working with sesame and soybean isolates, observed differences in conidial width. The authors analyzed the variance between hosts and isolates and found that the host plant affected the width of the conidia produced by C. cassiicola. However, when two different isolates were inoculated on the same host, no significant difference in conidial width was observed. In germination studies by the same authors, there was no appreciable difference between sesame and soybean isolates.

Although, classically, C. cassiicola produces "target spot" symptoms on the suscept, the fungus can produce water-soaked foliar

lesions as well. Onesirosan et al. (17) reported water soaking as a common symptom on tomato seedlings when infection occurred prior to transplanting. As found in the "target spot" phase, foliage of infected tomato seedlings took on a blighted appearance in advanced stages of disease development.

As well as inciting a foliar disease, C. cassiicola was shown by Boosalis and Hamilton (4) to cause a root and hypocotyl rot of soybean. In some cases the entire base of the stem of the soybean plant was girdled by the lesion. The authors also observed that seedlings of susceptible soybean developed a root rot when seeds were inoculated with C. cassiicola before planting. However, Boosalis and Hamilton found that the underground lesions formed by C. cassiicola were of little economic importance since subsequent development of the disease was arrested by warmer temperatures encountered in early summer.

Norse (14) found C. cassiicola to cause a stem and fruit disease on papaya in Barbados. Seedlings of papaya developed stem break, while mature fruit became infected around the peduncle. Unlike several other hosts, foliar lesions caused by C. cassiicola on papaya were observed to cause very little damage to the plants.

Onesirosan et al. (17) reported the production of a toxin in C. cassiicola isolates pathogenic on tomato. Existence of the toxin resulted in tissue death far in advance of hyphal development. However, these workers found that sesame and eggplant were relatively insensitive to the toxin, although both species were highly susceptible to the toxin-producing isolates of C. cassiicola. Bliss et al. (3) and Renish et al. (18) have used this toxin successfully to identify

cultivars of tomato resistant to C. cassiicola. However, Onesirosan et al. (17) found that the toxin could not be used to screen a segregating population since the assay did not distinguish between moderately and highly resistant individuals.

At the time that C. cassiicola was first recognized in the United States, Olive et al. (15) distinguished two races of the fungus by their differential responses on soybean and cowpea. The first race (race 1) produced extensive lesions on cowpea leaves but only moderate spotting on soybean. Race 2 was reported to produce only light spotting on both cowpea and soybean. In later work, however, there were conflicting results. Stone and Jones (24) discovered isolates of C. cassiicola on sesame and soybean that did not correspond to the two reported races, and postulated that there was a third race of the fungus. Working with cotton, sesame, cowpea, and soybean, Jones (9) later found no differences between isolates. Spencer and Walters (23), working only with cowpea and soybean, found that their isolates corresponded to the races 1 and 2 originally described by Olive et al. (15).

In an attempt to evaluate the problem, Spencer and Walters (23) examined the responses of several C. cassiicola isolates on cowpea and soybean, and also tested pathogenicity of the fungus on several other species. Their results confirmed, by differential responses on cowpea and soybean, the existence of two physiological races of C. cassiicola. They also suggested the existence of a variant at the species level, different from the two races on soybean and cowpea. However, Onesirosan et al. (16) tested several isolates of

C. cassiicola and were unable to determine the existence of two races. These workers found isolates highly virulent on soybean, cotton, and sesame, but weakly virulent on cowpea, corresponding to neither of the reported races.

MATERIALS AND METHODS

Isolates of C. cassiicola

Four of the isolates of C. cassiicola used in experiments were obtained from Dr. Alleah B. Haley at the University of Wisconsin, Madison, Wisconsin. These isolates from cucumber were designated CC1, CC2, CC3, and CC5.[1] A fifth isolate received from the American Type Culture Collection, labeled ATCC 16718, was originally found on azalea by E. K. Sobers (22).

The isolate that prompted this study was obtained from tobacco field soil in Amelia, Virginia, and labeled VI. Spores of this isolate of C. cassiicola were found by Dr. J. A. Fox, while using a sugar flotation technique to assay for nematodes in tobacco field soil Dr. Martha K. Roane later identified the fungus as C. cassiicola. Color descriptions of isolates were indexed according to standards set by ISCC-NBS (10,11), Royal Horticultural Society (1), and Munsell Color Co. (13).

Media

Media used and their methods of preparation are listed in Table 1. Eleven solid and eight liquid media were employed. Three of the media were variations of potato dextrose, eight were variations of tobacco extract and eight others were variations based on V8 Juice.

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[1]Haley, personal communication.

Table 1. Media Used to Grow Corynespora cassiicola.

1. Potato Dextrose Agar (PDA)
   This formula was the same as that found in Riker and Riker (19).[a]

2. Acidified Potato Dextrose Agar (Acid PDA)
   The procedure was the same as that for PDA with the addition of 10 ml of 25% lactic acid per liter of PDA after the medium was autoclaved.

3. Tobacco Extract Agar (TEA)
   Thirty-five g fresh tobacco leaf was ground with 500 ml distilled water in a Waring Blender. The resultant mixture was strained through a double layer of cheesecloth and 16 g agar was added. The volume was then adjusted to one liter with distilled water and the medium is autoclaved.

4. Tobacco Extract Plus Dextrose Agar (TEDA)
   Prepared the same as TEA with the addition of 20 g dextrose before autoclaving.

5. Tobacco Extract Plus Yeast Extract Agar (TEYA)
   Prepared the same as TEA with the addition of 20 g yeast extract before autoclaving.

6. Tobacco Extract Plus Dextrose Plus Yeast Extract (TEDYA)
   Prepared the same as TEA with the addition of 20 g dextrose and 20 g yeast extract before autoclaving.

7. V8 Juice Agar (V8A)
   300 ml V8 juice was brought to a one liter volume with distilled water. Sixteen g agar was added and the mixture was autoclaved.

8. V8 Juice Plus Dextrose Agar (V8DA)
   Prepared the same as V8A with addition of 20 g dextrose before autoclaving.

9. Double V8 Juice Agar (V8PA)
   Prepared the same as V8A but using 600 ml V8 juice per liter of medium.

10. 30% V8 Juice Plus Dextrose Agar (1/3V8DA)
    Prepared the same as V8DA but using 100 ml V8 juice and 6.5 g dextrose per liter of medium.

