

ILLINOIS
Natural History Survey
BULLETIN

**A Comparative Study
of Two Components
of the Poinsettia
Root Rot Complex**

by **W. S. Perry**

NATURAL HISTORY SURVEY

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age 437, Figure 3
age 439, Figure 4
age 440, Figure 5
age 441, Figure 7

Should have caption from Fig. 5, page 440

Should have caption from Fig. 3, page 437

Should have caption from Fig. 4, page 439

Caption should read: The colony on the right
was exposed to constant light for 14 days,
while the colony on the left was maintained
in the dark for the same period.

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This paper is published by authority of the State of Illinois, IRS Ch. 127, Par. 58.12. It is a contribution from the Section of Botany and Plant Pathology of the Illinois Natural History Survey. Robert S. Perry is a graduate student in the Department of Plant Pathology, University of Illinois, and a Junior Professional Scientist in the Section of Botany and Plant Pathology, Illinois Natural History Survey.



Frontispiece. — Healthy poinsettias and plants showing disease symptoms. In the upper picture the plant in front was inoculated with *Thielaviopsis basicola* when about 90 days old by the stem-incision method. It shows the curling and browning of leaves and the loss of leaves resulting from the disease caused by this fungus. The other plants were not inoculated and remained healthy. In the lower picture the stunted plants on the right were inoculated with *T. basicola* when about 30 days old by the soil-probe method. The control plants on the left were not inoculated and show normal, healthy growth.

A Comparative Study of Two Components of the Poinsettia Root Rot Complex

Robert S. Perry

THE MOST IMPORTANT pathological problem encountered by commercial growers of poinsettias (*Euphorbia pulcherrima* Willd.) is caused by a complex of fungi composed of *Thielaviopsis basicola* (Berk. & Br.) Ferr., *Rhizoctonia solani* Kuhn, and *Pythium ultimum* Trow. Collectively these three organisms cause a condition referred to as the poinsettia root rot complex. Each of these fungi is capable of producing a disease, or they may occur in combination, producing a disease complex. *R. solani* and *P. ultimum* generally attack young poinsettia cuttings during the early stages of propagation, and early death usually occurs. Symptoms of the disease caused by *T. basicola* usually appear later in the season, often near poinsettia marketing time, resulting in root deterioration and leaf drop. The presence of symptoms of any of the diseases caused by these fungi is not restricted to a specific phase of the plant's life cycle but may appear anytime. Since the three pathogens involved respond differently to fungicides and environmental conditions, control of this complex is difficult.

T. basicola is restricted to attacking the roots of poinsettias, but it is capable of producing infection at almost any point on the roots. An extreme infection may cause the entire root ball to turn black and deteriorate. An advanced symptom of *Thielaviopsis* root rot is a longitudinal splitting of the stem near the ground line. *P. ultimum* commonly produces infection sites near the root tips and causes extensive root deterioration. In the later stages of the disease caused by this fungus, its effects and those produced by the *T. basicola* disease are indistinguishable. *R. solani* attacks poinsettias either at the ground

line or at the transition region between the roots and stem. A ground-line infection usually produces girdling of the stem. An infection at the root-stem transition region usually spreads downward through the root system and results in dead and decaying roots with intact white tips, a condition distinguishable from the other two root rots.

It has been shown that each fungus in this disease complex is strongly influenced by soil temperature (Bateman & Dimock 1959). *Thielaviopsis* root rot is favored by the 13° to 26° C. and *Pythium* root rot by the 13° to 21° C. temperature range. Although these two diseases are restricted by higher temperatures, *Rhizoctonia* root rot is favored by the 21° to 30° C. range. The temperature ranges favorable to these three pathogens coincide with the temperatures favorable to growing poinsettias. Therefore, poinsettias are susceptible to one or more members of the complex at all temperatures suitable for poinsettia growth.

Soil pH appears to have as much influence on the development of the disease complex on poinsettias as has temperature. *Thielaviopsis* (Bateman 1960) and *Pythium* (Bateman 1962) root rots are most destructive in alkaline, neutral, and slightly acidic soils, but they are considerably restricted in highly acidic soils. Soil pH, however, appears to have little, if any, influence on *Rhizoctonia* root rot (Bateman 1963).

The diseases caused by these fungi usually have been investigated individually rather than as a complex. Because of the complications involved in working with three pathogens that frequently occur in different population ratios, obtaining consistent, meaning-

ful data for the complex is difficult. Controls have been found for *P. ultimum* and *R. solani* (Tompkins & Middleton 1950; Raabe & Hurlimann 1970). Although a control has recently been suggested for *Thielaviopsis* root rot of poinsettias (Manning et al. 1970), a single control for the entire complex has not been found.

Since more research has been conducted on *P. ultimum* and *R. solani* than on *T. basicola*, the present research was originally designed to investigate some of the environmental factors affecting the growth of *Thielaviopsis* and the development of the root rot caused by it. However, another fungus, *Chalaropsis thielavioides* Peyronel, frequently was obtained in isolations from diseased greenhouse poinsettias. Since *C. thielavioides* had not been reported as being a part of the poinsettia root rot complex, the author decided to investigate its importance as a pathogen on poinsettias. Numerous similarities between *C. thielavioides* and *T. basicola* were evident. The object of this work was to compare the two fungi. Prior to undertaking such a study, the pathogenicity of *Chalaropsis* on poinsettias had to be established. A comparison of *Chalaropsis* and two isolates of *Thielaviopsis* was made to determine the effects of environment on the growth of the fungi and the ability of the two fungi to produce disease symptoms on poinsettias.

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I gratefully acknowledge the assistance of Dr. W. M. Bever, Professor of Plant Pathology and Head of Department, University of Illinois; Mrs. Betty A. Nelson; and Mrs. Mary A. Sponsky in the preparation of this report. Richard M. Sheets, Natural History Survey Technical Illustrator, drew the graphs, and Wilmer D. Zehr, Survey Technical Photographer, provided some of the photographs. The paper was edited by Robert M. Zewadski, Associate Technical Editor of the Survey.

LITERATURE REVIEW

Thielaviopsis basicola (Berk. & Br.) Ferraris is pathologically important as a member of a root rot complex and as the fungus responsible for the black root rot disease and the internal collar rot disease on more than 120 species of plants in 30 families. *T. basicola* has been reported as a component of a root rot complex on poinsettia (Baker, Davis, & Thomas 1953; Bateman 1962 and 1963; Bateman & Dimock 1959; Dimock 1951; Keller 1954; and Keller & Shanks 1955), citrus (Tsao & Van Gundy 1962), tobacco (Allison 1938;

Stover 1950), and cotton (Blank, Leyendecker, & Nakayama 1953; King & Presley 1942; Leyendecker 1952; Presley 1947; Sherbakoff 1940; and Staffeldt 1959). *T. basicola* is known to produce a root and crown rot on phlox (Peterson 1967). Keller & Potter (1954) have reported that *T. basicola* causes a general root decay of cyclamen, begonia, scindapsus, cineraria, and gerbera. Lloyd & Lockwood (1961 and 1963) reported this fungus on the roots of peas and beans. Ostazeski (1966) reported the stunting and death of bird's-foot trefoil resulting from root-deterioration caused by *T. basicola*.

The influence of various environmental factors on the growth of *T. basicola* in culture and the incidence of disease produced by this fungus have been studied extensively. Bateman (1963) suggested that pH is probably the most influential factor affecting the growth of the pathogen and definitely the most important factor affecting disease development. He reported that *Thielaviopsis* grew best in a range of pH 4.7-5.5 and that cultural growth was retarded on neutral or alkaline media. All of the reports indicate that *Thielaviopsis* is favored by a slightly acid condition. Lucas (1955) found pH to be more influential on the growth of the pathogen in culture than in soils and reported a pH range of 3.9-6.2 for the two media. Keller (1954) found the optimum pH range to be 6.2-7.2 for disease development on poinsettias.

Earlier findings by Rawlings (1940) do not agree with Bateman's suggestions on the importance of the pH factor. Rawlings found pH to exert little influence on the colony type. He reported that pH had a greater influence on the amount of growth (based on colony diameter) than on the colony type or components of the colony. Rawlings also stated that growth on basic agars (pH 7.0-8.0) was greater than growth on acidic agars (pH 4.0-5.0). More chlamydo spores were produced on acidic agars than on basic agars.

The effect of temperature on the

growth of *Thielaviopsis* in culture and on the development of the disease brought about by this organism have been studied by numerous investigators. Lucas (1955) and Tsao & Van Gundy (1960) reported that the best growth of the fungus in culture occurred at 21° to 28° C. Bateman & Dimock (1959) reported 21° to 24° C. the optimum temperatures for growth. King & Presley (1942) found the best growth to occur at 30° C., whereas an early report by Rawlings (1940) stated that *Thielaviopsis* grew better at 20° C. than at either 10° or 30° C.

Optimum temperatures for disease development are reported to be slightly lower than optimum temperatures for the growth of this fungus in culture. Peterson (1967) declared 16° to 20° C. to be the optimum range for the development of the disease. In this temperature range stem lesions developed in 4 weeks. At slightly higher temperatures lesions developed after 8 weeks. Keller & Shanks (1955) determined the approximate optimum temperature for disease development to be 10° C. Several researchers have attempted to correlate average soil temperatures with soil types in predicting the incidence of disease (Baker, Davis, & Thomas 1953; Bateman 1963; Johnson & Hartman 1919; and Staffeldt 1959). As a result of their work, it is known that certain types of soils are more conducive to *Thielaviopsis* root rot than are others.

There are few reports on frequency of occurrence of *Thielaviopsis* root rot in a field situation. Presley (1947) reported that in one field of cotton in Mississippi 20 percent of the plants were killed by *T. basicola*.

Reports in the literature concerning the pathogenicity of *Chalaropsis thielavioides* and the incidence of the disease it causes are scant. This fungus has been reported as a pathogen on the roots of roses (Baker 1953), walnut trees (Hamond 1935), Chinese elms (Wright 1942), agave (De La Isla 1962), and lupine (Longree 1940). In general, *Chalaropsis* has been reported

as a fungus that causes the failure of bud and graft unions. Often referred to as the "black mold fungus," *Chalaropsis* frequently produces a fungal mat over the cut surface of the stock to be used in a graft. The impeding presence of the mycelial mat prevents the successful fusion of the stock and scion. Other accounts contain reports of black mold causing grafting failures on roses (Baker & Thomas 1946; Longree 1940; and Milbrath 1946). Baker & Thomas (1946); Lamb, Wright, & Davidson (1935); Wright (1942); and Hamond (1935) stated that *Chalaropsis* is a wound parasite.

Studies on the effect of environmental factors are lacking. Baker & Thomas (1946) reported that the optimum temperature range for growth of *Chalaropsis* in culture was 18° to 30° C.

Boerema (1959) suggested that *C. thielavioides* is an occasional surface contaminant on carrots packaged in perforated polyethylene bags. Lloyd & Lockwood (1962) reported *Chalaropsis* to be a common contaminant on packaged carrots. Since carrot slices are used as an isolating medium for obtaining *Thielaviopsis* from diseased tissues, they warned against mistaking the contaminant *Chalaropsis* for *Thielaviopsis*. Longree (1940) reported numerous similarities between *Chalaropsis* and *Thielaviopsis*. She prepared a good morphological description of *Chalaropsis* and compared several of the different isolates reported prior to 1940.

MATERIALS

Fungi

Two isolates of *Thielaviopsis basicola* were used for testing the effects of environmental factors on the growth and pathogenicity of the fungus. One strain, referred to in this paper as TBT, was obtained from Dr. Peter Tsao of the University of California at Riverside. This culture had been isolated from citrus roots. A second isolate, TBB,

was received from Dr. D. F. Bateman of Cornell University. This culture had been isolated from tobacco and was known to be pathogenic on beans also.

A culture of *Chalaropsis thielavioides* was isolated while attempting to isolate *T. basicola* from diseased poinsettias exhibiting symptoms like those of a *Thielaviopsis* infection. Stock cultures were made by making single-spore isolations from the original cultures. They were maintained in culture by repeated transfer.

