RELATIONSHIP BETWEEN *TUBERCULINA PERSICINA* (DITM.) SACC. AND *PUCCINIA CACABATA* ARTH. & HOLW.

by

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I hereby recommend that this dissertation prepared under my direction by Emroy Laud Shannon

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SIGNED: Emery L. Shannon

DEDICATION

The Author Dedicates This Dissertation

To His Parents:

Alma Shannon and Roderick Shannon

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ABSTRACT

Tuberculina persicina (Ditm.) Sacc. was found growing in association with southwestern cotton rust, Puccinia cacabata Arth. & Holw., in numerous localities in Arizona and New Mexico from 1964 to 1973.

The fungus was successfully isolated and grown on culture media using several isolation techniques. Tuberculina persicina was grown on potato-dextrose agar (PDA), rose bengal agar, cornmeal agar, and other media; however, it grew poorly on defined media including Czapek's solution agar.

Color of the mycelium on agar media varied from white to brown depending upon the medium. Conidia in mass were tan to brown on most media but were pink to lavender on rose bengal agar. Additions of glucose to PDA did not appreciably increase growth or alter cultural characteristics. Optimum temperature for growth was 23 to 30 C.

Several carbon sources were utilized when the fungus was grown in liquid culture. Best growth was obtained at pH 5.0 to pH 6.5, and no growth was obtained at pH 8.0. No conidia were observed in liquid cultures.

Vegetative hyphae on PDA were hyaline and 4.5 to 7.0 μ in diameter. Most hyphae were filled with oil-like globules. Sporodochial hyphae were brown and 9.0 to 12.0 μ in diameter at the base of the sporodochium. Conidiophores produced on the sporodochial hyphae

were 4.5μ in diameter. Masses of conidia were produced on the sides and at the terminal ends of conidiophores as the result of "blown-out" cells. Conidia were oval and had a mean diameter of 9μ - 11μ. Hyphal swellings resembling chlamydospores were frequently found at the base of the sporodochium.

Puccinia cacabata spermagonia and aecia on cotton leaves were successfully inoculated by applying a conidial suspension of T. persicina to either the upper or lower surfaces of cotton leaves. Typical violet-to lavender-colored conidia on sporodochia appeared on spermagonia and aecia six days after inoculation. Tuberculina colonies on rust pustules reduced aeciospore production by 50% in one test and 75% in a second.

Orseillin BB + fast green or orseillin BB + cotton blue stains were helpful in distinguishing between the two fungi within cotton leaf histological tissues. Germ tubes of conidia were found penetrating spermagonial openings 24 hr after inoculation. Conidiophores and conidia of T. persicina were visible within both spermagonia and aecia six days after inoculation. Tuberculina persicina prevented normal development of spermagonia and aecia, thereby reducing the number of aeciospores produced, and blocked the exit of aeciospores from aecia.

Results of histological and inoculation studies indicate that T. persicina is a pathogen of P. cacabata. The close and constant association of T. persicina with P. cacabata in nature suggests that T. persicina may be a hyperparasite of P. cacabata.

INTRODUCTION

Southwestern cotton rust, caused by Puccinia cacabata Arth. & Holw., is an important disease in certain areas in the southwestern United States and in Mexico. The fungus is a typical macrocyclic and heterocious species. It overwinters as two-celled teliospores on a few species of Bouteloua in the United States and has been reported on a few species of Chloris and Cathesticum in South America (10). When summer rains begin, the teliospores germinate producing promycelia which bear four basidiospores that infect cotton (Gossypium sp.). Subsequently, spermagonial and aecial stages are formed. Aeciospores infect Bouteloua but not cotton (22).

Fungicides have been used to protect cotton from infection by basidiospores, but have not been entirely effective in controlling cotton rust as they must be applied before infection occurs (6).

One possible method of controlling cotton rust that has not been investigated is the use of biological agents. Tuberculina persicina (Ditm.) Sacc. offers a possible means of control. This fungus has been observed on the spermagonial and aecial stages of the cotton rust fungus by this author a number of times over the past several years. However, search of the literature suggests that the relationship between T. persicina and P. cacabata has not been thoroughly investigated. A basic understanding of this relationship is necessary before its use can be considered as a possible means of controlling the cotton rust fungus.

The objectives of this dissertation are to determine: (a) by histological studies, the relationship between the mycelia of P. cacabata and T. persicina; (b) the impact of T. persicina development upon aeciospore production by P. cacabata; (c) the growth characteristics of T. persicina when grown in association with P. cacabata; (d) whether T. persicina is a parasite or pathogen of P. cacabata; and (e) growth characteristics of T. persicina when grown in pure culture on artificial media.

LITERATURE REVIEW

The genus Tuberculina includes a few species of imperfect fungi that are associated with species of the Uredinales or rust fungi. Taxonomically, it is placed in the family Tuberculariaceae, order Moniliales, and in the class Fungi Imperfecti. Saccardo (25) established this genus in 1880 to include imperfect fungi associated with rusts and which have hyaline conidia, hyaline hyphae, and smooth or nearly smooth sporodochia. Hulea (14), in his study of fungi commensal with rusts in 1939, has listed fifteen species of Tuberculina.

According to Lechemere (20), Tuberculina persicina (Ditm.) Sacc. was first described by Ditmar as Tubercularia persicina Ditm. He mentioned, also, that Gobi, in 1885, and Morini, in 1886, both studied a fungus which they called Tubercularia persicina Ditm. The fungus was transferred to Tuberculina persicina (Ditm.) Sacc. by Saccardo (26) in 1886.

Tuberculina persicina has been reported growing in association with numerous rust fungi throughout the world. The fungus has been reported in Israel (2), England (26), Roumania (14), Italy (26), Germany (26), India (29), Russia (35), Portugal (34), and the United States (16). The reports suggest that the fungus is always associated with a rust fungus in nature.

Keener (16) first reported on the presence of T. persicina in Arizona, noting its association with spermagonia and aecia of Puccinia

poarum Niels. on Helenium hoopesii Gray. He did not discuss the extent of occurrence in Arizona, nor did he mention its possible value in controlling the rust.

Berkenkamp and Streets (5) first noted the association of T. persicina with the cotton rust fungus, P. cacabata, reporting that "a parasite attacking the aecia of this rust fungus (P. cacabata) has been tentatively identified as Tuberculina persicina." A brief report (7) also was published concerning the association of T. persicina with P. cacabata in Arizona, but they did not discuss its importance in any detail. Smith (27) reported widespread occurrence of T. persicina on the aecial stage of the cotton rust fungus in New Mexico during a severe outbreak of the rust fungus near Rodeo, New Mexico in 1959.

Tuberculina species have been of great interest for years because of their potential for biological control of rust fungi. Most attention has been given to the study of Tuberculina maxima Rostrup since it is a possible means of controlling the white pine blister rust fungus, Cronartium ribicola J. C. Fisch. ex. Rabenh. Tuberculina maxima was first described by Rostrup in 1890, who reported that the fungus "attacked" C. ribicola on pine trees in Denmark and Germany (31).