Table 1 (continued).

11. Difco Nutrient Agar (NutAg)
    37 g Difco nutrient agar was added to one liter distilled water and autoclaved.

12. Liquid Media
    All liquid media were prepared in the same manner as their solid counterparts, but without agar. The list of liquid media used included: tobacco extract broth (TEB), tobacco extract plus dextrose broth (TEDB), tobacco extract plus yeast extract broth (TEYB), tobacco extract plus dextrose plus yeast extract broth (TEDYB), V8 juice broth (V8B), V8 juice plus dextrose broth (V8DB), double V8 juice broth (V8PB), and potato dextrose broth (PDB).

[a]All media were autoclaved at 121 C and 15 psi for 20 min.

Maintenance of Cultures and Methods of Inoculating Media

For standard maintenance of isolates, cultures were kept on PDA and V8DA (Table 1) in 9 cm petri dishes. Isolates were transferred at 5-6 week intervals and compared periodically with cultures kept on PDA at 4 C. Inoculum for growth and pathogenicity studies was grown on V8DA at 25 C for 14 days.

In growth studies, solid media were kept in 9 cm wide petri dishes (20 ml per plate) while liquid media (50 ml per flask) were contained in 250 ml Erlenmyer flasks. Both solid and liquid media were inoculated with 3 mm plugs cut with a sterile cork borer from margins of two-week old cultures grown on PDA or V8DA at 25 C. In growth studies isolates were incubated in the dark, and all liquid cultures were maintained in a stationary position.

At the termination of each study in which liquid media were used the contents of each flask was poured onto a tared filter paper in a Buchner funnel to which a suction had been applied. Excess medium was washed from the mycelial mats with distilled water. The fungal mats were then allowed to air-dry overnight on the filter papers, after which they were individually weighed on a Mettler analytical balance.

Growth of Plant Species Employed in Pathogenicity Studies

Plant species used for host range studies are listed in Table 2. Plants were grown in sterile potting mixture (2:2:1 v/v/v weblite: vermiculite:peat moss) fertilized with Osmocote (14-14-14 controlled release fertilizer). All plants were grown under greenhouse conditions in 22 cm clay pots, with no monitoring of temperature and humidity.

Table 2. Cultivars of Tobacco and Selected Plant Species Used in Experiments.

Common Names	Latin Binomials	Cultivars
Bean	Phaseolus vulgaris L.	'Kentucky Wonder'
Broadbean	Vicia faba L.	
Cowpea	Vigna sinensis (Torner) Savi	'Early Ramshorn'
Pea	Pisum sativum L.	'Little Marvel'
Peanut	Arachis hypogaea L.	'Florigiant'
Soybean	Glycine max L.	'Lee'
Eggplant	Solanum melongena L.	
Tobacco	Nicotiana tabacum L.	'NC 95' 'VA Gold' 'Vesta 5' 'VA 115' 'Coker 187' 'Coker 86' 'NC 88' 'NC 2326' 'Speight G-28' 'Bel-W-3' 'Burley 21' 'Beinhart 1000'
Tomato	Lycopersicon esculentum Mill.	'Rutgers' 'Tiny Tim'
Pepper	Capsicum frutescens L.	'Emerald Giant'
Cucumber	Cucumis sativus L.	'Ashley'
Pumpkin	Cucurbita pepo L.	'Sugar Pumpkin'
Squash	Cucurbita pepo var. melopepo (L.) Alif.	'Straight Neck'
Watermelon	Citrulus vulgaris Scrad.	'Jubilee'
Carrot	Daucus carota L.	'Supreme Danner's'
Corn	Zea mays L.	'Silver Queen'
Oats	Avena sativa L.	'Windsor'
Sunflower	Helianthus annuus L.	

Standard Methods Used in Pathogenicity Studies

Three methods of inoculum preparation were employed in pathogenicity studies:

(1) For use in stem and root rot studies fungal slurries of C. cassiicola were prepared by grinding the contents of two-week old cultures in a Waring Blender with distilled water. Five plates were blended with 500 ml distilled water.

(2) For stem inoculation, 3 mm plugs were cut from two-week old cultures of C. cassiicola grown on V8DA.

(3) In seed germination tests and foliar inoculations, 5 two-week old cultures were ground in a Waring Blender with 500 ml distilled water and filtered through a double layer of cheesecloth leaving a suspension of conidia and mycelial fragments.

Four techniques were used for inoculation of plants:

(1) Fungal slurries, prepared as previously discussed, were poured into pans of water to which roots of plants were growing.

(2) One hundred ml of fungal slurry were applied to the potting mixture at the base of the stem of plants. The entire surface of the potting mixture was then covered with wet cheesecloth to provide a humid atmosphere.

(3) Wounds approximately 2 mm deep and 10 mm long were made with a sterile scalpel at the base of the stems of plants. Three-mm plugs of fungal inoculum were placed in the wound, secured with moistened cheesecloth and wrapped with

parafilm to seal the inoculations.

(4) Fungal slurries strained through cheesecloth were applied to foliage of plants as a spray. Prior to inoculation plants were wetted with a suspension of 1 ml mineral oil brought to a volume of 100 ml with distilled water. Both mineral oil and inoculum were applied with an atomizer to the foliage until it was thoroughly wetted.

All plants were incubated under greenhouse conditions after inoculation. Regardless of the technique utilized, plants were observed for symptom expression for a period of 30 days after inoculation.

The criteria for evaluation of symptom development were as follows:

(1) In root pathogenicity studies, roots were considered suspect when they were stunted or exhibited dark lesions.

(2) Basal stem inoculations were evaluated by observing for symptoms of wilting and lesion development.

(3) In studies where stems were wounded and mycelial plugs were inserted, plants were rated positive for disease development if the lesions were 1 cm or longer.

(4) Foliar inoculations with strained fungal slurries were evaluated for the presence of leaf lesions.

(5) Seed germination was evaluated by measuring the growth of radicles and hypocotyls at the termination of the incubation period.