Since *C. thielavioides* has been reported to be a contaminant on packaged carrots (Boerema 1959; Lloyd & Lockwood 1962), the *Chalaropsis* may have come from the diseased poinsettia roots or from the carrot slices used as the isolating medium. A check was made on the isolation technique in an attempt to locate the source of the *C. thielavioides*. From every package of carrots used, several sterilized carrot slices were placed in petri dishes. These petri dishes were exposed to the same conditions as were the dishes in which pieces of diseased poinsettia roots had been placed on sterilized carrot slices. *Chalaropsis* was never found on any of the carrot slices that had not been brought into contact with diseased poinsettia root material. In every instance in which *C. thielavioides* grew on the carrot slices, a piece of root tissue was present. Consequently, the fungus must have come from the diseased poinsettia roots.

Media

Several media were used in an attempt to find the most suitable medium for the growth of the two fungi. Commercially prepared Difco media used were lima bean agar, prune agar, malt agar, cabbage infusion agar, cornmeal agar, Noble agar, and potato-dextrose agar. Freshly prepared potato-dextrose agar and carrot-dextrose agar were also used in growth tests. Six media were prepared from living poinsettias. These poinsettia media consisted

of leaf-and-stem-dextrose agar, root-dextrose agar, and entire-plant-dextrose agar. The remaining three were of the same components without the dextrose.

Six flowering poinsettias, each approximately 30 cm tall, were cut between the upper root and lower stem regions in preparing the root agar and the leaf-and-stem agar. The root sections were first thoroughly washed and then macerated in a Waring Blendor with 400 ml of distilled water for 5 minutes. The macerated material was added to 1,600 ml of distilled warm water in which 40 grams of agar had been dissolved. This volume was then divided into two 1-liter portions. Twenty grams of dextrose were added to one 1-liter portion of the mixture. This medium was autoclaved at 121° C. for 45 minutes and then poured into petri dishes. The leaf-and-stem agar was similarly prepared, except that the aboveground portions of six poinsettia plants were used. The entire-plant agar was prepared in the same way, except that the medium contained both the roots and the aboveground portions of the plants.

Two broth cultures were used to test growth on a liquid medium. Potato-dextrose broth and V-8 juice broth were made in the same manner as the counterpart agars were made, except that the agar ingredient was omitted. Into 125-ml flasks were poured 100 ml of each broth. The flasks were plugged and sterilized. Seeding was done by dropping fungus plugs into the broth.

Poinsettias

Nine varieties of poinsettias, Barbara Ecke Supreme, Elisabeth Ecke, Mikkell Pink, Mikkell Dawn, Paul Mikkelsen, Stop Light, Snow Flake, Snow Cap, and Ecke White, varying in flower color from dark red, to pink, to white, were used in establishing and maintaining the pathogenicity of *Chalaropsis thielavioides* and *Thielaviopsis basicola*. Six of the varieties were varieties marketed commercially and three were experimental varieties.

METHODS

Microscopic Examinations

Samples of the fungi were obtained by cutting plugs from colonies on agar and by making needle scrapings of diseased host tissues. Microscopic examinations of the fungi were made with a phase microscope. When samples containing only endoconidia were desired, a petri dish containing a fungus colony on agar was flooded with distilled water and slightly agitated. Drops of the liquid containing the spores were then removed from the dish and placed on microscope slides. Needle scrapings of host tissues were placed in drops of lactophenol on slides. The scrapings were usually composed of both mycelium and spores. Measurements of the fungus structures were made.

pH Studies

A pH range was prepared by altering the components of fresh potato-dextrose agar and V-8 juice agar. Two solutions were prepared: a 25-percent lactic acid solution (pH 2.0) and a sodium hydroxide solution made by dissolving 4 grams of sodium hydroxide in 1 liter of water (pH 12.0). A recorded number of drops of these solutions was then added to each of a group of sterilized petri dishes. Basic media were prepared by adding 5, 10, 15, or 20 drops of the sodium hydroxide solution to petri dishes, each of which contained 20 ml of liquified potato-dextrose agar or V-8 juice agar. The dishes were swirled to insure uniform distribution of the solution and the media. Readings of the pH values were taken with a Beckman pH meter before the seedings and after 10 days of growth. The media, after the addition of the sodium hydroxide solution, varied from pH 5.7 to 9.2.

The lactic acid solution was added in portions of 1, 3, 5, 8, 10, or 20 drops to dishes containing 20 ml of V-8 juice agar. These media, after the addition of the lactic acid, had a pH range of 7.1-2.7. The agars were solidified and the

dishes then inverted to prevent moisture condensation on the petri dish lids.

Tests on the influence of pH on the growth of the isolates were made on samples of the two fungi grown on V-8 juice agar and potato-dextrose agar. These groups consisted of the various pH plates seeded and kept in constant darkness. The plates were maintained in an incubator at a constant 24° C. Diameters of the colonies were measured every 24 hours for 10 days. Each plate was seeded with a plug approximately 2 mm square taken from actively growing areas in stock culture plates. Three replicate samples of each isolate were tested at each pH level.

Temperature Studies

Seeded plates of standard V-8 juice agar were placed in unlighted incubators set at 5°, 10°, 15°, 20°, and 30° C. Linear growth was measured directly along a consistent diameter bisect every 24 hours for 13 days. Three plates of each of the three isolates at each temperature were measured.

Light Studies

Each of six V-8 juice agar plates was seeded with a plug of TBB isolate, six with a plug of TBT isolate, and six with a plug of *Chalaropsis*. Three plates seeded with TBT, three with TBB, and three with *Chalaropsis* were placed in an unlighted incubator at 24° C. The remaining three plates of each isolate were placed at 24° C. in an incubator lighted by a constant light source of 95 footcandles. Daily measurements of the diameters of the growing colonies were made. Samples were taken from the fungal colonies on the 2nd, 7th, 10th, and 14th days of the testing period for microscopic observation.

Placed in each of thirty-six 125-ml flasks were 100 ml of potato-dextrose broth. In another thirty-six 125-ml flasks were placed 100 ml of V-8 juice broth. For each isolate, 12 flasks of potato-dextrose broth and 12 flasks of V-8 juice broth were used. Eighteen

flasks of each broth type were seeded and wrapped in a double layer of aluminum foil. Nine wrapped flasks of each broth were placed on a 144-revolutions-per-minute shaker. Nine flasks of each broth were seeded, placed on the shaker, and exposed to constant light. The remaining 18 flasks of each broth, 9 wrapped in foil and 9 not covered, were placed in the light but not on the shaker. This experiment was conducted at room temperature. Seedings were made by dropping a 2-mm-square plug from an active colony into the broth. Observations were made every 3 days for 15 days.

Pathogenicity Tests

Nine varieties of poinsettias were used in testing the pathogenicity of the two fungi. Cuttings were rooted in sand and then were potted in a soil composed of equal portions of sand, loam, and vermiculite. Poinsettias in the vegetative and flowering stages were inoculated. The vegetative poinsettias averaged 20 cm in height and were growing singly in standard 4-inch pots. The flowering poinsettias varied from 20 to 50 cm tall. These were grown three plants per three-fourth size, 6-inch diameter pot (a pan).

All poinsettias were maintained in a greenhouse at the University of Illinois Floriculture and Ornamental Horticulture Division. The air temperatures varied from 16.7° to 18.3° C. Soil temperatures varied from 15° to 18.7° C. In one brief experiment, the soil temperatures were raised to the 19° to 25° C. range by regulating the air temperature. The plants were watered twice daily and fertilized once daily with a mixture of equal parts (.68 kg, or 1.5 pounds) of ammonium phosphate and potassium phosphate dissolved in 379 liters (100 gallons) of water. Artificial lighting was not used. Shading was used on exceptionally hot days. Most of the pathogenicity tests were conducted during the winter months.

Inocula of each of the three isolates

were prepared by the following method. Four petri dish colonies of the isolate (including the V-8 juice agar, mycelium, and spores) were added to 1 liter of sterile, distilled water and blended for 5 minutes in a Waring Blendor.

Three different methods of placing the fungi in contact with the poinsettias were used. In the soil-drench method, the suspensions were poured over the soil surface in portions of 50 ml per plant (or 150 ml per pan). After the suspension had soaked in, 100 ml of distilled water were poured over the soil.

A second method is referred to as the soil probe. Prior to drenching the soil with 50 ml of inoculum as in the soil-drench method, the root balls of the poinsettias were probed six times with a handled scoopula. This probing served to break some of the roots. The soil around the check plants was drenched with 50 ml of sterile distilled water in place of the inoculum.

The third method of placing the fungi in contact with the poinsettias involved digging the soil away from the crown area of the poinsettias. The exposed upper root-lower stem region was washed with sterile water; the surface was disinfested with 75-percent ethanol; and it was rinsed with sterile, distilled water. A sterilized scalpel was used to make a 1-cm slit into the pith region of the stem. From an actively growing agar colony, a mass of fungus mycelium and spores was removed with a sterilized transfer needle and transferred into the slit. The slit was then covered with petroleum jelly and the soil was replaced. The lower stems of the check plants were disinfested with ethanol, slits were made, the slits were covered with petroleum jelly, and the soil was replaced.

The success of the inoculations was determined from disease symptoms exhibited by the plants and recovery of the fungi from the plants. Comparisons with check plants with regard to general vigor, leaf fall, chlorosis, dwarfing,

premature flowering, lesions, and reduced root growth were considered in evaluating the intensity of the diseases.

Isolations from diseased roots and lower stem lesions were made to recover the pathogens. Although potato and sweet potato slices and non-diseased poinsettia stems and roots were also tried, Yarwood's carrot-slice isolation technique (Yarwood 1946) was the only method successfully used in reisolating the pathogens. Packaged carrots were peeled and sliced into discs 40-70 mm thick. Four of these discs were placed in each sterile petri dish. The dishes were stacked under a bell jar. On top of the stack was placed an open dish containing 10 ml of a gaseous sterilant, propylene oxide (Klarman & Craig 1960). The bell jar was sealed for 24 hours to maintain the concentration of the propylene oxide. The jar was then removed and the unopened dishes were allowed to air for 24 hours to disperse the propylene oxide. Root balls of poinsettias showing symptoms of disease were opened and infected root segments approximately 1 cm long were removed. These segments were surface disinfested by placing them in a 5-percent Clorox solution for 2 minutes and then rinsing them in sterile distilled water. The root segments were placed on the sterilized carrot slices in petri dishes, 10 ml of water were added to each dish, and the dishes were kept on a laboratory table. Daily observations were made for the appearance of fungus growth.

RESULTS

Morphology of *Thielaviopsis basicola*

Thielaviopsis basicola produced both reproductive and vegetative stages abundantly in culture as well as on the host. Characteristically, there were two types of vegetative mycelium (Fig. 1B). The most common type was hyaline, thin-walled, and septate. The younger hyphae varied from 1.9 to 2.6 μ wide. In culture under favorable

conditions,¹ the hyaline mycelium constituted about 90 percent of the vegetative growth and was relatively dense. A second type of mycelium was found in the older areas of the colony, especially in the center, or on the agar surface below the aerial growth. The older hyphae were wider, 2.6–3.9 μ , with dark brown pigmentation.

Both types of mycelia produced spores. The hyaline mycelium produced endoconidiophores in which endoconidia were produced (Fig. 1D). During this investigation, growth under mildly unfavorable conditions resulted in an increase in the amount of thick-walled mycelium. A reduction in the growth of both types of mycelia was caused by intensely unfavorable conditions.

This fungus produced chlamydospores and endoconidia in its reproductive phase. The endoconidia were produced abundantly both on the host and in culture. They were single celled and stoutly cylindrical to barrel shaped with rounded corners (Fig. 1D). The cellular contents appeared to vary with conditions. Under favorable conditions they were composed of a thin, hyaline cell wall enclosing granular contents and two conspicuous vacuoles, one in each end of the cell.

Endoconidial cells produced under unfavorable pH or temperature conditions appeared to be inconsistent in contents. In some cases the contents appeared to be entirely granular or entirely vacuolated, while in others the two vacuoles appeared to be the sole constituents of the cells.

Aging of the endoconidia produced a slight change in shape. The ends of the individual cells transformed from flattened with rounded corners to semi-ovoid. During maturation these cells widened slightly, whereas *Chalaropsis*

endoconidia appeared to elongate slightly.