Spalding (28) studied T. maxima as a control agent of C. ribicola in several localities including England, Denmark, Belgium, and Germany and reported, in 1929, that T. maxima caused a marked reduction in C. ribicola aeciospore production.

Tubeuf (32), working in Germany in 1930, appears to be the first researcher to artificially inoculate white pine blister rust with spores of T. maxima. He collected conidia of T. maxima in a paper bag

and made transfers to aecia of C. ribicola on naturally infected pine trees. He also reported that T. maxima greatly reduced or entirely inhibited the production of aeciospores of the rust in certain regions of Germany.

Hubert (13), working in Idaho, also inoculated white pine blister rust cankers in 1934 with spores of T. maxima which he had collected from naturally infected white pine blister rust cankers. However, he only had limited success with artificial inoculations and suggested that the possibilities of use of T. maxima as a biological control agent were remote. There was little further activity in this area for about thirty years.

Quick and Lamoureaux (23) reported in 1967 that they successfully artificially inoculated C. ribicola cankers on western white pine in California with T. maxima spores collected from naturally infected trees. They concluded that T. maxima suppresses but may seldom eradicate C. ribicola in affected blister rust cankers. Leaphart and Wicker (19) made observations of C. ribicola cankers on western white pine which had been naturally infected by T. maxima. They reported, in 1968, that T. maxima is definitely implicated in reduction of activity of C. ribicola cankers.

Van der Kamp (33), in 1970, reported that T. maxima appeared to inhibit aeciospore production on cankers of Peridermium pini (Pers.) Lev. on Scots pine which had been naturally infected by T. maxima. Van der Kamp also noted (33) that swelling of host tissue was less pronounced or almost absent when T. maxima was present and speculated that

T. maxima may have been responsible for the low number of infections by P. pini in a 44-year-old stand of pine trees.

Tuberculina persicina and other Tuberculina species are found growing only in association with various rust fungi. Although Tuberculina species have generally been referred to as hyperparasites to describe the close relationship that they have with rust fungi, some authors prefer other terms in order to more closely describe the mutual relationship. These terms include commensalism, antagonism, parasitism, mycoparasitism, as well as hyperparasitism.

Commensalism implies that two or more species live in close association with one another without apparent harm or benefit to one another. Hulea (14), in his study of T. persicina and other so called rust parasites, concludes that there is no relationship of parasitism but one of commensalism. He explains, further, that T. persicina and the mycelium of rust develop independently, and that the development of T. persicina hinders in a purely mechanical manner the development of the rust spores, without any physiological influence or parasitic relationship.

Krstic (18), in his review of biological control in forest pathology, frequently uses the term "antagonism" to designate a relationship whereby one fungus is detrimental to the growth or survival of another. Antagonism would be a suitable term to use when describing the relationship that exists between Tuberculina species and rust fungi, as it is generally accepted that Tuberculina species are detrimental to the growth of rust fungi by reducing aeciospore production (17,30,37,38,39).

In his review of hyperparasitism, Boosalis (8) concludes that hyperparasitism, mycoparasitism, direct parasitism, and interfungus parasitism are terms used in reference to the phenomenon whereby one fungus is parasitic upon another. He further adds that the pathogen in this type of parasitism is known as the hyperparasite, the mycoparasite, or simply as the parasite. He did not list Tuberculina as a hyperparasite or mycoparasite of rusts or other fungi, nor did he place it in another category.

Mycoparasitism is a term first used by Butler (9) to designate the parasitic relationship between two fungi. This is a suitable descriptive term when it has been definitely shown that a parasitic relationship exists. Such relationships have been extensively reviewed by Barnett (3). He states that the greatest number of known species of mycoparasites belong to the Chytridiales, Mucorales, and Fungi Imperfecti. Barnett points out that true haustoria are produced by some Mucorales, that many fungi produce unspecialized hyphae which penetrate the host hyphae, and that they grow within the host fungus. Barnett further states that other fungi are completely external, making close contact with their hosts by means of special branches or entwining hyphae. Barnett, evidently did not consider Tuberculina species as mycoparasites, as he did not list them as such on rust fungi in his review.

Numerous workers have undertaken studies to determine how Tuberculina affects its "host" rust parasite. Lechemere (20), investigating T. maxima on white pine blister rust, concluded that T. maxima is restricted to the spermagonial and aecial structures entirely, that

it does not penetrate the pine host tissue, and that it does not attack or destroy the rust mycelium. Hubert (13) found that T. maxima frequently develops upon the pycnial stroma of C. ribicola beneath the pine epidermis or along the bark cracks, but he does not record the extent of injury to the blister rust fungus.

Wicker and Woo (40) studied the affect of T. maxima upon white pine blister rust on western white pine. They found that T. maxima infected sporulating blister rust cankers and that T. maxima invaded the pine cortex only after such tissue had been invaded by C. ribicola. They also found that once invasion by T. maxima caught up with invasion by C. ribicola it progressed no further and subsequently died. They concluded, therefore, that T. maxima is associated only with rust cankers.

Thirumalachar (30) made microtome sections to determine the relationship of Tuberculina sp. (probably T. costaricana Syd.) with Uromyces hobsoni Vize. on Jasminum grandiflorum Linn. Uromyces hobsoni produces telia, spermagonia, and aecia on J. grandiflorum. He found that Tuberculina sp. formed knots in the intercellular spaces on the side of the spermagonium, and that there was a gradual disintegration of the spermagonium. He found that in later stages, spermagonia were completely surrounded by mycelia which extensively developed into sporodochia. He stated that Tuberculina sp. was found to enter the aecial cup and "attacked" the spore mother cells, the spores, the peridial cells, and even the basal cells. When aecia were infected by

Tuberculina sp., telial development was completely suppressed thereby preventing development of the overwintering spore in the life cycle of U. hobsoni.

Barkai-Golan (2) studied T. persicina on the aecial and spermagonial stages of four different rust fungi in Israel and found that spermagonia were not invaded by T. persicina. He made histological sections to study the development of T. persicina on the aecia. In the first developmental stage, Tuberculina conidiophores covered only a small part of the aecium, but in more advanced stages it spread over the aecium. The leaves of the host plant appeared as if they were infected exclusively with Tuberculina but a few aeciospores, thinner than normal, and a few peridial cells could be found. The mycelium of T. persicina spread throughout the aeciospores and prevented normal growth. Most of the mycelium of T. persicina was located in the vicinity of the aecium, but a few hyphae penetrated to the intercellular spaces of the leaf tissue.