In all of the studies, pathogenicity was expressed as the percentage of plants exhibiting symptom development. Attempts were made to

isolate the fungus from plants exhibiting symptoms. Suspect tissue was rinsed with distilled water, dipped in 10% chlorox for 30 sec, then plated out on acid PDA. Disease development was expressed as the percentage of plants with symptoms, but no severity ratings were made.

Growth and Morphology of the Virginia Isolate

The VI isolate of C. cassiicola was examined on several media for characteristics of growth and morphology. In order to ascertain the macro- and microscopic colony characteristics of the VI isolate the fungus was grown on all of the media listed in Table 1, with the exception of acid PDA. The colonies were evaluated macroscopically for size, shape, color, and sporulation. Colonies of the isolate grown on V8DA were observed in the light microscope, and photographs were used to record conidial characteristics at 40 X and 125 X. Size, shape, color, septation, wall width, and the hilum of spores was carefully recorded.

The VI isolate was grown on several tobacco extract media at different temperatures to observe mycelial dry weight increases and radial growth. To determine mycelial dry weight production, the fungus was grown on four media (TEB, TEDB, TEYB, and TEDYB) at four temperatures (15, 20, 25, and 30 C) in incubators. Flasks containing 50 ml of media were inoculated as previously described. The fungus was harvested at 5, 10, 15, and 20 days after inoculation, dried on tared filter papers, and weighed on a Mettler analytical balance as mentioned previously. There were four replications for each time-

temperature-medium combination used in the experiment.

The VI isolate was grown on solid media in order to compare radial growth to dry weight accumulation. The media used were: TEA, TEDA, TEYA, and TEDYA. Petri dishes containing the solid media were inoculated as described previously and incubated for a period of 20 days at 15, 20, 25, and 30 C. There were four replications of each medium-temperature combination. At termination of the incubation period, radial growth of the fungus was measured (in mm) by drawing two lines perpendicular to one another crossing through the center of each petri dish, and taking an average of the distance the mycelium grew in four directions.

Comparison of Growth and Morphology of Six Isolates of C. cassiicola

Six isolates (CC1, CC2, CC3, CC5, ATCC 16718, and VI) of C. cassiicola were grown on several media to compare dry weight accumulation and radial growth. A temperature of 24 C was selected for all tests based on previous findings.

Dry weight accumulation was determined on four liquid media. These were: V8B, V8DB, V8PB, and PDB. Fifty ml of medium was contained in each 250 ml Erlenmyer flask. Flasks were inoculated with mycelial plugs and placed in incubators at 24 C for a period of 15 days. There were four replications for each isolate-medium combination. At the end of the incubation period the mycelial mats were harvested and weighed as previously described.

To compare radial growth, the six isolates of C. cassiicola were grown on four solid media (V8DA, V8PA, 1/3V8DA, and NutAg). After

inoculation of the media, the cultures were incubated for a 15 day period at 24 C. At the termination of the growing period radial growth was measured according to the method described for the VI isolate grown on tobacco extract media.

Pathogenicity of the Six Isolates

Pathogenicity on Tobacco and Tomato Roots

Three isolates (CC3, ATCC 16718, and VI) of C. cassiicola were tested for their ability to infect roots of three cultivars (VA. Gold, NC 95, and Vesta 5) of flue-cured tobacco and one cultivar (Rutgers) of tomato. Tobacco seedlings were transplanted into muffin pans containing sterile potting medium. The muffin pans were placed in metal pans filled with water and holes were cut in the depressions (cups) of the muffin pans as described by Troutman et al. (25), allowing the roots of tobacco and tomato seedlings to grow downward into the water. Five-week old tomato seedlings and tobacco seedlings at the eight-leaf stage were inoculated by pouring 100 ml of fungal slurry into the water in the dissecting pans. One pan of control plants for each cultivar of tobacco and tomato were grown under the same conditions but not inoculated. Plants in three pans were inoculated with each isolate. Plants were observed for a period of 30 days for symptom development. At this time plants were visually evaluated for wilting and root lesions. Suspect root lesions were plated out on acid PDA as previously described for verifying the presence of the fungus. The portion of the study dealing with tobacco was repeated twice while the tomato cultivar was tested once.

Basal Stem Inoculation of Selected Plant Species

Two isolates (CC3 and VI) of C. cassiicola were evaluated for their ability to infect the basal portion of stems of selected plant species. These plant species included: cucumber, squash, watermelon, NC 95 tobacco and Rutgers tomato. Plants were grown in 22 cm clay pots in sterile potting mixture. Inoculations were made at the eight-leaf stage for tobacco, six weeks after seeding for tomato, and three weeks after seeding for other species. Fungal slurries were prepared, poured onto the surface of the potting medium, and covered with cheesecloth as previously described. There were four replications of each species-isolate combination and four control plants for each species. Control plants were uninoculated but covered at the base of the stem with moistened cheesecloth. Plants were observed for a period of 30 days for symptoms of wilting and stem rot development. At the end of this period, lesions development was evaluated and symptomatic tissue plated out on acid PDA to verify results. This study was repeated once for each isolate-species combination.

Stem Wounding Inoculation

The six isolates of C. cassiicola were evaluated for their ability to incite stem lesions on twelve cultivars (NC 95, VA Gold, Vesta 5, VA 115, Coker 187, Coker 86, NC 88, NC 2326, Speight G-28, Bel-W-3, Burley 21, and Beinhart 1000) of tobacco. Plants were grown in 22 cm clay pots in sterile potting medium. Wounds were made and mycelial plugs inserted into the stems of plants at the eight-leaf stage as previously described, with four plants used for each isolate-cultivar combination. The two uninoculated controls for each

cultivar were wounded and wrapped. This test was repeated four times each for NC 95, and VA Gold, and twice for the other cultivars. After inoculation, plants were observed for a period of 30 days, after which lesion development was evaluated and isolations made from suspect tissue.