Endoconidia of the *T. basicola* isolate from citrus averaged 7.59 x 2.37 μ (Table 1). The endoconidia of this isolate varied from 4.56 to 13.11 μ in length and from 2.28 to 2.85 μ in width. Mean dimensions of the endoconidia of the isolate from tobacco were 7.96 x 2.85 μ , with a range of 5.20–11.70 μ long and 2.28–3.42 μ wide. Except for these slight deviations in measurements, the endoconidia from the two isolates were identical. Germ tubes developed from the ends of the cells. In this study germ tubes were not observed developing from the middle region of the cell in either *Chalaropsis* or *Thielaviopsis*.

Thielaviopsis endoconidia were indistinguishable from those of *Chalaropsis*. Generally, the biguttulations of *Thielaviopsis* endoconidial cells were more prominent, but this characteristic was not sufficiently consistent to permit separation of the two fungi.

Thielaviopsis endoconidia were produced within endoconidiophores similar to those of *Chalaropsis*. They were composed of tapering terminal cells (phialides) and one to four basal cells. In the isolates of the fungi studied in this investigation, basal cells varied in length from 6.3 to 9.1 μ and averaged 2.6 μ wide. In the *Chalaropsis* isolate the basal cells of individual endoconidiophores frequently varied slightly in size in such a way that the largest cell was at the end opposite the spore-producing cell. There was a progressive reduction in size of the remaining cells toward the terminal cell.

In the two isolates of *Thielaviopsis* this condition occasionally occurred, but usually all of the basal cells were of the same size. This condition made it difficult to distinguish the termini of the hyphae and the origins of the endoconidiophores. However, endoconidiophores arising from short, lateral branches were easy to differentiate. The tapering terminal cells varied in length from 15.6 to 32.4 μ . Endoconidiophores

¹Throughout this paper references to unfavorable conditions indicate pH, temperature, or light conditions, individually or combined, that were not conducive to the best growth of the fungi. Favorable conditions are the pH, temperature, and light requirements indicated by this study as being most conducive to the growth of the two fungi.

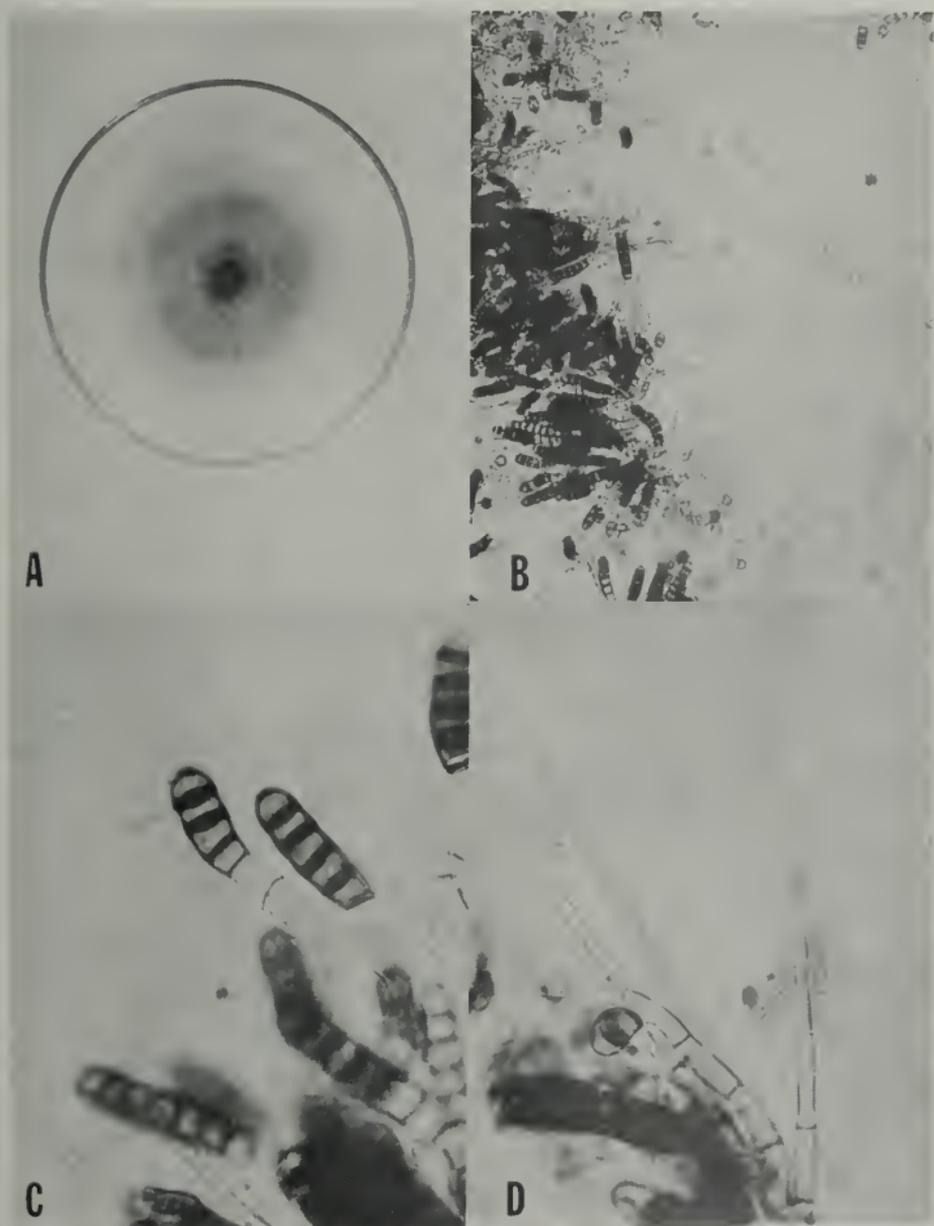


Fig. 1. — *Thielaviopsis basicola*: A, 10-day-old colony on V-8 juice agar; B, colony components — two kinds of spores and two types of mycelia (X 130); C, chlamydospore chains (X 520); and D, endoconidiophores with endoconidia (X 520).

were produced individually or in clusters on terminal tips of hyphae or on stubby lateral branches. Endoconidiophores produced in clusters were of various lengths, because they did not

arise from a common point but from various subbasal, hyphal cells.

Conidia were produced endogenously by the phialides of the conidiophore. Although these spore-producing cells

Table 1. — Comparative measurements, in microns, of spores of the two isolates of *Thielaviopsis basicola* used in this investigation.

| Isolate | Host | <i>Endoconidia</i> | | | | | | <i>Chlamydospore Chains</i> | | | | | | Average Length of Spores | | |
|---------|---------|--------------------|------|---------|---------|------|---------|-----------------------------|-------|---------|---------|------|---------|--------------------------|------|------|
| | | Length | | | Width | | | Length | | | Width | | | Spores Per Chain | Mean | |
| | | Maximum | Mean | Minimum | Maximum | Mean | Minimum | Maximum | Mean | Minimum | Maximum | Mean | Maximum | | | Mean |
| TBT | Citrus | 13.11 | 4.56 | 7.59 | 2.85 | 2.28 | 2.37 | 28.30 | 17.10 | 20.40 | 7.27 | 5.70 | 5.80 | 7 | 5 | 4.41 |
| TBB | Tobacco | 11.70 | 5.20 | 7.96 | 3.42 | 2.28 | 2.85 | 26.00 | 13.00 | 18.20 | 6.50 | 4.80 | 5.70 | 6 | 4 | 3.99 |

produced conidia individually, several conidial cells in different stages of development could be seen within a single conidiophore. Mature cells were extruded singly from the apex. Conidia not dispersed after expulsion from the terminal cells occasionally created chains by adhering to one another end to end. Chains of conidia containing 15 or more cells were observed.

The thick-walled, dark brown, segmented mycelium produced chlamydospore chains. Most of the thin-walled hyphae remained hyaline and did not change. However, some of this mycelium in certain areas of the colony became dark brown, remained segmented, and developed thick walls. The terminal tips of the thicker hyphae developed into single, hyaline, bulbous cells. Other than the enlarged tip, no apparent specialized structures were observed. As the tip cell became more ovoid to elongately ovoid to club shaped, the walls thickened and the entire structure became darkly pigmented. Cross walls developed as the cell elongated to produce a final structure of two to seven chlamydospores (Fig. 1C).

One hundred chlamydospores and 100 chlamydospore chains of each isolate, TBT and TBB, were measured (Table 1). Chlamydospore chains of the TBT isolate averaged 20.4 x 5.8 μ . The largest chain measured 28.3 x 7.27 μ and contained seven spores. The smallest chain measured 17.1 x 5.7 μ and consisted of four spores. The mean length of an individual spore was 4.41 μ .

Chlamydospore chains of the TBB isolate averaged 18.2 x 5.7 μ . The largest chlamydospore chain measured 26.0 x 6.5 μ and was composed of six spores. The smallest measured 13.0 x 4.8 μ and was composed of three spores. The individual spores averaged 3.99 μ in length.

Chlamydospore chains were produced at the ends of long strands of hyphae. Clusters of chlamydospore chains were the result of clusters of hyphal strands rather than the branching of a single

strand. Mature chlamyospore chains were released from the parent mycelium by fragmentation. The chlamyospore chains were released intact with a short fragment of hypha still attached to the basal cell.

Some controversy has developed among plant pathologists over the germination of *Thielaviopsis* chlamyospores. Christias & Baker (1967) reported that before germ tubes could develop, the individual spores comprising the chain had to be separated. Each spore was then believed capable of germination. Their work appeared to demonstrate the function of chitinase in breaking the spores apart so that germination could take place. However, Papavizas & Adams (1969) later demonstrated that intact chlamyospore chains, as well as individual chlamyospores, were capable of germination. They found that the breaking up of chlamyospore chains into individual spores by chitinase was not a necessary prelude to germination. I did not study the germination of chlamyospores. There is no record of a necessary maturation or resting period before germination of *Thielaviopsis* chlamyospores as has been noted in *Chalaropsis* chlamyospores (Longree 1940).

Colony Characteristics of *T. basicola*

Observations were made and data were recorded on the development of *Thielaviopsis basicola* colonies on V-8 juice agar and potato-dextrose agar. More extensive development of vegetative and reproductive structures occurred on V-8 juice agar. Growth of *T. basicola* on V-8 juice agar was luxuriant but slow. A 15–16 day growth period under favorable conditions was required for both of the isolates studied to completely cover the agar surface in a 90-mm diameter petri dish. However, measurable growth was recorded 2 days after seeding.

The first growth appeared as hyaline, vegetative hyphae radiating outward from the point of seeding. Throughout

the development of the colony, the outermost perimeter was composed of these hyaline, nonsporulating hyphae appressed to the agar surface. On the 4th day after seeding, aerial, hyaline mycelia began to be produced around the seeding point and to develop progressively outward. By the 5th day after seeding, endoconidiophores and endoconidia could be found in the aerial hyphae.

Within the next 48 hours the hyphae around the seeding plug began to darken. Microscopic examinations of the fungus in this region revealed that the darkening was brought about by the production of darkly pigmented, thick-walled hyphae. These dark strands predominated the fungal mass but remained close to the agar surface. Pigmentation appeared to vary between dark gray to black and golden brown. Pigmentation did not appear to be consistent with the isolate, as both isolates produced both color types but not within the same colony.

The dark, thick-walled hyphae gave rise to the terminal chlamyospore chains. The chlamyospores were also thick-walled and darkly pigmented in colors corresponding to those of the hyphae from which they had developed. As chlamyospore production continued to increase, endoconidial production decreased.

A fully developed colony consisted of a series of concentric circles of visible differences in growth type (Fig. 1A). The innermost region around the point of seeding was a mass of compacted, semi-aerial, dark-walled hyphae. Thick-walled, dark hyphae and chlamyospore chains composed approximately 95 percent of the fungus material in this region, but occasional hyaline hyphae were found. This center region, about 10 percent of the entire colony area, had a black, matted appearance. The next growth ring constituted about 40 percent of the colony area and was composed of approximately 75 percent dark hyphae and chlamyospore chains

and 25 percent hyaline hyphae and endoconidia. Because of the presence of fewer dark hyphae in relation to the mass of hyaline hyphae and the more aerial nature of the hyaline hyphae, this region had a gray, wool-like appearance.