The exact manner in which Tuberculina enters the "rust host" or "plant host" has not been fully explained. Hulea (14) believed that fungus "parasites" penetrate the host plant through ruptures provided by the rusts. He was not able, however, to observe the exact point of penetration of rust "parasites" i.e., Tuberculina sp. and Darluca sp. Barkai-Golan (2) was unable to infect healthy leaves with conidia of T. persicina or those which had been wounded by a needle. The literature gives no accounts of Tuberculina growth in nature without the presence of a rust fungus.

Spore germination studies of T. persicina were conducted by Barkai-Golan (2). He found that germination of conidia in a hanging drop slide, with or without the presence of aeciospores, was high in both cases; thus, proving that the presence of aeciospores is not required for germination of conidia. At optimum temperature, 23 - 25 C, germination started after three hours. The greatest percentage of germination was obtained from conidia collected and checked the next day. The numbers of conidia germinating decreased gradually among those collected from leaves kept three days or more, but viability remained for many months. There was no significant difference in the percentage of conidia germinating in daylight or in darkness.

Some workers experienced difficulty in growing Tuberculina on artificial media. Hubert (13), in 1934, cultured T. maxima by placing small strips of bark bearing the fungus on malt agar but the fungus did not develop independently upon malt agar and soon died. However, he was able to transfer and grow T. maxima on malt agar from a culture that he obtained from the Netherlands which was grown on cherry agar. Sporulation of T. maxima took place on both media. Vladimirskaya (35), in 1939, obtained abundant germination of T. persicina conidia when they were placed on slices of carrot, seeds of pea, soybean, maize, rice, and on milk and beer wort agars. He also reported that media most favorable for mass cultivation of T. persicina were those containing a large proportion of sugars and little protein.

Villanueva (34), in 1955, gave an excellent report concerning growth of T. persicina in liquid and on solid agar media. He found that T. persicina could utilize a wide range of carbon and nitrogen

compounds. Pigment production in his cultures of T. persicina was directly related to the amount of Vitamin B_1 present in the original culture. He concluded that thiamine is an essential vitamin for growth of T. persicina on artificial media.

Wicker (36), in 1968, reported that he cultured T. maxima on 3% potato dextrose agar but gave no specific details of growth on this medium.

Previous citations suggest that T. persicina is distributed throughout the world and is found in association with numerous rust fungi. However, the relationship between rust fungi and T. persicina has not been satisfactorily explained and cultural characteristics and descriptions of the morphology of T. persicina on agar media are lacking.

METHODS AND MATERIALS

Isolation of T. persicina in Pure Culture

Cotton leaves with spermagonia and aecia of P. cacabata that had been invaded by T. persicina were collected on numerous dates from several localities in Arizona and New Mexico. The leaves were placed in a plastic bag after collection, temporarily stored in a refrigerated auto or refrigerated room for a few hr, then stored in a refrigerator at approximately 5 C until isolation procedures could be started.

Several isolation techniques and numerous growth media were used since isolation of T. persicina from P. cacabata had not been previously cultured on synthetic media (Table 1).

Trial No. 1, Bowie Collection, September 14, 1964: Aecia with T. persicina colonies were removed from cotton leaves after being refrigerated for 3 days and placed in a 70% ethyl alcohol solution for a few seconds. The tissue was then transferred to a 0.525% NaOCl solution for 5 min, then placed in Petri dishes containing glucose-yeast-extract agar (GYEA) and incubated at 26 C.

Trial No. 2, Continental Collection, October 1, 1964: Aecial pustules from cotton petioles refrigerated for 7 days, were placed in a 0.525% NaOCl solution for 3 min, then rinsed in sterile distilled water (SDW). Aeciospores and T. persicina conidia were scraped from the aecial pustules and agitated in a tube containing 10 ml of SDW. A series of four dilution tubes containing 9 ml of SDW was used to dilute the resulting spore suspension. One ml of the spore suspension from

TABLE 1. Summary of *Tuberculina persicina* isolation attempts.

Trial No.	Collection Date and Site	Time Material Refrig. (days)	Isolation Technique[a]	Medium[b]	*T. persicina* isolated
1	9-14-64, Bowie, Ariz.	3	70% alcohol + 0.525% NaOCl	GYEA	No
2	10-1-64, Continental, Ariz.	7	0.525% NaOCl + SDW + dilution tubes	GYEA + V-8 juice	No
3	10-1-64, Continental, Ariz.	14	Washed tissue + dilution tubes	GYEA	No
	10-1-64, Continental, Ariz.	14	Washed tissue + dilution tubes	GYEA + rose bengal	No
	10-1-64, Continental, Ariz.	14	Washed tissue + dilution tubes	GYEA + V-8 juice	No
4	9-14-64, Bowie, Ariz.	30	Washed tissue + dilution tubes	GYEA	Yes
	9-14-64, Bowie, Ariz.	30	Washed tissue + dilution tubes	GYEA + rose bengal	Yes
	9-14-64, Bowie, Ariz.	30	Washed tissue + dilution tubes	GYEA + V-8 juice	Yes
5	9-14-64, Bowie, Ariz.	32	Washed tissue + dilution tubes	GYEA + aecial extract	Yes
6	11-17-64, Univ. of Ariz. Greenhouse	0	Dilution tubes	GYEA	No
7	9-11-66, Silver City, N.M.	2	70% alcohol + 0.525% NaOCl	PCDA	No

TABLE 1 (Continued)

Trial No.	Collection Date and Site	Time Material Refrig. (days)	Isolation Technique[a]	Medium[b]	T. persicina isolated
8	9-10-66, Lordsburg, N.M.	3	70% alcohol + 0.525% NaOCl	PCDA	No
9	9-14-71, Elfrida, Ariz.	3	0.525% NaOCl + SDW + dilution in SDW	PCDA	Yes
10	9-5-72, Bowie, Ariz.	10	0.525% NaOCl	Cornmeal agar	Yes
11	9-14-72, Robles Junction, Ariz.	1	Flaming with alcohol	Cornmeal agar	Yes

[a] Cultures in trials 1-9 were incubated at 26 C and trials 10 and 11 were incubated at 23 C.

[b] GYEA is the abbreviation for glucose-yeast-extract agar and PCDA is the abbreviation for potato-carrot-dextrose agar.

each of the above dilution tubes was transferred to three Petri dishes containing GYEA which had been amended with V-8 juice (1 liter of GYEA + 6 oz V-8 juice). The cultures were incubated at 26 C.

Trial No. 3, Continental Collection, October 1, 1964: Cotton petioles with aecial pustules containing T. persicina sporodochia and conidia refrigerated for 14 days were placed in a one liter beaker and covered with a wide mesh screen. A stream of tap water was allowed to run into the beaker for 2-4 hr each day for 3 successive days. The petioles were placed in a refrigerator at 5 C between washings. The top layer of aeciospores and T. persicina conidia were scraped from the rust pustules with a scalpel and placed in a test tube containing 10 ml of SDW. The aeciospores-T. persicina conidia mixture was agitated to obtain a spore suspension. A series of four dilution tubes was used to dilute the spore suspension. One ml from each of the dilution tubes was transferred to each of four Petri dishes containing the following agar media: GYEA, GYEA + V-8 juice, GYEA + rose bengal. After draining liquid from the dishes, the cultures were incubated at 26 C.