Foliar Inoculations

The six isolates of C. cassiicola were tested for their ability to produce foliar disease symptoms on four cultivars (NC 95, VA Gold, NC 2326, and Coker 86) of tobacco, cowpea, soybean, Rutgers and Tiny Tim tomato, pumpkin, oats, squash, cucumber, pepper, watermelon, peanut, sunflower, eggplant, and pea. Inoculations were made at the eight-leaf stage for tobacco, six weeks after seeding for tomato, and three weeks after seeding for other species. The leaves were wetted with mineral oil and inoculated with strained fungal slurries (containing conidia and mycelial remnants) as previously described. There were four replications for each cultivar-isolate combination and two uninoculated control plants for each cultivar. Controls were left uninoculated but sprayed with mineral oil. Plants were observed for 30 days for lesion development on the foliage and isolations were made from margins of suspect tissue. This study was run once for all cultivar-isolate combinations with the exception of CC3 and VI on NC 95 and Coker 86, and all six isolates on squash, pumpkin, watermelon, cucumber, sunflower, cowpea, and soybean, which were repeated once each.

Statistical Analysis

Data for growth studies and seed germination tests was analyzed as summarized in Table 3. Analysis of variance of growth data was done using computerized Statistical Analysis System (SAS 76) programs. All parameters used in growth experiments were independent of one another necessitating use of factorial procedures for the analyses. In addition to the analysis of variance, parameters labeled factor (A) in Table 3 were compared in each growth experiment to determine if there were statistical differences in the means, by using Duncan's Multiple Range Test for Variable Yield at a significance level of 5%.

Seed germination experiments were analyzed using t-tests to determine significant differences between the means of inoculated and uninoculated seed. The level chosen to test significance was set at 5% using statistical tables from Rohlf and Sokal (20).

Table 3. Summary of Procedures for Analysis of Variance of Growth Study Data and Significance Tests for Seed Germination Studies.

Title of Experiment	Parameters and Tests
1. Growth of C. cassiicola (VI) on four liquid media at four temperatures.[ab]	Three way factorial with factors: A = Temperature B = Medium C = Time period of growth
2. Growth of six isolates of C. cassiicola on liquid media.[ab]	Two way factorial with factors: A = Isolate B = Medium
3. Growth of six isolates of C. cassiicola on solid media.[ab]	
4. Growth of C. cassiicola (VI) on four solid media at four temperatures.[ab]	Two way factorial with factors: A = Temperature B = Medium
5. Effect of C. cassiicola (VI and CC 3) on seed germination.	T-tests were used to determine significant differences between inoculated and uninoculated seed at the 0.05 level.

[a]Factor (A) was analyzed for differences between means at the 5% level of significance using Duncan's Multiple Range Test for Variable Yield.

[b]Procedures for Analysis of Variance and Duncan's Multiple Range Test taken from Barr, A. J., J. H. Goodnight, J. P. Sall and Jane T. Helwig. 1976. A User's Guide to SAS 76. Sparks Press, Raleigh, N.C.

RESULTS

Growth and Morphology of the Virginia Isolate

The following is a description of the VI isolate of C. cassiicola. Colonies are grayish-green to greenish-black (R.H.S. 199 A, ISCC-NBS 95 or Munsell 8.0 GY 2.2/3.6) with a mycelium that grows mostly submersed and does not appear hairy on most media. Conidiophores are hyaline to pale brown (R.H.S. 153 A, ISCC-NBC 100 or Munsell 9.2 Y 5.9/9.2), straight, and grow erect. Conidia are straight to slightly curved, thick-walled, pale-brown to olive green (R.H.S. 152 C, ISCC-NBS 100 or Munsell 9.2 Y 5.9/9.2), 4-20 pseudoseptate, 350-450 μ long, 12-20 μ thick at the widest point, and filiform to obclavate in shape. The hilum is dark and pronounced. Radial growth of the isolate is extremely slow (1.0-1.5 mm per day) but regular, forming circular colonies on solid media. Sporulation and subsequent spore maturation give colonies a dark coloration on most media. When grown on media in the absence of an abundant carbon source, mycelial growth is extremely sparse and sporulation practically non-existent.

Colony Characteristics of CC1, CC2, CC3, and CC5

Colonies are gray to grayish-brown (R.H.S. 199 A, ISCC-NBS 95 or Munsell 8.0 GY 2.2/3.6), thinly hairy, with a mostly submersed mycelium. Conidiophores are hyaline to pale brown (R.H.S. 152 C, ISCC-NBS 100 or Munsell 9.2 Y 5.9/9.2), straight, and erect on V8DA medium. Conidia are straight to slightly curved, moderately thick walled, hyaline to pale brown, 4-20 pseudoseptate, 350-500 μ in

length, 8-15 μ thick at the widest point, and filiform in shape. The hilum at the base of the conidium is not particularly evident.

Isolates CC1, CC3, and CC5 show regular, rapid (5-6 mm per day) radial growth on solid media, forming circular colonies. CC2 grows irregularly radially forming roughly elliptical colonies with uneven edges. The CC2 isolate may be differentiated macroscopically from CC1, CC3, and CC5 based on this attribute. All of these isolates form concentric rings of gray (R.H.S. 197 A, ISCC-NBS 110 or Munsell 8.0 Y 3.6/2.0) and brown (R.H.S. 199 A, ISCC-NBS 95 or Munsell 8.0 GY 2.2/3.6) caused by differential spore maturation.

Colony Characteristics of ATCC 16718

Colony morphology of ATCC 16718 is very similar to CC1, CC2, CC3, and CC5 of C. cassiicola. The basic difference between this isolate and the others involves conidial characteristics, which will be discussed later. The ATCC 16718 isolate grows very rapidly and shows regular growth, forming circular colonies in which the mycelium is slightly submersed in the medium. Coloration of the colonies is usually grayish-brown (R.H.S. 199 A, ISCC-NBS 95 or Munsell 8.0 GY 2.2/3.6) depending upon the medium in which the fungus is grown.

Comparison of Spore Morphology of Isolates of Corynespora cassiicola

All six isolates (CC1, CC2, CC3, CC5, ATCC 16718, and VI) of C. cassiicola examined microscopically show the typical variability in conidial shape described by Wei (26) and Ellis (5). Table 4 is a summary of the conidial characteristics of each isolate.

Table 4. Comparison of Conidial Characteristics of Six Isolates of Corynespora cassiicola.