In the remaining concentric growth patterns, the percentages of dark hyphae and chlamyospore chains comprising the fungal colony decreased progressively as the distance from the point of seeding increased. The proportion of hyaline hyphae and endoconidia increased progressively as the distance from the seeding point increased. Apparently no standard number of concentric growth rings made up a colony. Common borders between the growth rings were not sharp and well defined but were transition zones gradually integrating one ring with the next. Additional characteristics of typical *T. basicola* colonies observed during this study were consistent with those described by Johnson & Valleau (1935).

T. basicola on Poinsettias

Thielaviopsis endoconidia were found on almost all external surfaces of diseased areas on poinsettias. Abundant endoconidia were found on decaying root segments, in lesions on lower stems, and on the inner walls of the lower pith cavities of the stem. These spores were always found on the external or internal surfaces but never embedded within the host tissue. As did those of *Chalaropsis*, the thin-walled, hyaline hyphae ramified within the infected host tissue. Endoconidiophores developed from the hyaline hyphae that were either on the surface of the host or embedded in the diseased tissues. Endoconidial production seemed to be influenced little by environmental factors or host conditions. Endoconidia were found either on or within the host on internal surfaces at any time when symptoms of *Thielaviopsis* infection were present.

Thielaviopsis chlamyospores were produced much less frequently on the host than were the endoconidia. However, the *Thielaviopsis* chlamyospores

were found more frequently on the host than were *Chalaropsis* chlamyospores. Chlamyospore production of *Thielaviopsis* seemed to be related to environmental conditions and the development of the disease. The development of longitudinal lesions on the lower stems of the poinsettia host was a symptom of an advanced stage of *Thielaviopsis* infection. Chlamyospores were found in these lesions. During my investigation, chlamyospores were detected only within the stem lesions, probably because chlamyospore production required light. Masses of thick-walled, darkly pigmented hyphae with multi-spored, terminal chlamyospore chains developed within these lesions.

Morphology of *Chalaropsis thielavioides*

The vegetative stage of *Chalaropsis thielavioides* consisted of septate hyphae that varied in width from 1.2 to 2.6 μ . In culture most of the hyphae were thin and hyaline. As they aged, the cells became slightly wider, and the walls darkened. The reproductive structures were produced on this type of mycelium. Even under favorable conditions the mycelia were sparse. Most of the growth consisted of endoconidiophores arising from the older hyphae. Under unfavorable conditions the mycelia became even more sparse than that produced under normal conditions. The darkening of the mycelium as it aged varied inversely with the intensity of the unfavorable conditions. Under highly unfavorable conditions the mycelium failed to darken and remained thin walled, and vacuoles predominated the segmental contents. The hyphal cells sometimes enlarged to produce bead-like strands of mycelium.

This fungus produced two types of asexual spores, endoconidia and chlamyospores (Fig. 2B). The endoconidia were produced within endoconidiophores (Fig. 2D), each composed of two or three basal cells and a spore-producing cell (phialide). The basal cells varied from 6.5 to 9.1 μ in length and were about 2.6 μ wide. The spore-pro-

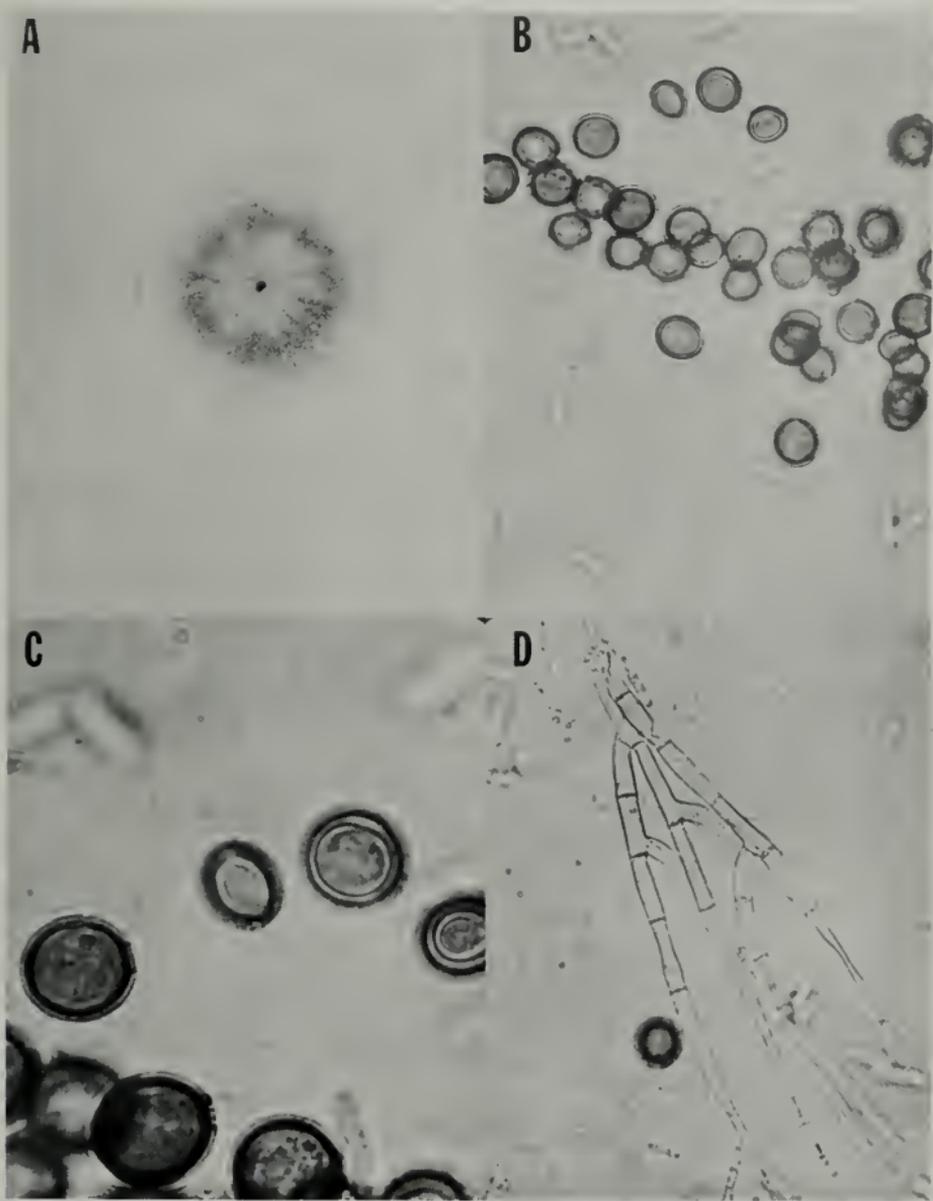


Fig. 2. — *Choloropsis thielavioides*: A, 10-day-old colony on V-8 juice agar; B, components of a typical colony (X 520); C, chlamydospores (X 1235); D, endoconidiophores with endoconidia (X 520).

ducing terminal cell varied from 11.7 to 27.3 μ in length. The tapered phialides produced endoconidia singly. Although several endoconidia in various stages of formation were seen within a terminal cell, the mature endoconidia were extruded from the apex individually. A

stubby side branch occasionally developed from a mature strand of mycelium. One to five endoconidiophores developed from this branch. It was not uncommon to see several conidiophores on one lateral branch (Fig. 2D).

The endoconidia of *C. thielavioides*

Table 2. — Comparative measurements, in microns, of spores of *Chalaropsis thieloviioides* occurring on and isolated from various host plants.

| Authority | Host | <i>Endoconidia</i> | | | | | | <i>Chlamydosporae</i> | | | | | |
|-------------------------------|----------------|--------------------|--------------|------|--------------|--------------|------|-----------------------|--------------|------|--------------|--------------|------|
| | | Length | | | Width | | | Length | | | Width | | |
| | | Maxi- mum | Mini- mum | Mean | Maxi- mum | Mini- mum | Mean | Maxi- mum | Mini- mum | Mean | Maxi- mum | Mini- mum | Mean |
| Peyronel ^a 1916 | Lupine | 55.0 | 8.0 | ... | 4.5 | 3.0 | .. | 20.0 | 10.0 | ... | 15.0 | 8.0 | ... |
| Hamond 1935 | Walnut | 40.0 | 7.5 | 14.7 | 5.5 | 2.0 | 3.7 | 22.0 | 10.0 | 16.5 | 16.0 | 8.0 | 12.1 |
| Hamond 1935 | Carrot | 24.0 | 9.0 | 14.2 | 6.0 | 3.0 | 3.6 | 16.0 | 11.0 | 12.9 | 14.0 | 10.0 | 12.1 |
| Longree 1940 | Rose | 32.6 | 7.0 | 9.5 | 7.4 | 2.6 | 3.3 | 17.0 | 9.6 | 13.5 | 15.4 | 8.0 | 11.4 |
| Longree 1940 | Chinese Elm | 35.4 | 9.5 | 14.7 | 5.1 | 2.6 | 3.9 | 17.6 | 10.2 | 14.4 | 16.0 | 9.9 | 12.9 |
| Perry ^b 1969 | Poinsettia | 13.7 | 3.4 | 7.5 | 2.3 | 1.1 | 1.7 | 8.6 | 6.3 | 7.6 | 8.6 | 6.3 | 7.6 |

^aMeasurements taken from Longree (1940).^bMeasurements rounded off to coincide with measurements from other investigators.

were produced in great abundance on the host and in culture. These cells were found clinging together in chains of a dozen cells or more. Endoconidia germinated at any time after they were released from the conidiophores. Germ tubes were produced from the ends or the sides of the spores. Individual endoconidia were hyaline and biguttulate. They were barrel-shaped to cylindrical with rounded corners. As these cells aged, the ends became slightly more rounded. The endoconidia varied from 3.42 to 13.68 μ long and 1.14–2.28 μ wide, with mean dimensions of 7.50 x 1.67 μ . The endoconidia of minimal dimensions were almost always found in chains. Spore measurements of the isolate used in this work and the spore measurements of isolates used by other workers are compared in Table 2.

A second type of asexual spore, the chlamyospore (Fig. 2C), was produced in fewer numbers than were the endoconidia. Longree (1940) referred to these as macroconidia. These cells were produced only in the older parts of a colony. Short lateral branches developed from cells of the older, pigmented mycelium. The tips of these lateral branches and the terminal tips of the hyphae became bulbous. Longree (1940) reported that macroconidia and macroconidiophores were frequently found clustered at the base of an endoconidiophore. In my investigations I did not find this condition. Macroconidia from the *C. thielavioides* used in this work were always produced individually and not at the bases of endoconidiophores.

Maturing chlamyospores were thin walled and hyaline for a short while, but soon became thick walled and darkly pigmented. The mature chlamyospores were subglobose to ovoid with small flattened bases. Mature chlamyospores were freed from the colony without any portions of the conidiophores still attached to them. Spores freed shortly before maturity often still had a short fragment of conidiophore

attached. Freed, hyaline chlamyospores sometimes had a fragment of conidiophore equal in length to several times their diameters.

The diameter of the chlamyospore varied with the isolate. Mature chlamyospores from the isolate used in this investigation were spherical, with a mean diameter of 7.64 μ . They varied from 6.27 to 8.55 μ in diameter. As indicated by the data in Table 2, other reports of this fungus show the chlamyospores to be more ovoid than spherical.

These spores did not germinate readily. None of the chlamyospores observed showed any evidence of germination. However, the cultures were not maintained for more than a month. Longree (1940) stated that the macroconidia either require a resting period or mature slowly. She found that 2-month-old macroconidia germinated poorly, whereas the percentage of germination was greater in 3-month-old spores. Chlamyospores were produced only after the mycelium had reached a certain stage of maturity or when the mycelium was subjected to unfavorable conditions.

Colony Characteristics of *C. thielavioides*

Chalaropsis thielavioides grows luxuriantly on only a few agar media. Although it does grow on most common agars, the fungus sporulates abundantly and produces an extensive colony on only a few. Good growth was produced on V-8 juice-dextrose-yeast extract agar (Fig. 2A), but even this growth was slow. On V-8 juice agar *Chalaropsis* covered the agar surface in a 90-mm diameter petri dish in 10–12 days under favorable conditions. If a plug of agar with fungal spores and mycelium was placed on agar in a petri dish, measurable growth was observed after 2 days. This first growth appeared as non-sporulating hyphal strands living on or slightly below the agar surface. The vanguard of hyaline mycelia continued outward, preceding the subsequent

colonial development. If a single point of seeding was made on the agar, a circular colony was almost always produced.