Trial No. 4, Bowie Collection, September 14, 1964: Cotton rust pustules with T. persicina were selected from refrigerated specimens stored for 30 days. The aecia were placed in a beaker covered with a wire screen and washed with running tap water for 3 hr. Subsequently, they were then rinsed in 500 ml of SDW. Tuberculina persicina conidia were scraped from the cleaned pustules with a sterile scalpel and added to 10 ml of SDW. Other isolation procedures and media used for culturing are the same as those described in Trial 3.

Trial No. 5, Bowie Collection, September 14, 1964: A medium containing cotton rust aecia was used in this isolation trial. Since T. persicina from cotton rust pustules had not been isolated to this date, substances in cotton rust aecia were thought to be essential for T. persicina growth. Aecial pustules were obtained by inoculating cotton leaves with basidiospores of P. cacabata using a technique described by Blank and Leathers (6). When aecial pustules started to form at 13 days, ten leaves heavily infected with aecia were selected and ground with mortar and pestle. Fifty ml of SDW were added while grinding the plant tissues. The resulting suspension was filtered through a Hercules filter (0.1 filter pad) and added to 325 ml of melted sterile GYEA. The medium was poured into nineteen Petri dishes.

Three cotton rust pustules with obvious conidia and sporodochia of T. persicina were selected from refrigerated specimens stored for 32 days and used for isolation purposes. These were cleaned and spore suspensions were prepared as described in Trial 4. One ml of the spore suspension from each of the dilution tubes was transferred to each of four Petri dishes containing the aecia - GYEA medium. After draining liquid from the dishes, the cultures were incubated at 26 C.

Trial No. 6, University of Arizona Plant Pathology Greenhouse Collection, November 17, 1964: Non-refrigerated T. persicina conidia were scraped from cotton rust aecia and added to 10 ml of SDW. One ml of the spore suspension was transferred to a series of three other dilution tubes. One ml of the spore suspension from each dilution tube was added to Petri dishes containing GYEA. The dishes were placed in an incubator at 26 C.

Trial No. 7, Silver City, New Mexico, September 11, 1966: (T. persicina was associated with Puccinia schedonnardi Kell. et Swing. on Sphaeralcea coccinea (Pursh) Rydb.). The technique used to isolate T. persicina from tissue stored in a refrigerator for 2 days was the same as that used for Trial 1 except that potato-carrot-dextrose agar (PCDA) was used as the culture medium. Each liter of PCDA contained: soluble extracts from 200 g potatoes and 50 g carrots; dextrose 20 g; agar 20 g; $MgSO_4$, 0.3 g; $CaCO_3$, 0.2 g; and distilled water to make 1.0 liter.

Trial No. 8, Lordsburg, New Mexico Collection, September 10, 1966: This material from cotton leaves was subjected to the same isolation techniques used in Trial 7.

Trial No. 9, Elfrida, Ariz. Collection, September 14, 1971: Rust pustules from cotton with obvious T. persicina conidia and sporodochia were selected from specimens refrigerated for 3 days. These were surface sterilized in 0.525% NaOCl solution for 5 min and then washed in SDW. Conidia of T. persicina were scraped from the aecial pustules and agitated in a 250 ml Erlenmeyer flask containing 40 ml of SDW. The flask was agitated to suspend the conidia in water. One ml of the spore suspension was transferred to PCDA dishes and incubated at 26 C.

Trial No. 10, Bowie Collection, September 5, 1972: Ten days after collecting and storing at 5 C, rust pustules with T. persicina were removed from cotton leaves and surface sterilized in 0.525% NaOCl for 10 min. The tissue was transferred to Difco cornmeal agar and incubated at 23 C.

Trial No. 11, Robles Junction Collection, September 14, 1972: Green cotton bolls with aecia of P. cacabata and T. persicina sporodochia

and conidia were stored at 5 C for 24 hr following field collections. The bolls were then surface sterilized by dipping briefly into 95% ethyl alcohol and then flamed. Fragments of tissue about 1 mm in size were transferred to Petri dishes containing Difco cornmeal agar and incubated at 23 C.

Growth of T. persicina on Agar Media

Two tests were conducted with several commonly used agar media to determine which might be best for culturing T. persicina. Difco media were used in both tests and were prepared according to label directions. A basal medium similar to one described by Lilly and Barnett (21), also was used in both tests. The composition is described in Test No. 1, below. All media were autoclaved, cooled, and 25 ml were poured into Petri dishes. The pH of each medium was determined with Hydrion pH test papers after autoclaving.

Test No. 1: The six agar media used in this test are summarized in Table 2. The composition of the basal medium is given below:

Difco agar	20.0 g
asparagine monohydrate	2.0 g
sucrose	10.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
biotin	5.0 mg
thiamine	100.0 mg
microelement solution	2.0 ml
distilled water to make	1 liter

TABLE 2. Growth of Tuberculina persicina on agar media, Test No. 1[a]

Medium	pH after Autoclaving	Mean diameter of 10 colonies at 18 days (mm)	Type of Growth
Difco Czapek Dox Agar	7.3	3.7	Flat
Basal Medium Agar	6.5	7.9	Flat
Difco Bean Pod Agar	5.6	12.2	Raised (2 mm)
Difco Potato-Dextrose Agar	5.6	13.4	Raised (4 mm)
Difco Cornmeal Agar	6.0	15.2	Flat
Potato-Carrot-Dextrose Agar	6.0	17.5	Raised (4 mm)

[a] Hyphal tips of T. persicina were placed in the center of media poured into Petri dishes and incubated at 26 C.

The basal medium was buffered with Sorenson's phosphate buffer solution (11) composed of 18.8 ml of 0.1M KH_2PO_4 and 28.1 ml of 0.1M K_2HPO_4 per liter (pH of 6.5 - 7.0).

The microelement solution was prepared as follows:

H_3BO_3	2.5 g
$MnCl_2$	1.5 g
$ZnCl_2$	0.1 g
$CuCl_2$	0.05 g
MoO_3	0.05 g
distilled water to make	1.0 liter

Each medium was poured into ten Petri dishes (except PCDA, of which four dishes were used). Hyphal tips of T. persicina grown on PCDA were placed in the center of the media. The cultures were incubated at 26 C for 18 days.

Test No. 2: Six commonly used agar media were selected for this test, plus the basal agar medium described in Test No. 1. However, the latter was modified by using 15 g of agar and 25 g of glucose. Difco agar, alone, also was used (Table 3).

A culture of T. persicina from Bowie, Ariz. collected on Sept. 5, 1972 was used to treat the media. Conidia were transferred to a tube of SDW and agitated to obtain a conidial suspension. Conidia were added to four places in each of the six agar medium dishes used in this test. Each treated area received 0.008 ml of the conidial suspension, containing approximately 200 conidia. Dishes then were sealed with masking tape and incubated at 23 C for 39 days.