Isolates	Presence of a Pronounced Hilum	Length in Microns[a]	Secondary Conidial Formation	Spore Color
CC1	NO	350-500	NO	Olive
CC2	NO	350-500	NO	Olive
CC3	NO	350-500	NO	Olive
CC5	NO	350-500	NO	Olive
ATCC 16718	YES	350-500	YES	Hyaline
VI	YES	350-450	YES	Olive

[a]Spore length was based on a minimum of 35 measurements for each isolate.

The conidia of all isolates are extremely long, ranging from 350-500 μ in length. However, the VI isolate (Fig. 1) is generally slightly shorter (350-450) and less filiform than the others. Both the VI isolate and ATCC 16718 exhibited the ability to form intercalary secondary conidia. This phenomenon was not observed for CC1, CC2, CC3, (Fig. 2) or CC5. In addition, VI and ATCC 16718 both possess a dark, pronounced hilum as previously described. Conidia of all isolates showed a pale-green (R.H.S. 152 C, ISCC-NBS 100 or Munsell 9.2 Y 5.9/9.2) to brown (R.H.S. 199A, ISCC-NBS 95 or Munsell 8.0 GY 2.2/3.6) coloration at maturity with the exception of ATCC 16718 which remains hyaline to slightly gray (R.H.S. 197 A, ISCC-NBS 110 or Munsell 8.0 GY 2.2/3.6) through maturation.

Radial Growth of the VI Isolate

Table 5 gives a summary of the results of radial growth study of the VI isolate on solid tobacco extract media. Both parameters used in the test (temperature and medium) were found to have a statistically significant effect on growth at the 0.1% level as analyzed by analysis of variance. The average radial growth at all four temperatures was found to be similar for TEDA, TEYA, and TEDYA. However the fungus grown on TEA medium which lacked the additional carbon sources showed less radial growth than the others. For all of the media average radial growth was greatest at 25 C.

Radial Growth of Six Isolates

The results of radial growth studies on the various V8 media and nutrient agar, based on an average of four replications (in cm),

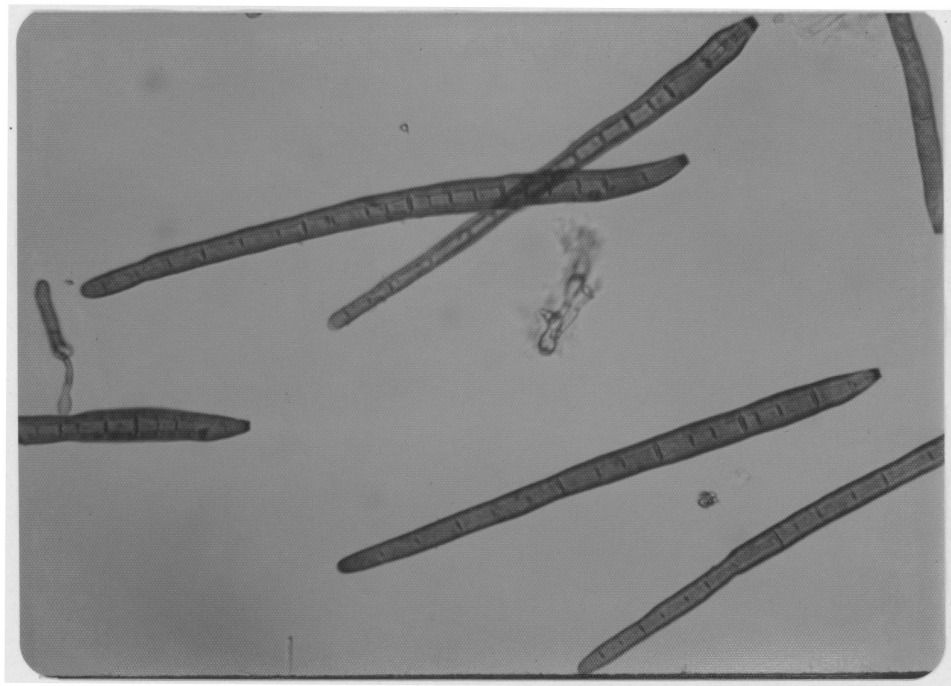

Figure 1. Conidia of the VI isolate (magnification 40 X).

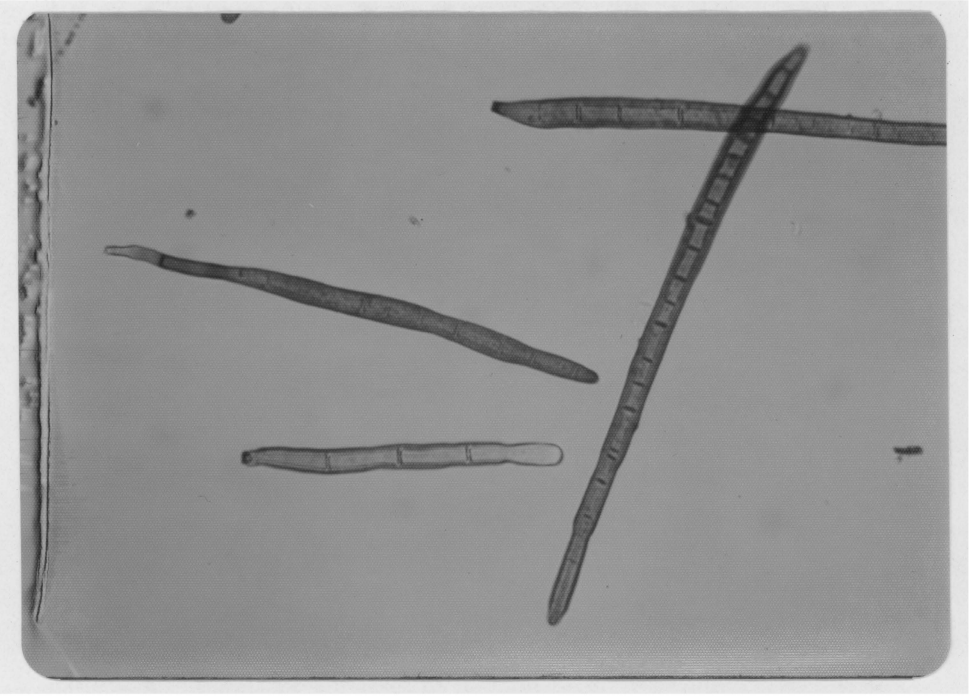

Figure 2. Conidia of the CC3 isolate (magnification 40 X).