For the first few days the colony appeared hyaline, and then it began to whiten. The change to white was brought about by the production of endoconidiophores and endoconidia. These reproductive structures grew with a concentric perimeter slightly (about 5 mm) within the enlarging hyaline perimeter. After 5-6 days of growth under favorable conditions, *Chalaropsis* produced a colony that covered about two-thirds of a 90-mm diameter petri dish. By this time a small, circular halo immediately surrounding the initial plug had turned black. This blackened area consisted of developing and mature chlamydo-spores. The mycelium was almost entirely on the surface of the agar or slightly submerged, and it was pigmented a golden brown. The black color in the colony was due to the dark-brown-to-black pigmentation of the numerous chlamydo-spores. Although the mycelium in the chlamydo-spore area remained on the surface, the surrounding mycelium became more aerial and fluffy. This caused the center area to appear sunken in the white mycelium. After 8-10 days a light gray area constituting about one-half of the colony began to appear in the center of the colony. The gray color was due to the intermingling of maturing chlamydo-spores and the accompanying abundant endoconidia.

The fully developed colony appeared as a series of progressively lighter colored, concentric circles. The innermost circle, about one-tenth of the total area, was black; sunken; and composed of many chlamydo-spores, few endoconidia, and much thick-walled mycelium.

The next zone was fluffy and whitish gray and accounted for about one-half of the colony area. This area consisted of abundant endoconidia, much thin-walled mycelium, and abundant chlamydo-spores. When this area was

scraped from the agar and the surface of the agar was examined, a dark surface growth of thick-walled mycelium was seen radiating outward from the center. This surface growth appeared to terminate after enlarging to about one-half of the area of the entire colony. Chlamydo-spores which grew up through the aerial endoconidial mycelium and caused the graying of this region were produced from this growth.

The next outermost circle was white and, although definitely aerial, not quite as fluffy as the adjacent, inner region. This white area constituted about one-third of the colony and was composed almost entirely of endoconidia, endoconidiophores, and thin-walled hyphae. The surface growth below this region frequently was grayish and contained few chlamydo-spores.

The outermost concentric region made up about one-tenth of the total area. It consisted entirely of surface and subsurface, hyaline, thin-walled hyphae with no spores of either type. Under ideal conditions, the margin of the colony was regular. Abnormal or adverse conditions frequently produced lobed or irregular margins. A 10-day-old *Chalaropsis* colony on V-8 juice agar grown under favorable conditions is shown in Fig. 2A.

C. *thielavioides* on Poinsettias

Signs of *Chalaropsis* were more difficult to find on the poinsettia plant than were signs of *Thielaviopsis*. *Chalaropsis* endoconidia were found in moderate numbers on the external surfaces of the diseased areas of the roots. These spores were also found externally in the areas of the stem where bark had been loosened or sloughed off near the ground line. All of the mycelium produced within the infected host tissues was thin walled and hyaline. This mycelium was difficult to locate in the decaying tissues. It appeared to produce endoconidiophores that protruded through the epidermis of the host tissue and extruded the endoconidia externally.

Chlamydo-spores of *Chalaropsis* were

not produced abundantly on poinsettias. Environmental conditions or host influence may have been responsible for the lack of abundant chlamydo-spores. Chlamydo-spores from the isolate used in this experiment were found only in the pith cavities of the lower stem region of the host. These spores appeared to be restricted to the lowest pith chamber, since they were not found in the upper chambers of the stem. Thick-walled, darkly pigmented hyphae developed on the walls and septations of the lowest chamber. Individual terminal chlamydo-spores were produced from the thick-walled hyphae. No chlamydo-spores were found embedded in the host tissues, on diseased roots, or on any diseased surface exposed to light. Light may have been the critical factor that determined the location of *Chalaropsis* chlamydo-spore production.

Comparison of Growth of *Chalaropsis thielavioides* and *Thielaviopsis basicola* on Various Media

Various media were tested to determine the one most satisfactory for culturing the three isolates. Of the media tested, V-8 juice agar was the most satisfactory. Prepared as suggested by Miller (1955), this agar had a pH range of 7.0-7.25. Both fungi produced vegetative and reproductive structures abundantly on this medium.

Both fungi grew and sporulated equally well on carrot-dextrose agar and V-8 juice agar, but the carrot-dextrose agar was not used because of its more involved method of preparation. V-8 juice can be easily purchased, whereas carrot juice is not so readily available. A juice was easier to use in the media preparation than macerated carrot tissue.

Potato-dextrose agar was not as satisfactory as the V-8 juice agar. Hyaline mycelia and endoconidia were produced on potato-dextrose agar, but chlamydo-spores and dark hyphae were produced infrequently and sporadically. Colonies of both fungi on potato-dextrose agar were sparse and usually occurred be-

low the surface or appressed to the surface.

Testing of the poinsettia agars proved interesting but not too beneficial. No growth was made by either fungus on agar derived from poinsettia tops only, either with or without dextrose. Growth of *Thielaviopsis* on agar derived from the entire poinsettia plants was poor. This growth consisted of light gray, random mycelia on the agar surface. Sporulation did not occur. *Chalaropsis* produced a sparse, aerial mycelium with some endoconidia. Again, the presence or absence of dextrose had little effect. All three isolates grew well on the poinsettia-roots-only agar. Both types of vegetative and reproductive structures were produced abundantly. Growth of all three isolates was noticeably better on the dextrose-supplemented agar. Fungal growth on these two media was comparable to growth on V-8 juice agar. The poinsettia-roots medium was not used, however, because of the extra time and effort required in its preparation.

Growth on lima bean agar, prune agar, malt agar, cabbage infusion agar, cornmeal agar, and Noble agar was negligible. Of all the agars tested, only potato-dextrose agar and V-8 juice agar were used in subsequent experiments.

Two liquid media were tested. Potato-dextrose broth and V-8 juice broth supported good growth of both fungi. *Chalaropsis* produced a complete surface mat in 7 days on V-8 juice broth. *Thielaviopsis* produced a complete mat in 10 days. Both fungi failed to produce a complete mat on potato-dextrose broth.

Neither fungus produced any growth in either of the two broths in shake cultures.

Comparison of Growth Rates

Daily measurement of the colonial enlargement of the three isolates indicated that *Chalaropsis* was the fastest growing. The colony increased at the rate of 8.0 mm in diameter per day. The colony produced was dense and

contained both coarse and fine hyphae as well as both types of spores.

The colonies of both isolates of *Thielaviopsis* increased 5.9 mm in diameter daily. This fungus grows somewhat slower than *Chalaropsis*. The colonies produced were similar to those produced by *Chalaropsis*. They were dense and contained both types of hyphae and both types of spores. The rate of growth of each isolate is shown graphically in Fig. 5.

Effect of pH

Although temperature influenced the amount of growth, pH influenced the type of growth of the fungi reported on here. Conspicuous variations in the morphology of the fungi accompanied variations in the pH of the media. Fig. 3 shows the effect of pH on colony growth.

C. thielavioides had a narrower favorable pH range than either isolate of *T. basicola*. *C. thielavioides* produced some growth at pH 3.9 and fair growth at pH 9.2. In my opinion, based on the amount of growth and quality of the colony, the optimum pH range for *Chalaropsis* was 6.7-8.0 (Fig. 3A). Although colonies on agars with a pH in excess of 8.0 produced normal daily colonial enlargement, the hyaline hyphae were distorted in shape and the endoconidia were nearly oval. An increase in pH to above 8.0 produced an intensification of the distortions. Chlamydo spores and dark hyphae were sparse or absent in all colonies on agar with a pH greater than 8.0. Colonies on agars with a pH between 8.0 and 8.7 were appressed to the agar surface. Colonies on agars with a pH higher than 8.7 were almost entirely submerged and definitely hyaline.

Chalaropsis grew better on basic agar than on acid agar. Although the growth of reproductive and vegetative structures was retarded on agars in the lower pH range, the deforming of structures was not as extensive as it was on the basic agars. Growth was reduced on agars with a pH of less than 7.0.

Colonies varied from dark gray at pH 6.8 to whitish gray at pH 3.8. On none of the acidic agars did the colonies appear hyaline. Their color was due to the dark, thick-walled hyphae intermingled with some hyaline hyphae and endoconidia. At the lower end of the pH range, there were fewer chlamydo spores. The colony produced at pH 3.8 contained occasional thick-walled hyphae but no chlamydo spores. An abundance of endoconidiophores was responsible for the whitish color of this colony.

The type of growth also varied as pH values varied. A decrease in pH produced an increase in the mass of aerial hyphae. Colonies on highly acidic agars appeared fluffy in comparison with the wooly colonies grown at pH 7.0.

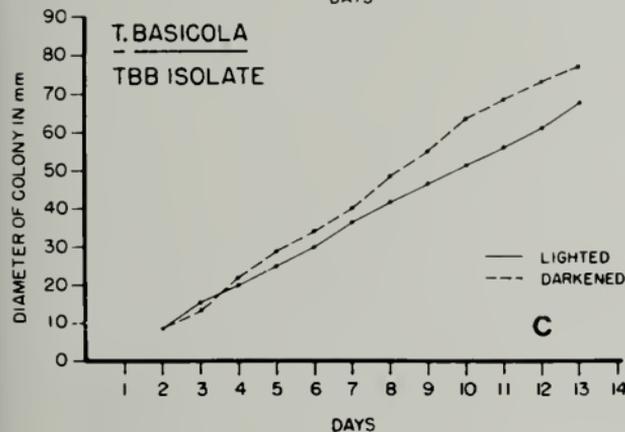
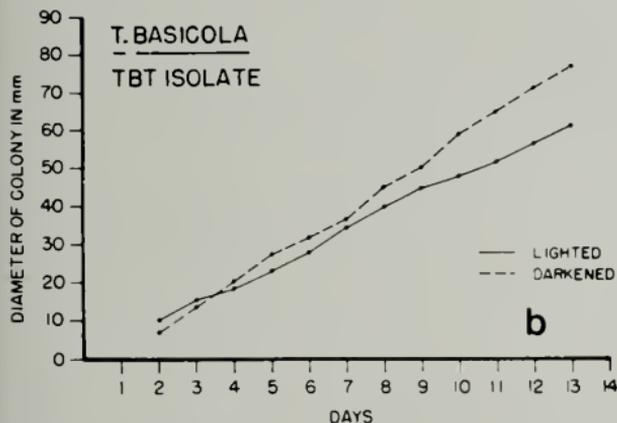
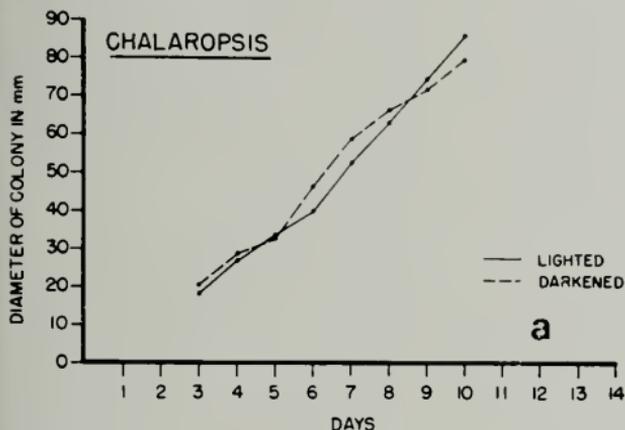
Variations in pH influenced the growth of isolate TBT more than that of isolate TBB. Neither isolate grew at a pH lower than 3.2. Although the basic limit was not defined, a progressive decline in the growth and formation of typical structures of the colonies at pH 9.0 indicated that the maximum was somewhere near that point. As Fig. 3C shows, after 10 days of growth the diameters of TBB colonies on agars with various pH values ranged from 18 to 62 mm. A variation of 27-74 mm in the diameters of the TBT isolate colonies occurred on these agars (Fig. 3B). The optimum for both isolates was between pH 5.9 and 6.7. The colonies on agars outside of the optimum pH range produced more vegetative structures than reproductive structures.