TABLE 3. Growth of Tuberculina persicina on agar media, Test No. 2[a].

Medium	pH after Autoclaving	Mean Diameter of 24 Colonies at 39 days (mm)	Mycelia Growth Habit	Mycelia Color	Conidia Color in Mass
Difco Agar	5.2	0.0	-	-	-
Difco Czapek Solution Agar	7.3	(less than 1 mm)	Flat	Light gray to tan	No conidia
Basal Medium Agar	5.2	(less than 1 mm)	Flat	-	-
Difco Malt Extract Agar	4.6	15.5	Raised	Tan	Yellow to tan, brown areas
Difco Cornmeal Agar	6.0	18.4	Flat	Light gray	Brown
Difco Lima Bean Agar	5.6	19.0	Upright, cottony growth. Tan droplets form on mycelium	White (tan at edges of culture)	Only traces of conidia
Difco Potato-Dextrose Agar	5.6	19.5	Upright	Brown at edges, light gray at center	Tan, conidia very abundant
Difco Cook Rose Bengal Agar	6.0	20.6	Raised	Tan	Pink to lavender, conidia abundant

[a] Tuberculina persicina conidia were added to media in six Petri dishes and incubated at 23 C. Conidia were added to four equispaced sites in each dish.

Growth of T. persicina at Various Carbon Levels

A test was conducted to determine the influence of sugar upon the growth of T. persicina using Difco PDA as the basic medium. Streptomycin sulfate (100 ppm) was added to one treatment to determine its affect upon growth of T. persicina since it might be useful in future isolation studies to prevent growth of bacterial contaminants. A summary of treatments is given in Table 4.

Difco PDA was added to distilled water at the rate of 39 g per 1000 ml of water. Following autoclaving and cooling, 23 ml of each medium was poured into each of 10 Petri dishes. The pH after autoclaving was 5.6.

A culture of T. persicina collected at Elfrida, Arizona on September 14, 1971 and grown on a liquid basal medium was used as the fungus source for treatment. Hyphae of T. persicina were added by pipetting 0.05 ml of the suspension to the center of each dish. The ten Petri dishes from each treatment were placed in a plastic bag to retain moisture and incubated at 23 C.

Growth in Liquid Media

Two tests were conducted to determine growth characteristics of T. persicina in liquid media. The first test was used to determine whether T. persicina could utilize varied carbon sources for growth, and the second to determine the influence of pH upon growth.

Carbon Source Test: The basal liquid medium used in this test is a modification of the medium used by Lilly and Barnett (21) described previously. A basal medium containing asparagine was prepared and 10 g of one of the following carbon sources were added per liter: D^{+} maltose

TABLE 4. Growth of Tuberculina persicina on various quantities of glucose[a].

Medium	Mean Diameter of 10 Colonies at 65 Days
PDA only	56.4
PDA + Streptomycin Sulfate (100 ppm)	55.9
PDA + 1% Glucose	58.1
PDA + 1.5% Glucose	56.9
PDA + 2% Glucose	59.8
PDA + 3% Glucose	59.8
PDA + 4% Glucose	62.5

[a] Hyphal tips of T. persicina were centrally placed on media in Petri dishes, and incubated at 23 C.

hydrate, D⁻ mannose, galactose, sucrose, lactose, L sorbose, D⁻ levulose, starch, or cellulose. The control medium contained asparagine as the primary source of carbon. Twenty-five ml of each of the carbon source solutions was poured into four 250 ml Erlenmeyer flasks. Cotton stoppers were inserted in the mouth and a cap was placed over the stopper. All solutions were autoclaved at 20 psi for 15 min.

After cooling, 0.5 ml of a T. persicina spore suspension containing approximately 500 conidia was added to each culture flask. The T. persicina conidia added to the flasks were taken from a culture isolated from cotton rust aecia collected at Elfrida, Arizona on September 14, 1971 and grown on PCDA. Cultures were incubated at 26 C.

pH Test: The basal medium used here was the same as that used for the carbon nutrition study. The carbon source consisted of 10 g of sucrose per liter. The medium was autoclaved at 20 psi for 15 min. Individual treatments were adjusted to the following pH levels with NaOH or HCl after autoclaving: 5.0, 6.0, 6.5, 7.0, 7.5, and 8.0. One hundred ml of each pH treatment were poured into four 250 ml Erlenmeyer flasks which had been previously plugged with cotton, capped, and then sterilized. To each flask was added 0.5 ml of a T. persicina spore suspension; this volume contained approximately 650 conidia. The conidia were taken from a culture isolated from cotton rust aecia collected at Elfrida, Arizona on September 14, 1971 and grown on PCDA. Cultures were incubated at 26 C. Each culture was examined at 2, 6, and 8 weeks after conidia were added and estimates were made of growth. Final dry weights of mycelia were made at 25 weeks.

Comparison of Conidia and Hyphae for Subculturing

Since conidia and hyphae offered potential for subculturing T. persicina on various media, each of these was tested to determine if differences in growth would result.

Conidia and hyphae were taken from a culture of T. persicina collected on September 3, 1972 at Bowie. Conidia were transferred to a tube of SDW and agitated to obtain a suspension. Hyphae also were selected from the edge of the culture and added to a second tube of SDW and then agitated. One ml of the conidial suspension was added to one ml of the hyphal suspension in a third tube. Petri dishes containing 20 ml of PDA were treated with propagules of T. persicina at four areas in each dish at equal distances from each other, using eight dishes per treatment. Each treated area received 0.007 ml of the fungus propagules. The treatments consisted of: (1) conidia only (75 conidia per treated area), (2) conidia plus hyphae (38 conidia plus 10 hyphae with an average length of 100μ), and (3) hyphae alone (20 hyphae with an average length of 100μ). Each dish was sealed with masking tape and incubated at 23 C for 44 days.

Influence of Temperature upon Growth of T. persicina

A culture from Bowie, Arizona collected on September 5, 1972 was used as the fungus source. Conidia were transferred to a tube of SDW and agitated to obtain a conidial suspension. Petri dishes containing 25 ml of PDA were treated, in the manner previously described, in four places on each dish using four dishes for each treatment. Each area received 0.009 ml of the spore suspension which contained approximately 200 conidia. Each dish was sealed with masking tape and incubated at

23 C for 6 days. At the end of the 6 day growing period four dishes were placed in incubators at each of the following temperatures: 9 C, 17 C, 23 C, 27 C, 30 C, 34 C, and 37 C. Colony diameters were measured 40 days after treatment.