Table 5. Radial Growth of the VI Isolate of Corynespora cassiicola Grown at Four Temperatures on Solid Media.

Growth Media	Temperatures (C) 15	20	25	30
TEA	1.45 [a]	1.51	1.49	1.44
TEDA	1.68	1.88	1.91	1.61
TEYA	1.68	1.91	1.94	1.68
TEDYA	1.75	2.05	2.31	1.68
AVERAGE	1.64 C[b]	1.84 B	1.91 A	1.60 C

[a]Growth measured in cm after a 20 day period, as an average of four replications.

[b]Averages followed by the same letter are not significantly different.

are in Table 6. Analysis of variance showed both the isolate and media parameters to have a significant effect on growth at the 0.1% level. The average growth for all isolates was found to be greatest on 1/3V8DA. Growth of the VI isolate was significantly less than all other isolates.

Dry Weight Accumulation of the VI Isolate

Results of growth of the VI isolate on liquid tobacco extract have been summarized in Table 7. All parameters used in the test (temperature, medium, and growth period) were found to have a statistically significant effect on growth at the 0.1% level, according to the analysis of variance. At all four temperatures employed the greatest mycelial dry weight increases were observed in the TEDYB as compared to all other media. Over the whole range of media used, growth was found to be best at 20 and 25 C. When the effect of the temperature parameter was analyzed with Duncan's Multiple Range Test, there was no statistical difference between growth at 20 and 25 C. However, growth at these two temperatures was significantly greater than at 15 or 30 C.

Growth of Six Isolates on Liquid Media

Dry weight accumulation of the six isolates is summarized in Table 8. As measured by analysis of variance both parameters (isolate and medium) used in the test were found to have a statistically significant effect on growth at the 0.1% level. Growth was found to be greatest on V8PB when comparing average dry weight accumulation of all isolates. There was no significant difference between isolates

Table 6. Radial Growth of Six Isolates of Corynespora cassiicola at 24 C on Solid Media.

Growth Media	Isolates					
	CC1	CC2	CC3	CC5	ATCC 16718	VI
V8DA	3.33[a]	2.28	3.30	3.11	2.90	1.45
V8PA	3.39	2.76	3.30	3.58	2.92	1.41
1/3V8DA	3.44	2.82	3.49	3.02	3.16	1.51
V8TA	3.44	2.37	3.32	3.56	2.55	1.42
NutAg	2.44	2.11	1.93	1.72	1.85	1.45
AVERAGE	3.21 A[b]	2.47 D	3.07 AB	3.00 B	2.68 C	1.45 E

[a]Growth in cm after a 15 day period, as an average of four replications.

[b]Averages followed by the same letter are not significantly different.

Table 7. Average Growth by Dry Weight Accumulation of the VI Isolate of Corynespora cassiicola in Tobacco Extract Media.

Days After Inoculation	Temperatures (C) 15	20	25	30
	Tobacco Extract Broth (TEB)			
5	88[a]	102	130	89
10	83	107	135	61
15	182	217	287	178
20	180	233	341	180
	Tobacco Extract plus Dextrose Broth (TEDB)			
5	225	214	285	132
10	263	322	343	122
15	419	634	559	222
20	364	682	587	232
	Tobacco Extract plus Yeast Extract Broth (TEYB)			
5	150	227	178	122
10	166	273	234	163
15	130	302	297	163
20	126	310	298	183
	Tobacco Extract plus Dextrose plus Yeast Extract Broth (TEDYB)			
5	422	426	353	187
10	428	675	545	158
15	645	1245	753	154
20	1046	1345	985	298
AVERAGE	307 B[b]	440 A	394 A	165 C

[a]Weight in mg as an average of four replications.

[b]Averages followed by the same letter are not significantly different.

Table 8. Growth by Dry Weight Accumulation of Six Isolates of Corynespora cassiicola at 24 C in Liquid Media.

Growth Media	Isolates					
	CC1	CC2	CC3	CC5	ATCC 16718	VI
V8B	723[a]	239	450	478	292	135
V8DB	596	674	716	668	582	209
V8PB	737	1188	1109	1170	943	457
PDB	467	464	395	66	291	71
AVERAGE	631 A[b]	641 A	668 A	595 A	527 A	218 B

[a]Dry weight in mg after a 15 day period, as an average of four replications.

[b]Averages followed by the same letter are not significantly different.

CC1, CC2, CC3, CC5, and ATCC 16718. These five isolates, however, showed statistically greater dry weight accumulation than the VI isolate.

Pathogenicity on Tobacco and Tomato Roots

Table 9 is a summary of the results of root pathogenicity tests with CC3, ATCC 16718, and VI, expressed as the percentage of plants exhibiting lesion development on the roots. Control plants were not symptomatic. Small, dark lesions were found on the roots of VA Gold and NC 95 tobacco, and Rutgers tomato inoculated with the CC3 isolate. The fungus was subsequently isolated from lesions on roots of NC 95 tobacco and the tomato cultivar but not from VA Gold. The only above ground symptom expression observed was on the tomato cultivar, which exhibited moderate wilting.

A small percentage (8.3%) of the tomato plants inoculated with ATCC 16718 exhibited root lesions, but attempts to isolate the fungus from root tissue failed. ATCC 16718 failed to initiate symptoms on any of the other plant species. The VI isolate did not produce above-ground symptoms on any of the species tested. Attempted isolation from root tissue failed to establish the presence of the fungus in the diseased tissue.

Basal Stem Inoculation of Selected Plant Species

Stem lesions were produced on cucumber, squash, and watermelon by the CC3 isolate and successful reisolation of the fungus was made from symptomatic tissue (Table 10). Lesions produced by the CC3 isolate were often superficial and small in size, having little effect

Table 9. The Ability of Three Isolates of Corynespora cassiicola to Cause Root Rot on Selected Tobacco and Tomato Cultivars.

Cultivars	Isolates			
	CC3	ATCC 16718	VI	Control
Tobacco				
cv. VA Gold	8.3[ab]	0.0	0.0	0.0
cv. NC 95	16.7[c]	0.0	0.0	0.0
cv. Vesta 5	0.0	0.0	0.0	0.0
Tomato				
cv. Rutgers	25.0[b]	8.3[a]	0.0	0.0

[a]Fungus was not reisolated from symptomatic tissue.