TBB and TBT colonies on agars within the optimum pH range were white for the first 2-3 days, due to the abundant production of thin-walled hyphae and endoconidiophores. After several days the colony, except for a narrow white perimeter, developed shades of gray. The gray color resulted from the production of chlamydo spores and thick-walled hyphae. Daily diameter enlargement varied between 5.8 and 7.4 mm.

Morphologically, the colonies on the

more basic media tended to be light gray to whitish. As the basic condition was intensified, chlamydospore production was lessened and endoconidial production became greater. At pH 8.0 the colonies of both isolates consisted almost

entirely of masses of endoconidiophores with few other structures. These colonies were characterized by alternate rings of dense and sparse masses of endoconidiophores. Occasionally chlamydospores and some dark hyphae were



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Fig. 3. — The effect of variations in pH and medium type upon the growth of: A, *Chalaropsis thielaviaoides*; B, the TBT isolate of *Thielaviopsis basicola*; C, the TBB isolate of *T. basicola*.

observed in random clumps, usually within the denser mycelial circles. Colonies of both isolates of *Thielaviopsis* on agar with a pH value of 8.6 were composed almost entirely of hyaline hyphae appressed to the agar surface. A few endoconidia were present. Each colony on agar more basic than pH 8.6 consisted only of thin, distorted, hyaline hyphae embedded in the agar. The daily diameter growth of the colonies growing on media with pH values above 6.7 varied from 3.6 to 6.5 mm.

Acid media induced the formation of a different type of colony. Each colony on an acid agar was composed mainly of aerial mycelium. Concentric circles within the colony were produced on agars with pH values of 7.0-4.5. However, the mycelium in each of these circles was black to intermediate gray. Growth on media with a pH as low as 4.5 resulted in an increase in the production of chlamydo spores. This increase produced a corresponding darkening of the colonies to almost entirely black.

Colonies of both isolates grown on agars in the pH range 4.5-4.1 were lighter in color than colonies on slightly less acidic agars. Chlamydo spores and dark hyphae were produced in clumps. They were not produced on strongly acidic agars. No chlamydo spores were produced on agars with pH 3.7 or below. Fluffiness (or the amount of the aerial mycelium) increased as the pH of the agar decreased down to pH 3.7. Colonies on the pH 3.7 agar were almost entirely aerial and composed of elongated strands of hyaline hyphae. A few endoconidia and endoconidiophores were found. Colonies on these highly acidified agars were dense enough to form visible margins. These margins were characteristically reticulately lobed. Margins of colonies produced on the highly basic agars were characteristically smooth. Daily increases in diameters of the colonies on acid media varied from 1.8 to 6.2 mm. Both isolates of *Thielaviopsis* failed to grow on agars with a pH of 3.2 or lower.

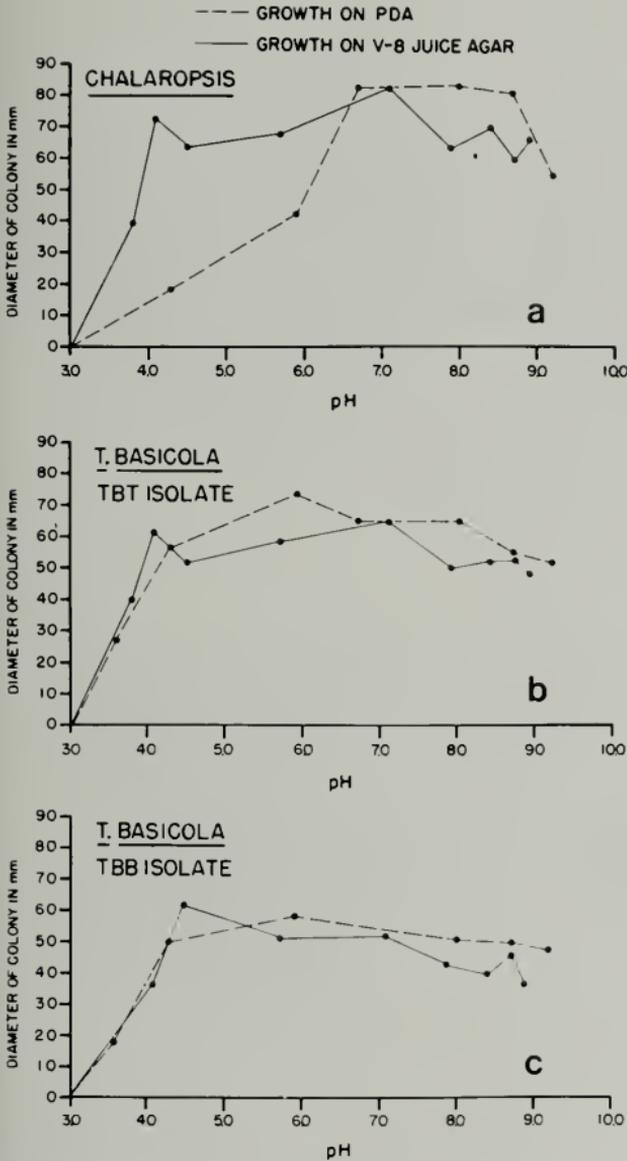
The effect of fungus growth on the pH of the media was not determined. Before the agar plates were seeded, the pH was adjusted by adding a predetermined number of drops of acid or base solution to the liquified media. After the 10-day testing period, the media were remelted and tested for pH with a pH meter. The changes in pH of most media were so slight as to be negligible. Fluctuations that may have taken place during the 10-day period were not determined.

Effect of Temperature

Temperature exerted an effect on the rate of growth but not on the type of growth of the fungi reported on here. In all tests where temperature did not severely restrict growth, the three isolates produced colonies characterized by a dense composite of both types of hyphae and both types of spores. The only variation was in the diameters of the colonies.

As shown in Fig. 4A, the greatest amount of growth of *Chalaropsis* occurred at 30° C. Daily enlargement of colonies growing at this temperature varied between 6.4 and 8.5 mm. An abrupt decrease in growth at 35° C. resulted from the thermal death of many mycelial cells. Although growth at 10° C. was slight, it was measurable and averaged about 1.2 mm per day. Growth was evident at 5° C.; however, it was restricted to the seed plug and too limited to be measured.

The optimum temperature for growth of both isolates of *Thielaviopsis* was 25° C. (Fig. 4B and C). The TBB isolate grew slightly better at 30° C. than did the TBT isolate. Colonies maintained at 20° C. and 15° C. produced both types of spores and both types of hyphae but were smaller than the colonies grown at 25° C. The TBT isolate produced no growth at either 5° C. or 10° C. TBB, however, produced visible growth at 10° C., but it was confined to the seed plug and was too limited to be measured. This isolate produced no growth at 5° C.



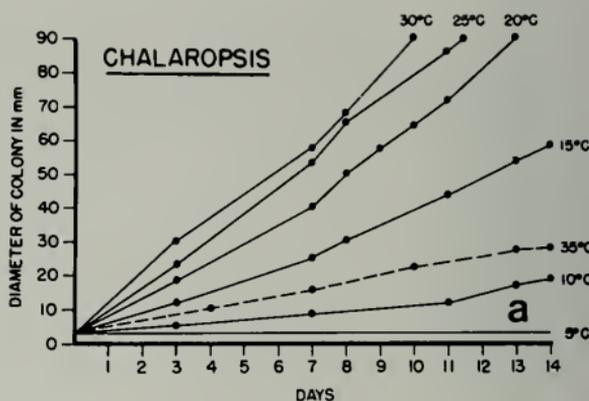
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Fig. 4. The effect of temperature upon the growth of: A, *Chal-aropsis thielavioides*; B, the TBT isolate of *Thielaviopsis basicola*; C, the TBB isolate of *T. basicola*, all on V-8 juice agar.

Effect of Light on Growth Rates

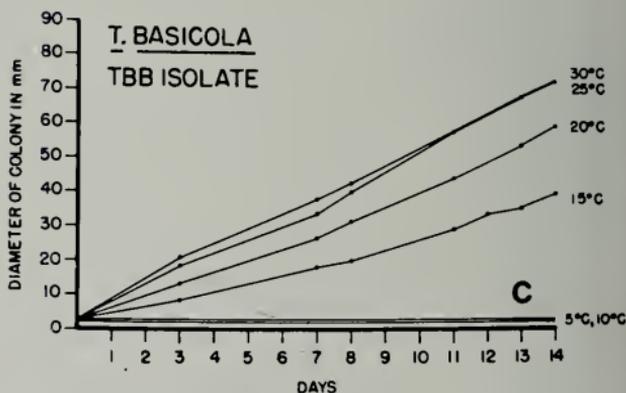
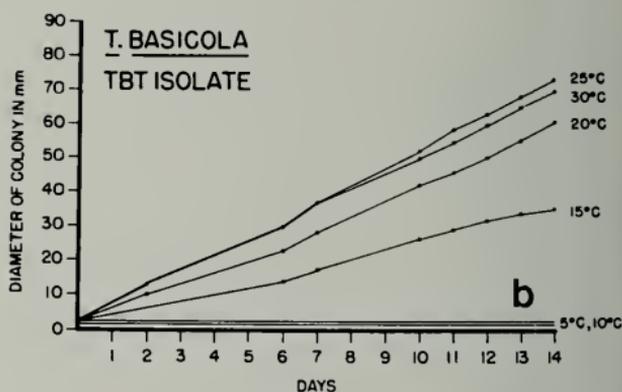
As shown in Fig. 5A, *Chalaropsis* grew slightly more rapidly in the light than in the dark. While the growth of the colony in the light averaged 8.6 mm in diameter per day for a 10-day period, the growth of the colony in the dark averaged only 8.0 mm per day. During the first half of the test period, growth in the dark slightly exceeded growth in

the light. Numerous chlamydospores were produced in the dark after 7-10 days, after which mycelial growth was retarded (Fig. 6). Since few chlamydospores were produced in the light, mycelial production was not noticeably retarded. Microscopic examination of the colonies after 14 days revealed that the colony grown in the light consisted mainly of hyaline hyphae and endoconidia. The colony grown in the dark



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Fig. 5. — The effect of light upon the growth of: A, *Chalaropsis thielavioides*; B, the TBT isolate of *Thielaviopsis basicola*; C, the TBB isolate of *T. basicola*, all on V-B juice agar.



was generally darker gray and composed of thick-walled, dark hyphae and chlamydospores as well as hyaline hyphae and endoconidia.