Conidial Germination Affected by Water

The effect of water upon germination of T. persicina conidia was studied. A culture from Elfrida, Arizona collected on September 14, 1971 was used as source of the fungus. Conidia from two-month-old cultures were suspended in 10 ml of SDW. One ml of the suspension then was transferred to each of six tubes, each containing 9 ml of SDW. These tubes were agitated for 2 min after which the conidia were allowed to soak for: 15 min, 2 hr, 6 hr, 24 hr, 48 hr, or 72 hr. At the end of each treatment, 0.5 ml (containing approximately 200 conidia) of a given suspension was transferred to two Petri dishes containing 2% water agar and to four PDA dishes. The dishes were incubated at 26 C. Germination counts were made at 24 hr and again at 6 days by counting 100 conidia in each dish and recording the no. of conidia with germ tubes.

Survival of T. persicina in Culture

Measurements were made of the length of time that T. persicina remained viable as conidia and mycelium on an agar medium. Cultures selected for this study were of the following ages: 12.5 months, 10.5 months, 8 months, 6 months, and 1.5 months. All cultures were grown on PDA at 23 C, allowed to dry as growth proceeded, and four cultures were randomly selected from each age group. Conidia from the four dishes of each age group were combined and transferred to SDW, then 0.2 ml of the conidial suspension (approximately 250 conidia) were transferred

to four Petri dishes of PDA and four Petri dishes of 2% water agar. The dishes were placed in an incubator at 23 C and germination counts were taken at 6 days. Germination counts were made by counting 100 conidia in each dish and recording the number of those with germ tubes. Attempts were made to induce growth in the dried cultures by pouring cooled PDA on the cultures and incubating for 2 weeks at 23 C.

Survival of T. persicina on Plant Tissue

Measurements were made to determine germination percent of T. persicina conidia stored for various time periods on original host tissue. The source of T. persicina conidia was from cotton rust aecia on cotton leaves collected at Deming, N. M. on September 30, 1969 and Bowie, Arizona on September 5, 1972. Conidia also were collected at Robles Junction, Arizona from cotton rust aecia on green cotton bolls. Conidia were tested for germination on 2% water agar and PDA at various time periods after collection and storage at conditions recorded below for each collection. The number of conidia and number of dishes used for each test is also given below. Germination counts were made at 3 days by counting 100 conidia in each dish and recording the number of conidia with germ tubes.

Deming, New Mexico Collection: Cotton leaves with approximately 20 mature cotton rust pustules invaded by T. persicina were collected from the field and allowed to dry on the lab table at 24 C for 7 days. The leaves then were placed in a sterile Petri dish and stored on the lab table for 2.5 years. At the end of the storage period the leaves were washed under tap water for a 2 hr period to remove dust and other foreign material. Tuberculina persicina conidia from four rust pustules

(several thousand conidia) were transferred to 10 ml of SDW. One ml of the conidial suspension was added to each of four dilution tubes. One-half ml of the suspension from each dilution tube was pipetted to a Petri dish containing 2% water agar and to each of two dishes containing PDA. The cultures were incubated at 26 C for 3 days, then germination counts were made.

Six rust pustules with T. persicina were randomly selected from the stored material, washed in tap water for 2 hr, then surface sterilized in 0.525% NaOCl for 3 min. The tissue was transferred to four PDA dishes and incubated at 26 C for one month.

Bowie, Arizona Collection: Cotton leaves with aecia invaded by T. persicina were collected at Bowie, Arizona on September 5, 1972 and stored in a refrigerator at 5 C.

Seven days after storage at 5 C four leaves containing 20 rust pustules were placed in a Petri dish and allowed to dry on a lab shelf for 41 days. Conidia were scraped from 8-10 pustules and placed in the center of two Petri dishes each with 2% water agar. One ml of SDW was added to each dish, then placed in an incubator at 23 C for 3 days.

Eight days after storage a second germination test was conducted. Conidia were scraped from 10 aecia and suspended in 10 ml of SDW. One ml of the suspension was pipetted to each of four Petri dishes containing 2% water agar. Dishes were sealed with masking tape and placed in an incubator at 23 C for 3 days, after which germination counts were made.

Fourteen days after storage at 5 C, a third germination test was conducted. After placing 8-10 aecia in an aqueous solution of 100 ppm

streptomycin sulfate for 5 min, conidia of T. persicina were scraped from the aecia and added to 10 ml of SDW amended with 100 ppm streptomycin sulfate for 5 min. One ml of the suspension was added to a second tube containing 9 ml SDW, then one ml of the conidial suspensions in tubes one and two were separately added to each of two Petri dishes containing 2% water agar. The dishes were incubated at 23 C for 3 days.

For the fourth germination test, four cotton leaves with 8-10 pustules per leaf were collected from the same Bowie field but on September 23. These were allowed to dry at 22 C for 23 days. Conidia were scraped from the pustules and conidial germination was determined on 2% water agar in four Petri dishes by using the same techniques described in the first germination test.

Robles Junction Collection: Green cotton bolls with cotton rust pustules which had been invaded by T. persicina were collected west of Tucson, Arizona (Robles Junction) on September 14, 1972, and temporarily stored in an incubator at 5 C. Twenty-four hours later, the viability of Tuberculina conidia was determined as follows: several thousand T. persicina conidia were scraped from the pustules and suspended in 10 ml of SDW. One ml of the conidial suspension was added to a second tube containing 9 ml of SDW. One ml of the conidial suspension from each tube was pipetted to each of two Petri dishes containing 2% water agar. The cultures were placed in an incubator at 23 C for 3 days.

For the second germination test, aeciospores and T. persicina conidia were scraped from approximately fifty rust pustules after 24 hr at 5 C. The resulting mixtures of spores was divided into four equal

parts and placed in four separate Petri dishes. Dishes were placed at -16 C, 17 C, 22 C, and in a desiccator at 25 C. Dishes were removed after 30 days later and conidia were prepared for germination as follows: dry conidia of T. persicina plus adhering aeciospores, were lifted with sterile forceps and transferred to the centers of two Petri dishes containing 2% water agar. One ml of SDW was added and each dish was rotated so that the conidia were evenly dispersed. Each dish contained approximately 10,000 T. persicina conidia. Dishes were incubated at 23 C for 3 days, after which viability determinations were made.

For the third germination check, bolls with T. persicina were removed after 4 days of refrigeration and placed in an aqueous solution of 100 ppm streptomycin sulfate for 5 min. Tuberculina persicina conidia were scraped from two rust pustules and suspended in 10 ml of the 100 ppm streptomycin sulfate, after which one ml was pipetted to a second dilution tube. One ml of the spore suspension from each of the two tubes was pipetted to each of two Petri dishes containing 2% water agar. There was a minimum of 500 conidia per dish. Dishes were incubated at 23 C for 3 days.

Four days after being placed in temporary storage at 5 C, the fourth germination test was started. Four of the green bolls with aeciospores and T. persicina conidia were randomly selected and placed in a freezer at -16 C for 26 days. Subsequently, conidia were scraped from the aecia and deposited in the centers of two Petri dishes containing 2% water agar. Following the addition of one ml of SDW, the conidia were incubated for 3 days at 23 C.