[b]Numbers given are the percentage of plants exhibiting symptom development.

[c]Fungus was reisolated from symptomatic tissue.

Table 10. Pathogenicity of the CC3 and VI Isolates of Corynespora cassiicola on Tobacco and Selected Plant Species Inoculated at the Base of the Stem.

Plant Species	Isolates		Uninoculated Check
	CC3	VI	
Cucumber	75[ab]	0	0
Squash	75[a]	75[a]	0
Watermelon	75[a]	0	0
Tobacco cv. NC 95	0	0	0
Tomato cv. Rutgers	0	50	0

[a]Fungus was reisolated from symptomatic tissue.

[b]The percentage of plants exhibiting symptoms after days.

on overall growth of the plants. The VI isolate produced stem lesions on squash, and appeared to cause wilting on tomato, but could only be reisolated from squash. Although no controls containing agar medium were used in the experiment, the lack of pathogenicity from the VI isolate indicates that no other symptom-producing contaminant existed in the inoculum source.

Stem Wounding Inoculation

Most plants inoculated by the stem wounding method produced lesions 1 cm or longer, as shown in Table 11. Five of the tobacco cultivars tested exhibited 100% lesion development from all six of the isolates of C. cassiicola. Most lesions were found to be 1.5-3.5 cm in length although some were much longer. Lesions were usually superficial. No plant death was observed for any of the isolate-cultivar combinations, although slight wilting was found with greater lesion development. Figures 3 and 4 show advanced lesion development on the stems of NC 95 tobacco plants inoculated with the VI and CC3 isolates, respectively.

Foliar Inoculations

Results of foliar spore inoculations are summarized in Table 12. Pathogenicity was confirmed (symptom development plus reisolation of the fungus) for: CC1 on cowpea, soybean, tomato cv. Tiny Tim; and squash; CC2 on cowpea, soybean, and tomato cv. Tiny Tim; CC3 on cowpea, soybean, tomato cvs. Rutgers and Tiny Tim, squash, cucumber, watermelon, sunflower, and tobacco cv. Coker 86; CC5 on cowpea, soybean, tomato cv. Tiny Tim, and cucumber; and VI on tomato cv. Tiny Tim, pumpkin,

Table 11. Lesion Development on Tobacco Cultivars Wounded and Inoculated with Six Isolates of Corynespora cassiicola.

Cultivar	Isolates						Control
	CC1	CC2	CC3	CC5	ATCC 16718	VI	
NC 88	100[ab]	100	88	100	75	100	0
NC 95	100	100	100	100	75	75	0
VA Gold	100	100	100	100	100	100	0
Vesta 5	75	75	100	100	75	100	0
VA 115	100	100	100	100	100	100	0
Coker 187	75	75	100	100	100	100	0
Coker 86	75	100	100	100	100	100	0
NC 2326	75	88	100	75	100	88	0
Speight G-28	100	100	100	100	100	100	0
Bel-W-3	100	100	100	100	100	100	0
Beihnart 1000	100	100	100	100	100	100	0

[a]The percentage of plants exhibiting stem lesions one centimeter or longer after 30 days.

[b]The pathogen was reisolated for all cultivar-isolate combinations.

Figure 3. Typical lesion caused by the VI isolate of Corynespora cassiicola when inoculated into a wound on the stem of an NC 95 tobacco plant.

Figure 4. Typical lesion caused by the CC3 isolate of Corynespora cassiicola when inoculated into a wound on the stem of an NC 95 tobacco plant.

Table 12. Pathogenicity of Spore Suspensions of Six Isolates of Corynespora cassiicola on Foliage of Selected Plant Species.

Plant Species or Cultivar	Isolate						
	CC1	CC2	CC3	CC5	ATCC 16718	VI	Control
Cowpea	50[ab]	38[b]	75[b]	50[b]	0	0	0
Pea	0	0	0	0	0	0	0
Peanut	0	0	0	0	0	0	0
Soybean	100[b]	25[b]	75[b]	50[b]	0	0	0
Eggplant	0	0	0	0	0	0	0
Tobacco							
cv. NC 95	0	0	0	0	0	0	0
cv. VA Gold	0	0	0	0	0	0	0
cv. NC 2326	0	0	0	0	0	0	0
cv. Coker 86	0	0	50[b]	0	0	0	0
Tomato							
cv. Rutgers							
cv. Tiny Tim	38[b]	25[b]	100[b]	25[b]	0	100[b]	0
Pepper	0	0	0	0	0	0	0
Cucumber	38[b]	0	68[b]	25[b]	0	0	0
Pumpkin	0	0	0	0	0	89[b]	0
Squash	13[b]	0	88[b]	0	0	38[b]	0
Watermelon	0	0	63[b]	0	0	0	0
Oats	0	0	0	0	0	0	0
Sunflower	0	0	68[b]	0	0	50[b]	0

[a]The percentage of plants exhibiting symptoms after a 30 day period.

[b]Pathogen was reisolated from host tissue.

and squash. Isolate ATCC 16718 produced no symptoms on the plant species tested.

With any of the six isolates tested, lesion development resulting to tomato, squash, and cucumber foliage was severe, with tissue necrosis usually resulting. However, spots on soybean and cowpea foliage initiated by CC1, CC2, CC3, and CC5 were only chlorotic in nature and rarely resulted in death of the plant tissue. Only small chlorotic spots were seen on tobacco cv. Coker 86 inoculated with CC3. The VI isolate was found to cause severe stem and foliar necrosis on pumpkin sometimes resulting in death of the plants.

DISCUSSION

Colony characteristics and spore morphology of isolates of C. cassiicola examined in this study matched descriptions in the literature by Wei (26) and Ellis (5), with one possible exception, isolate ATCC 16718, from the American Type Culture Collection. Spores of ATCC 16718 were found to be hyaline to buff colored, in comparison to the brown to greenish-black conidia of the other isolates examined. If spore color is disregarded, however, the isolates in Table 8 may be divided into two groups: those having both a pronounced hilum and secondary conidia, and those having neither of these characteristics. Isolates ATCC 16718 and VI fall into the former category and CC1, CC2, CC3, and CC5 into the latter classification.