Both of the *Thielaviopsis* isolates produced more growth in the dark than in the light (Fig. 7). The TBB isolate averaged 5.9 mm of diameter growth per day in the dark and 5.2 mm per

day in the light. As Fig. 5C shows, growth in the dark was consistently greater than growth in the light. Production of chlamydospores and dark-walled hyphae seemed to be favored by light. Fourteen-day-old colonies grown in the light contained many more chlamydospores and dark hyphae than the colonies grown in the dark. These

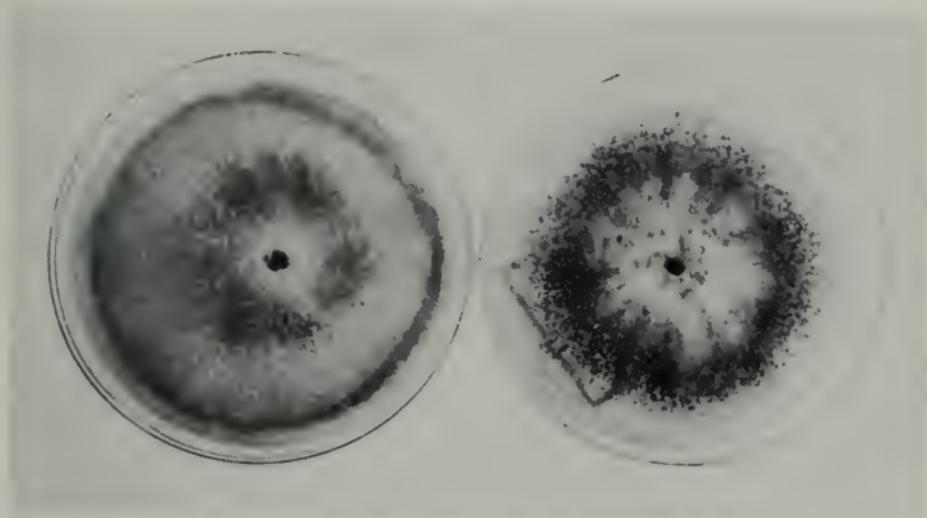
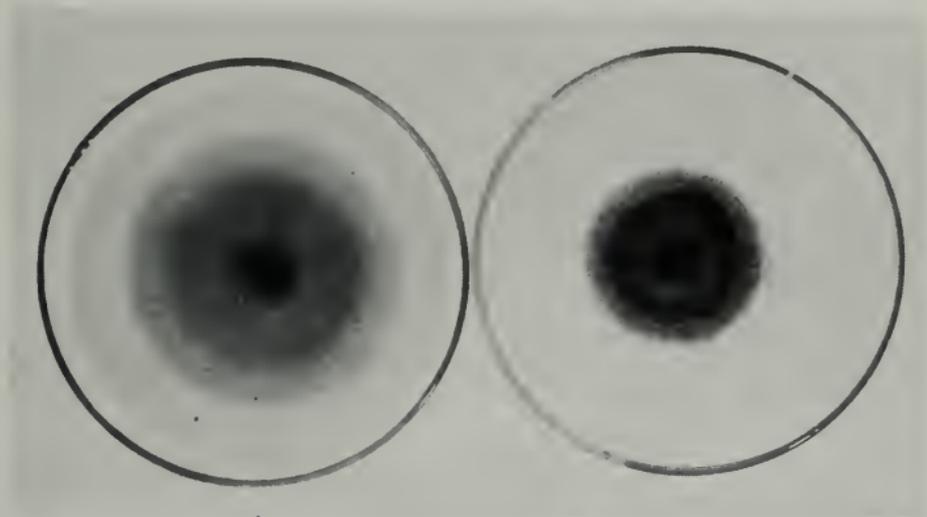


Fig. 6. — The effect of light on colony development of *Choloropsis thielavioides* grown on V-8 juice agar. The colony on the left was exposed to constant light for 14 days, while the colony on the right was maintained in the dark for the same period.



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Fig. 7. — The effect of light on colony development of *Thielaviopsis basicola* grown on V-8 juice agar. The colony on the left was exposed to constant light for 14 days, while the colony on the right was maintained in the dark for the same period.

two structures were evident in the 2- to 3-day-old lighted colonies, which produced chlamydo spores and dark hyphae throughout the test period. The *Thielaviopsis* colonies produced under light were dark gray to black; the colonies produced in the dark varied from dark gray through light gray to whitish.

Colony color, morphology, and other characteristics of TBT and TBB isolates of *T. basicola* grown in the light were almost identical, as were the characteristics of the same isolates grown in the dark. Apparently the reaction to light is a characteristic of the fungus rather than of the individual isolates. Like the

TBB isolate, the TBT isolate averaged 5.9 mm of daily diameter growth in an unlighted situation. Light appeared to be more influential in retarding the growth of TBT than in slowing that of TBB. The diameter growth of TBT cultures in the light averaged only 4.7 mm per day. The average rates of growth of these colonies are shown in Fig. 5B.

Colony components, growth rate, and growth type of the *T. basicola* isolates when grown on liquid media in the light were comparable to those produced on agar media in the light. Likewise, the colonies grown on liquid media in the dark were similar to those grown on agar media in the dark. Although direct measurements of growth were not made, daily observations and frequent microscopic examinations were used to observe the development of the isolates. Growth on lighted and unlighted potato-dextrose broth was not as profuse as growth on V-8 juice broth. *Chalaropsis* produced more chlamydospores and thick-walled hyphae in unlighted cultures. Both isolates of *Thielaviopsis* produced abundant chlamydospores and thick-walled hyphae in the lighted cultures. In all cases the three isolates produced limited amounts of hyaline hyphae and endoconidia within the liquid and profuse amounts on the surface. The production of chlamydospores and dark hyphae was limited to the surface mat.

Results of Pathogenicity Tests

Evaluations of the success of the infection tests were based on these criteria: (i) production of external and internal disease symptoms on or in the host plants, (ii) microscopic confirmation of the presence of fungal pathogens, and (iii) reisolation of the fungus from the poinsettias. As shown in Fig. 8, the stem-incision inoculation method was the most efficient in producing infection. The host-parasite relationship created by this method resulted in external symptoms on the aerial portions of the host and various amounts of root decay.

The soil-probe and soil-drench methods of introducing the pathogen into the root environment resulted in the production of disease symptoms on a small percentage of host plants. The results are based on 10 separate inoculation attempts, each performed at different times but under similar conditions. Twenty poinsettia plants were used each time.

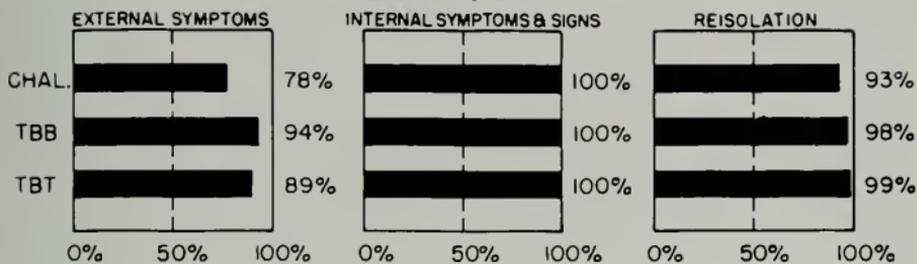
Infection from direct introduction of *Chalaropsis thielavioides* into the host plant through an incision resulted in external symptoms in 78 percent of the plants inoculated while in the vegetative stage. External symptoms were not pronounced and did not develop immediately. The most common symptom was general chlorosis and stunting that became evident only when comparisons were made with the check plants (Fig. 9A). Epinastic and curling leaves (Fig. 9B) were frequent symptoms that indicated root disorders. A general reduction in the sizes of the root balls of the inoculated plants was evident upon removing the diseased plants from the soil (Fig. 9C). In a few cases decaying roots and roots with small lesions were found. The inner surface of the lowest pith chamber of the stem (the inoculation site) was blackened and soft (Fig. 9D).

External symptoms were not produced on poinsettias inoculated while in the flowering stage. Occasionally a small area of diseased tissue was found on the inner surface of the inoculated pith chamber of the flowering plants, but these areas failed to become extensive.

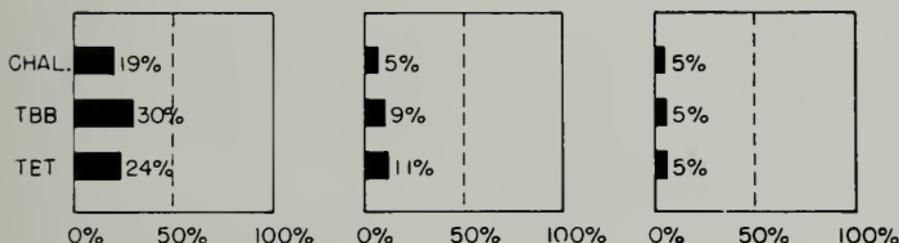
Microscopic examinations of scrapings of the inner surfaces of the stems of plants inoculated in the vegetative stage revealed an abundance of chlamydospores, endoconidia, and mycelium of *Chalaropsis* in the decaying host tissue. *Chalaropsis* was cultured consistently from the diseased inner surfaces of the pith chambers.

Quite similar results were obtained when the *Thielaviopsis* isolates were used in stem-incision inoculations. The

STEM INCISION



SOIL PROBE



SOIL DRENCH

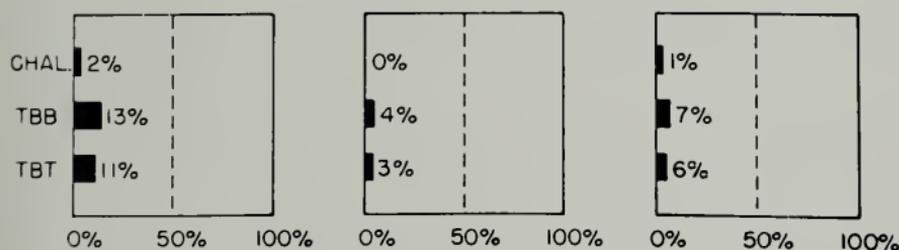


Fig. 8. — A comparison of the disease incidence resulting from three methods of initiating host-pathogen association.

TBB isolate produced external symptoms on 94 percent and the TBT isolate on 89 percent of the plants inoculated in the vegetative stage.

Two groups of symptoms developed on poinsettias that had *Thielaviopsis* introduced into stem incisions while the plants were in the vegetative stage. One group of symptoms appeared soon after the fungus had been implanted, but the other group did not appear until the

plants flowered. Soon after the fungus was introduced, many of the plants became stunted and chlorotic (Fig. 10A), but many others appeared healthy and exhibited no external symptoms until the time of flowering. The presence or absence of external symptoms appeared to vary with the poinsettia varieties. At approximately the time the upper bracts of the poinsettias began to change color, the lower leaves of the inoculated



Fig. 9. — Symptoms of *Chalaraopsis* root rot on poinsettias: A, stunting of a diseased 120-day-old plant (right) compared to a healthy 120-day-old plant (left); B, wilting of leaves from natural infection on a 110-day-old plant; C, slightly reduced root ball of diseased plant on right and normal-sized root ball of healthy plant on left; D, stem with check incision (left) and stem with incision inoculation (right).

plants began to fall. Leaf fall was progressive, beginning with the lowest leaves and working upward until only the flower bracts were left at the tips of naked stems (Fig. 10B). Occasionally wilting preceded leaf fall, but often unwilted leaves were dropped. The root ball was reduced in size. Numerous blackened and decaying roots were conspicuous within the root balls (Fig. 10C). Longitudinal sections of the inoculated stems, crowns, and upper root areas of the poinsettias revealed general internal decay throughout the region adjacent to the inoculation slit (Fig. 10D). Endoconidia and hyphae were in the stem lesions and on the outer surfaces of the root lesions. *Thielaviopsis* was isolated from the diseased tissues around the inoculation lesions of

most plants. However, the symptoms discussed here did not develop on the plants inoculated while in the flowering stage.

The soil-probe method of placing *Chalaraopsis* in contact with the poinsettia roots resulted in symptom production on 19 percent of the plants tested. Stunting and chlorosis became evident only after 30–45 days.

Root decay was more extensive on the vegetative poinsettias than on the flowering poinsettias inoculated by the soil-probe method. The aerial portions of flowering poinsettias did not show disease symptoms. Typical symptoms resulting from inoculations by the soil-probe method were extensive root decay and lack of new root development. Reisolation of *Chalaraopsis* from

the diseased roots was occasionally successful, but interference from soil contaminants usually prevented successful reisolations.

The application of both isolates of *Thielaviopsis* to the roots of poinsettias by the soil-probe method resulted in symptoms slightly different from those produced by *Chalaropsis*. Of the plants that had a soil-probe application of the TBB isolate 30 percent developed external symptoms, and of those receiving an application of the TBT isolate 24 percent developed such symptoms.

No symptoms were produced by *Thielaviopsis* on the aerial parts of poinsettias inoculated while in the flowering stage. Vegetative poinsettias inoculated by applying *Thielaviopsis* to probed soil became slightly chlorotic and stunted within 30–60 days. Infected poinsettias also began to flower 7–10 days earlier than did healthy plants.

Lesions began to develop on the crown area of the stem after about 30 days. The outer epidermis and cuticle around the lesion loosened and sloughed off. This was followed by the formation of additional lesions, loosening of the epidermis, and production of callus tissue around the lower stem. Numerous chlamydospores were produced in the lesions. The destruction of roots was extensive. Small areas on the roots or root stubs became discolored and soft. The decay progressed up the root, and in time the entire root became a dark, slimy mass. Endoconidia and hyphae were produced in great abundance on the decaying roots. The entire root balls of most diseased plants decayed and only the lower stem was left in the soil. In such cases the poinsettias usually toppled and died. The amount of disease that developed in a heavily infected greenhouse crop was similar to the re-



Fig. 10. — Symptoms of *Thielaviopsis* root rot on poinsettias: A, stunted 120-day-old plant (right) and a healthy plant; B, leaf drop and stunting of a diseased 95-day-old plant (right) and a healthy 95-day-old plant (left); C, root balls of poinsettias — diseased roots on left and healthy ones on right; D, stem with check incision (left) and stem with incision inoculation (right).

sults reported by Presley (1947) on the amount of disease in a field situation.