Inoculation of Cotton Rust with T. persicina

Inoculation studies were conducted to determine whether P. cacabata could be infected with T. persicina, to study the development of T. persicina on rust pustules, and to provide tissue for histological studies.

Trial No. 1: Cotton plants of the 1517C cultivar were grown in six-inch greenhouse pots and thinned to 3-4 plants per pot. At the two-leaf stage, plants were exposed to basidiospores of P. cacabata. Basidiospores were obtained: (1) by soaking grama grass (Bouteloua barbata Lag.) containing teliospores of P. cacabata in water for a few hours, and (2) by placing the telial material on a wide mesh screen above the cotton plants in a humidity chamber for 12-18 hr (Fig. 1). Spermagonia appeared on the upper surface of cotton leaves 5 days after inoculation. Individual leaves had 100 to 250 spermagonia on the upper leaf surface.

Prior to inoculating plants with T. persicina, one leaf from each pot was taped with a 3/4" square piece of Scotch Brand transparent tape on the upper surface and a second leaf was taped on the lower leaf surface. The purpose of taping was to provide a barrier for T. persicina to determine whether T. persicina infection takes place on the upper or lower leaf surface.

The T. persicina culture used as inoculum was isolated from cotton rust aecia collected at Bowie, Arizona on September 5, 1972 and grown on PDA. Tuberculina persicina inoculum was prepared by transferring conidia from the culture to a tube of SDW and agitating to obtain a conidial suspension.

Fig. 1. Humidity chambers used to inoculate cotton with basidiospores of Puccinia cacabata.--Basidiospores were produced by teliospores on grama grass plants suspended on a wire screen above the cotton plants for 12-18 hr.

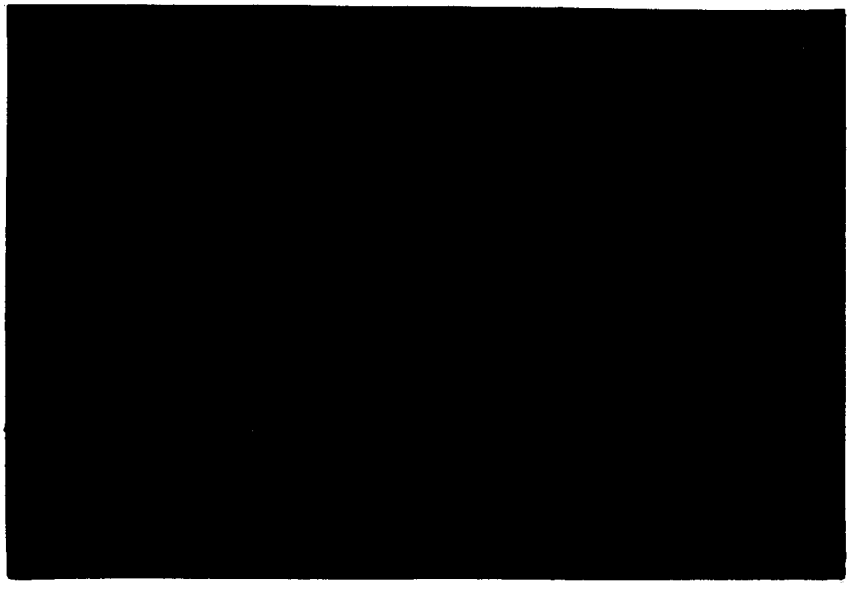

Fig. 2. Technique used to inoculate spermagonia of Puccinia cacabata.--A conidial suspension of Tuberculina persicina was brushed onto cotton leaves. Plants were then covered with a polyethylene bag for 24 hr to maintain high humidity.

Cotton rust spermagonia were inoculated 2 days after they were first observed by applying the inoculum with a fine camel hair brush. The portion of leaves inoculated is as follows: Pot #1, both leaf surfaces; Pot #2, upper leaf surface; Pot #3, bottom leaf surface; Pot #4 (check pot), both surfaces of leaves treated with SDW. Four cotton leaves free of spermagonia also were inoculated with the conidial suspension. The number of T. persicina conidia in the spore suspension was calculated to be 12,500/ml. Two ml were used to inoculate the test plants. When tested on 2% water agar, nearly 100% of the conidia germinated.

Following inoculation, two wet paper towels were placed at the inner edge of each pot. The plants then were covered with transparent plastic bags for 24 hr to insure 100% humidity (Fig. 2).

Plants were maintained in growth chambers from the time they were inoculated with T. persicina conidia until final readings were taken. Uninoculated check plants were placed in separate chambers to prevent accidental spread of T. persicina. All chambers were regulated to provide a temperature of 24 C and a diurnal light cycle of 12 hr. A summary of treatments is shown in Table 5.

Trial No. 2: Plants of the cultivar Deltapine 16 were grown in six-inch greenhouse pots and thinned to four plants per pot. Plants were inoculated at the 3-4 leaf stage with basidiospores of P. cacabata in the same manner as in Trial No. 1. Inoculations were made on several successive days to obtain plants with rust pustules at different stages of development.

TABLE 5. Colonization of *Puccinia cacabata* aecia by *Tuberculina persicina* when inoculated with *T. persicina* conidia, Trial No. 1[a].

Pot No.	Leaf No.	Percent of aecial surface colonized by T. persicina[b]		
		7 days	14 days	24 days (all leaves)
1-upper & lower leaf surface inoc.	1	5	-	75
	2	15	50	
	3	5	-	
	4	15	35	
	5	20	35	
	6	10	-	
	7	5	35	
	8	0	-	
	9	5	-	
2-upper leaf surface inoc.	1	10	35	75
	2	15	50	
	3	5	35	
	4	25	50	
	5	15	-	
	6	5	35	
3-lower leaf surface inoc.	1	0	-	75
	2	25	35	
	3	10	-	
	4	0	35	
	5	0	35	
	6	5	35	
4-Uninoculated check		0	0	0

TABLE 5 (Continued)

[a] Cotton rust spermagonia were inoculated with T. persicina conidia 2 days after spermagonia were evident. Following inoculation, the plants were covered with transparent plastic bags for 24 hr to insure 100% humidity. Plants were maintained in chambers at 24 C.

[b] The percent figures are visual estimates of the aecial surface area colonized by T. persicina. Each cotton leaf contained approximately 20-25 aecial pustules on the lower leaf surface. Dash (-) indicates that leaves were previously removed for histological study.

One leaf in each pot was taped on the top surface and another on the lower surface with Scotch Brand transparent tape after the final group of plants was inoculated. The tape was placed parallel along the leaf until the leaf was completely covered. An additional layer of tape was applied at right angles to the other layer. The tape was applied in an effort to exclude T. persicina from the leaf surface to determine the sites of penetration.

Plants with rust spermagonia and aecia in this trial were inoculated with T. persicina conidia at the same time. Other check plants were not inoculated. Ten leaves free of spermagonia also were inoculated with T. persicina conidia.