It is interesting that the least pathogenic isolates, ATCC 16718 and VI, should be morphologically similar. Both Stone and Jones (24) and Spencer and Walters (23) have previously postulated the existence, within collections of C. cassiicola, of variants at the species level. It may be that ATCC 16718 and VI, while satisfying most of the morphological requirements for this species, should be placed in a different species because of morphological differences.

Growth of the VI isolate was shown to be much slower than that of the other isolates at 24 C, the optimum temperature for growth of all of these isolates in culture. It does not appear likely that slow growth could be correlated with a lack of isolate pathogenicity, since ATCC 16718 which gave negative results in all pathogenicity tests,

grew much better than VI and equally as well as the other isolates on liquid media. Although the reason for the slow growth was not pursued, a recent review by Hollings (8) gives a possible explanation for this phenomenon. The existence of mycoviruses (or virus-like particles) has been regularly confirmed in aberrant cultures of Helminthosporium spp. Since C. cassiicola is similar morphologically to this genus, the possibility exists that the VI isolate may harbor a mycovirus.

CC3, the most pathogenic isolate, is able to produce responses on nine of the species tested compared to only three for the VI isolate. The less pathogenic isolates tested have a fairly limited host range and fail to cause any reaction on three reported hosts (pepper, eggplant, and pea) of the fungus. A summary of the results obtained is presented in Table 13. The race differentiation of C. cassiicola confirmed by Spencer and Walters (23) based on the response of cowpea and soybean might be invalid, since two isolates, ATCC 16718 and VI, produce no reaction on either plant species. However, based on the work of Seaman et al. (21), the CC1, CC2, CC3, and CC5 isolates, which produce light spotting on both soybean and cowpea, correspond to race 2 of C. cassiicola.

Although all six isolates of C. cassiicola tested produced lesions when inoculated into wounds on several cultivars, only slight wilting and no plant death ever resulted. The possibility exists that C. cassiicola might enter into root rot complexes. The extreme conditions to which plants were subjected, in no way resembled field conditions. In addition, since the VI isolate has never been recovered from tissue of any of the cultivars, this particular isolate lacks

Table 13. Tobacco Cultivars and Other Plant Species Identified as Hosts of Corynespora cassiicola.

Plant Species	Isolates					
	CC1	CC2	CC3	CC5	ATCC 16718	VI
Cowpea	+[b]	+	+	+	-[c]	-
Pea	-	-	-	-	-	-
Peanut	-	-	-	-	-	-
Soybean	+	+	+	+	-	-
Eggplant	-	-	-	-	-	-
Tobacco						
cv. Coker 86	-	-	+	-	-	-
cv. NC 95	-	-	+	-	-	-
Tomato						
cv. Rutgers	-	-	+	-	-	±[d]
cv. Tiny Tim	+	+	+	+	-	+
Pepper	-	-	-	-	-	-
Cucumber	+	-	+	+	-	-
Pumpkin	-	-	-	-	-	+
Squash	+	-	+	-	-	+
Watermelon	-	-	+	-	-	-
Oats	-	-	-	-	-	-
Sunflower	-	-	+	-	-	±

[a]Species were identified as hosts based on symptom development and reisolation of the fungal isolate from either roots, stems, or leaves of the plants.

[b]'+' denotes sympton development and reisolation of the fungus.

[c]'-' denotes no symptom development

[d]'±' denotes symptom development without reisolation of the fungus.

the ability to parasitize tobacco roots and probably will not be a problem in Virginia. However, results of inoculations on wounded tobacco stems indicate that C. cassiicola may act as a wound pathogen. This would become important under field conditions where farm machinery causes mechanical damage to plants near the soil line.

The limited pathogenicity found in all of the isolates might be explained by repeated transfer of the fungus in culture over a period of time, or perhaps virus contamination. The isolates, however, have proven to be pathogenic on some plant species. A better explanation of the results of this study would seem to be that of Onesirosan et al. (16), i.e., C. cassiicola usually acts as a saprophyte.

Because this study was originally initiated in order to determine the pathogenic ability of the VI isolate of C. cassiicola and to determine its possible economic importance, there are two points that should be noted about the fungus. These are:

(1) Certain morphological dissimilarities exist between the VI isolate and four of the other isolates tested. This points out a possible problem in the classification or taxonomy of the species. The VI isolate also grows significantly slower than the other isolates tested.

(2) Pathogenicity of the VI isolate is limited. Because of its apparent avirulence this isolate of the fungus would be rather limited in its ability to cause a problem in the field unless ideal conditions existed. Even in the event that a perfect situation for disease development

materialized, it is doubtful that death of the plant would result.

Based on these two points concerning the VI isolate, it may be assumed that this isolate of the fungus will not become economically important on flue-cured tobacco in Virginia in the near future. The only doubt concerning this assumption is the possible entry of C. cassiicola as a wound parasite, which at this time is only speculative.

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GROWTH AND PATHOGENICITY OF SIX ISOLATES OF *CORYNESPORA CASSIICOLA* (BERK. & CURT.) WEI

by

James Cleveland Adams III

(ABSTRACT)

Corynespora cassiicola, the causal agent of Corynespora leaf spot on tobacco in Nigeria, was isolated from a stand of symptomatic flue-cured tobacco in Virginia. The Virginia isolate of *C. cassiicola* was compared to five other isolates of the fungus to determine relative morphology, growth rate, and pathogenicity on several cultivars of tobacco and selected plant species.

Macro- and microscopic differences were noted in colonies of the six isolates, including differences in colony appearance, spore color, and secondary conidial formation. The Virginia isolate (VI) of *C. cassiicola* was found to be extremely slow growing relative to the other isolates. All isolates tested were found to have limited host ranges. Isolate CC3 from cucumber was pathogenic on nine of the plant species examined, while isolate ATCC 16718 from azalea attacked none. The Virginia isolate was pathogenic on three species but had no effect on the tobacco cultivars tested. The data from growth studies and pathogenicity tests indicate that the Virginia isolate of *C. cassiicola* poses no threat to tobacco production in Virginia.

The possibility exists, however, that this fungus is a wound pathogen, and is currently going undetected in the field.