Reisolation from the decaying roots was successful in the few cases in which soil contaminants did not overrun the *Thielaviopsis* colonies.

The introduction of *Chalaropsis* into the poinsettia root environment by the soil-drench method resulted in only slight disease incidence. Symptoms were slight and hardly detectable. Only 2 percent of the inoculated plants appeared slightly chlorotic or stunted or had diseased roots. In a few cases *Chalaropsis* was reisolated, but soil contaminants usually overran the *Chalaropsis*. For every 100 poinsettia plants growing in soils infested with the TBB isolate, 13 developed disease symptoms. Many roots of the diseased plants became decayed and new roots did not develop. The TBT isolate applied in a soil drench produced similar results, but on only 11 percent of the treated plants. As with the previous inoculation method, attempts to reisolate both fungi were usually unsuccessful because of faster growing soil contaminants.

DISCUSSION

The results of pathogenicity tests conducted during this study have shown that *Chalaropsis thielavioides* is a weak pathogen on poinsettias. This fungus is probably not capable of producing its own infection site. However, it is capable of producing disease symptoms on poinsettias after it has entered a plant through wounds. Pathogenicity studies performed during this investigation suggest that poinsettias with intact roots grown under normal conditions will probably not exhibit a high incidence of disease from this fungus even in soil with large concentrations of *Chalaropsis* propagules. The amount of wounding proportionally increases the degree of disease incidence. An abnormal number of broken roots, which might result from rough handling during transplanting or intense watering,

may appreciably increase losses from the disease caused by *C. thielavioides*. More extensive root breakage from heavy infestations of insects or from inserting stakes in pots could result in extreme disease losses. Although the death of a few secondary roots from insufficient watering might not directly affect the plant, such a condition could provide entry sites for *Chalaropsis*.

Thielaviopsis is not restricted to entering poinsettias through wounds. Studies show that this fungus can produce an appreciable amount of disease in poinsettias without wounds. However, wounding increases the probability of disease.

Results of some of the cultural studies suggest that environmental conditions may also influence the amount of disease caused by *Chalaropsis* and *Thielaviopsis*. A pH range of about 6.0-8.0 and temperatures from about 20° to 30° C. were most favorable for growth of both fungi in culture. Similar pH and temperature conditions in the soil should result in good development and vigorous growth of the fungi. Since these conditions are not ideal for the growth of poinsettias, the combination of weakened hosts and vigorously growing pathogens should result in a greater disease incidence than would normally be expected.

No work was planned as a part of this investigation to test the effect of variations in soil temperatures on disease development. However, one related experiment did produce the anticipated results. The greenhouse air temperature was increased by 6° C. to induce vigorous flower production. This increase produced a corresponding increase in soil temperature. Using various poinsettia varieties, 150 rooted cuttings were potted in the warmer soils. The soil-probe method was used to inoculate 50 plants with *Chalaropsis* and 50 with *Thielaviopsis*; 50 were injected with distilled water. After 60 days, not a single plant had become diseased. This experiment indicated that increased soil temperatures may reduce

disease incidence by inhibiting fungal development. These results support the findings of Blank et al. (1953) in their work on *Thielaviopsis* root rot of cotton.

Light enhances the production of chlamydo-spores in *Thielaviopsis* and discourages their formation in *Chalaropsis*. An advanced symptom of *Thielaviopsis* infection is the formation of ground-line lesions in which chlamydo-spores are found in abundance. Although chlamydo-spores are occasionally found on diseased roots, their presence there is minor when compared with their presence in the exposed lesions. Colonies of *Thielaviopsis* grown in the light contained multitudes of chlamydo-spores, while colonies grown in the dark contained far fewer. Since all other factors were equal, the conclusion may be drawn that *Thielaviopsis* chlamydo-spore production is enhanced by, although not dependent on, the presence of light.

Chalaropsis produced symptoms different from those produced by *Thielaviopsis*. Although advanced *Chalaropsis* disease symptoms consisted of ground-line lesions and areas of sloughed-off epidermal tissues, chlamydo-spores were not found in these areas but rather on the internal surfaces of the same areas. In culture, *Chalaropsis* chlamydo-spores were found abundantly in colonies grown in the dark but seldom in colonies grown in the light. These results prove that chlamydo-spore production in *Chalaropsis* is favored by darkness.

Results of reisolation attempts prove that the development of *Chalaropsis* and *Thielaviopsis* in poinsettias is restricted to the tissues not containing chlorophyll. Attempts to reisolate the fungi from all parts of diseased poinsettias showed that both fungi grow only in the roots and in the ground-line stem region of the more mature plants. In no instance was either fungus isolated from leaves or upper stems. These results were anticipated after finding no growth of either fungus on the agar made from the aerial structures of

poinsettias and poor growth on the agar made from the entire plant. Apparently there is an unknown factor in the chlorophyll-containing tissues that does not exist in the other tissues and that prohibits the growth of *Chalaropsis* and *Thielaviopsis*.

SUMMARY

Poinsettias are subject to root rots that are caused by three fungi, *Thielaviopsis basicola*, *Rhizoctonia solani*, and *Pythium ultimum*, occurring either individually or in complexes. Infection by these fungi may result in stunting, leaf drop, premature flowering, dwarfed flowers, stem lesions, and occasionally the death of the root. Another fungus, *Chalaropsis thielavioides*, which has not previously been considered a member of the poinsettia root rot complex, was frequently obtained while attempting to isolate *T. basicola*. Instead, *C. thielavioides* has been considered to be a surface saprophyte on roots of many plants, especially carrots. There are few reports of it as a plant pathogen. During an investigation of *Thielaviopsis* root rot, *C. thielavioides* was repeatedly isolated from the roots of diseased poinsettias exhibiting *Thielaviopsis*-like symptoms. This led to the concept that *Chalaropsis* should be included as part of the root rot complex.

Some of the major characteristics of *Thielaviopsis basicola* and *Chalaropsis thielavioides* are presented for comparison in Table 3.

Two isolates of *Thielaviopsis basicola* and one isolate of *Chalaropsis thielavioides* were selected for comparison in cultural requirements, morphology, and pathogenicity. Nine varieties of poinsettias were used to determine and maintain the pathogenicity of the three fungal isolates. Various media were used to determine the pH requirements and the most suitable culture medium for each of the isolates.

Colony characteristics and morphology were compared for each of the iso-

lates grown in culture. The isolates were grown at six different temperatures to ascertain the optimum temperature for growing each in culture. Media comprising a pH range of 2.7-9.2 were used to determine the optimum pH requirement for each isolate. Cultures of each of the three isolates were grown on several different media both in the light

and in the dark to compare the morphological features and growth rates of each isolate under both conditions. Three methods of placing the pathogen in contact with the host were used to determine the pathogenicity of each isolate.

Morphologically, the two fungi compared were similar except for the pro-

Table 3. — Some of the major characteristics of *Thielaviopsis basicola* and *Chalaropsis thielavioides*.

| Characteristic | <i>Thielaviopsis basicola</i> | <i>Chalaropsis thielavioides</i> |
|--|---|--|
| Disease produced | Root rot | Root rot, grafting failure |
| Hosts | Numerous herbaceous plants | Relatively few herbaceous plants, some trees and shrubs |
| General disease symptoms on poinsettias | Chlorosis, stunting, small root balls, leaf drop, premature flowering | Chlorosis, stunting, small root balls |
| Advanced disease symptoms on poinsettias | Longitudinal lesions on stem near ground line, girdling of stem, death of host | Internal decay of stem near ground line |
| Pathogenicity | Strongly pathogenic | Weakly pathogenic |
| Morphology | | |
| Mycelium | Mostly thin walled and hyaline; some thick walled and dark | Mostly thin walled and hyaline; some thick walled and dark |
| Endoconidia | Stoutly cylindrical, hyaline, single celled; average measurements: 7.78 x 2.61 μ | Cylindrical, hyaline, single celled; average measurements: 7.6 x 1.7 μ |
| Chlamydo spores | Dark, multispored chains, terminal; average measurements: 19.30 x 5.77 μ ; club-shaped chains; unit breaks up into individual spores; no resting period required before germination | Dark, single spores, terminal; average measurements: 7.64 x 7.64 μ ; round unit remains intact; resting period required before germination |
| Environmental requirements | | |
| pH | Optimum range: pH 5.9-6.7 | Optimum range: pH 6.7-8.0 |
| Temperature | Optimum range: 24° to 27° C. | Optimum range: 20° to 30° C. |
| Light | Most growth in the dark | Most growth in the light |
| Growth rate on V-8 juice agar | Average 5.9 mm in diameter per day | Average 8.0 mm in diameter per day |
| Mode of entry into host | Capable of producing its own infection site | Requires a wound for entry |
| Location within diseased poinsettia | Mycelium ramifies host tissue; endoconidia on external and internal surfaces of lower stem and roots | Mycelium ramifies host tissue; endoconidia on external and internal surfaces of lower stem and roots |
| Site of chlamydo spore production on diseased poinsettias | In stem lesions near ground line | On internal surfaces of lower pith chambers of stem |
| Anticipated disease incidence on poinsettias in heavily infested soils | With no root damage: 12 percent With some root damage: 27 percent | With no root damage: 2 percent With some root damage: 19 percent |

duction of chlamydospores. Chlamydospores of *Thielaviopsis* were produced in club-shaped chains composed of three to seven spores. *Chalaropsis* chlamydospores were spherical and were produced individually.

Light enhanced the formation of chlamydospores in *Thielaviopsis* and inhibited their formation in *Chalaropsis*. An advanced symptom of *Thielaviopsis* infection was the formation of ground-line lesions in which chlamydospores were found in abundance. Culture colonies of *Thielaviopsis* grown in the light contained multitudes of chlamydospores, while colonies grown in the dark contained far fewer.

Advanced disease symptoms of *Chalaropsis* infection were characteristically ground-line lesions and areas of sloughed-off epidermal tissues. Chlamydospores were not found in these areas but rather on the internal walls of the stem pith chambers near the ground line. In culture, chlamydospores were found abundantly in *Chalaropsis* colonies grown in the dark but seldom in colonies grown in the light. These results confirmed that chlamydospore development in *Chalaropsis* is inhibited by the presence of light.

The results of isolation attempts proved that the development of these isolates of *Chalaropsis thielavioides* and *Thielaviopsis basicola* on poinsettias is restricted to nonchlorophyllous tissues. Both fungi were frequently isolated from the roots and ground-line stem tissues of diseased poinsettias but never from the upper stems, leaves, or flowers.

Both isolates of *Thielaviopsis* grew best in culture on media with a pH

range of 6.0–7.0. The *Chalaropsis* isolate grew best on agar media with a pH range of 7.0–8.0. The greatest growth of *Chalaropsis* occurred at 30° C. The optimum temperature for growth of both isolates of *Thielaviopsis* was 25° C.

The results of the pathogenicity tests showed *Chalaropsis thielavioides* to be a weak pathogen. This fungus was probably not capable of producing its own infection site. It was capable of producing disease symptoms on poinsettias similar to the symptoms produced by *Thielaviopsis* after it had entered the host plant through wounds.

Thielaviopsis was not restricted to wounds for entry. Pathogenicity tests showed that this fungus could produce appreciable amounts of disease on unwounded host plants, although wounding did increase disease incidence.

Similarities between *Chalaropsis thielavioides* and *Thielaviopsis basicola* were numerous. Morphology and colony characteristics indicated a possible relationship between these two genera. Growth factors, dimensions of comparable structures, similar vegetative and reproductive structures, and the production of similar disease symptoms on poinsettias were some of the characteristics that these two fungi had in common. Some specific factors such as the location of chlamydospore production and pathogenicity on poinsettias were points in which the two were different. *T. basicola* is important for its role as a component in the root rot complex of numerous plants, including poinsettias. *C. thielavioides* is not of major importance as a plant pathogen. The work presented here proves that *Chalaropsis* is a weak pathogen on poinsettias.

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