The number of spermagonia of P. cacabata varied from five to fifty per leaf. In order for each treatment to have approximately the same number of rust pustules, it was necessary to use a few more plants in certain treatments than in others. Each treatment contained approximately 50 aecial pustules.

Tuberculina persicina conidia used as inoculum were obtained from the same culture used in Trial No. 1. When tested on 2% water agar, conidial germination was nearly 100%. Ten ml of the suspension were used to inoculate all test plants; each ml contained approximately 30,000 conidia. Inoculum was applied with a fine camels hair brush to both leaf surfaces except for check plants which were not inoculated. Following inoculation with T. persicina, the plants were grown as in Trial No. 1. Visual estimates of the aecial surface area colonized by T. persicina were made at 7, 14, and 24 days following inoculation.

A summary of treatments at the time of inoculation with T. persicina conidia is summarized in Table 6.

Histological Studies

Histological studies were conducted so that the relationship between T. persicina and the cotton rust fungus could be better understood. Histological material for study was obtained by inoculating cotton leaves with P. cacabata (Fig. 1), and then inoculating the resulting cotton rust infected leaves with T. persicina conidia on several successive days (Fig. 2). All material was fixed in formalin-aceto-alcohol (FAA) (15). Cotton leaf tissues inoculated only with P. cacabata basidiospores also were studied for comparison. Tissues were dehydrated by using the Zirkle n-butyl dehydration method (15), then placed in one-half paraffin + one-half n-butyl alcohol in an oven at 62 C for 8-12 hr. Tissuemat paraffin with a melting point of 55 C was used to embed the tissues. Following two changes of paraffin over a 3-6 hr period at 62 C, the tissues were embedded in paraffin and prepared for rotary microtoming. Ribbons with 12 μ thick sections were floated on 4% formalin on slides coated with Haupt's adhesive (15). Slides were allowed to dry for one hr or more on a slide warmer. Paraffin was removed by moving the slides through two changes of xylol followed by a graded series of alcohol (15).

Sections were stained by using orseillin BB in 3% acetic acid counterstained with fast green (1), orseillin BB in 3% acetic acid counterstained with cotton blue (1), or pianeze counterstained with cotton blue (24). The stained sections were mounted in Canada balsam (15).

TABLE 6. Colonization of Puccinia cacabata aecia by Tuberculina persicina when inoculated with T. persicina conidia, Trial No. 2.

Age of spermagonia when inoculated with T. persicina conidia[a]	Leaf surface taped[b]	Percent Infection of Rust Pustules-Days after Inoculation with T. persicina[c]						
		6	9	10	13	17	22	29
17	Untaped	0	0	0	0	-	-	-
	Upper	0	0	0	3	-	-	-
	Lower	0	0	0	50	-	-	-
7	Untaped	1	10	10	10	50	60	75
	Upper	0	0	10	-	-	-	-
	Lower	0	10	10	10	-	-	-
6	Untaped	0	0	0	1	15	15	15
	Upper	0	0	0	0	15	15	15
	Lower	0	0	0	0	15	15	15
5	Untaped	0	0	1	1	1	1	1
	Upper	0	0	1	2	2	2	2
	Lower	0	0	1	10	10	10	10
1	Untaped	0	0	0	10	25	50	50
0	Untaped	0	0	0	10	10	25	50

[a] Each figure refers to the no. of days after cotton leaves were inoculated with basidiospores of P. cacabata. Spermagonia appeared on cotton leaves 4-5 days after inoculation with P. cacabata basidiospores.

[b] Leaves with spermagonia of various ages were taped on the upper or lower leaf surface with Scotch Brand transparent tape in an effort to exclude T. persicina conidia; other leaves were left

TABLE 6 (Continued)

untaped. Untaped leaves were inoculated on both upper and lower surfaces with T. persicina conidia using a fine camel hair brush.

[c] The percent figures are visual estimates of the aecial surface area colonized by T. persicina. Each treatment contained approximately 50 aecial pustules. Dash (-) indicates that further readings were not taken because leaves had begun to dry.

EXPERIMENTAL RESULTS

Isolation of T. persicina in Pure Culture

A problem existed in isolating T. persicina at first because cultural characteristics of the fungus have not been described in the literature. The only distinguishing character that could be used for identification was conidial size and shape (26).

Another problem in isolating T. persicina was the slow rate of growth in culture (Table 2, 3). Numerous cultures of T. persicina have probably been discarded in early isolation attempts because sufficient time was not allowed for growth. Two weeks sometimes were needed before the fungus produced sufficient mycelium for detection.

Results of various isolation attempts are given in Table 1. The results indicate that several techniques can be used in isolating T. persicina from spermagonial and aecial specimens.

A few of the isolation attempts failed because of excessive growth of Cladosporium sp. and Alternaria sp. These two fungi were common contaminants in most of the isolation dishes. It was necessary to make transfers of T. persicina as soon as mycelial growth could be detected to avoid these contaminants. It was difficult to make isolations of T. persicina from field collected material stored in a refrigerator for more than 2 weeks because other fungi and bacteria "overran" the plant tissue.

In successful isolation attempts, it was easy to obtain cultures of T. persicina from media treated with the third and fourth dilution

tubes when this isolation technique was used. The media treated with the first and second dilutions were usually overrun with contaminants.

Growth of T. persicina on Agar Media

Tuberculina persicina made satisfactory growth on most media used in these tests (Table 2, 3). However, it grew poorly on the basal agar medium and Difco's Czapek Dox Agar. No growth was recorded on Difco agar (Table 3). The best overall growth was obtained on PDA and PCDA at 26 C, and Difco's Cook Rose Bengal Agar and PDA at 23 C. Cultural characteristics and color of both mycelium and conidia differed between various media (Table 2, 3). Typical cultures of T. persicina are shown in Fig. 3, 4.

Vegetative hyphae of T. persicina grown on PDA are hyaline and measure 4.5 to 7.0μ in diameter with a mean diameter of 5.5μ. Oil-like globules are commonly found within hyphae, although some hyphae have no visible cell contents (Fig. 5). Spherical or elongate hyphal swellings resembling chlamydospores measuring 14.0 to 16.0μ in diameter are frequently found near sporodochia (Fig. 6).

Conidia measure 9 X 11μ and are born at the ends and sides of conidiophores as "blown-out cells" (Fig. 7). Hyaline conidiophores arise from specialized hyphae which make up the dark brown, dense sporodochium (Fig. 8). Hyphae at the base of the sporodochium measure 10.0 to 12.0μ in diameter and arise from a dense layer of hyphae (Fig. 8). Hyphae at the base of the sporodochium and hyphae that bear the chlamydospore-like structures appear to have clamp connections (Fig. 6, 8). Two nuclei could be found within the cells of hyphae when they were stained with orseillin BB and cotton blue (Fig. 9).

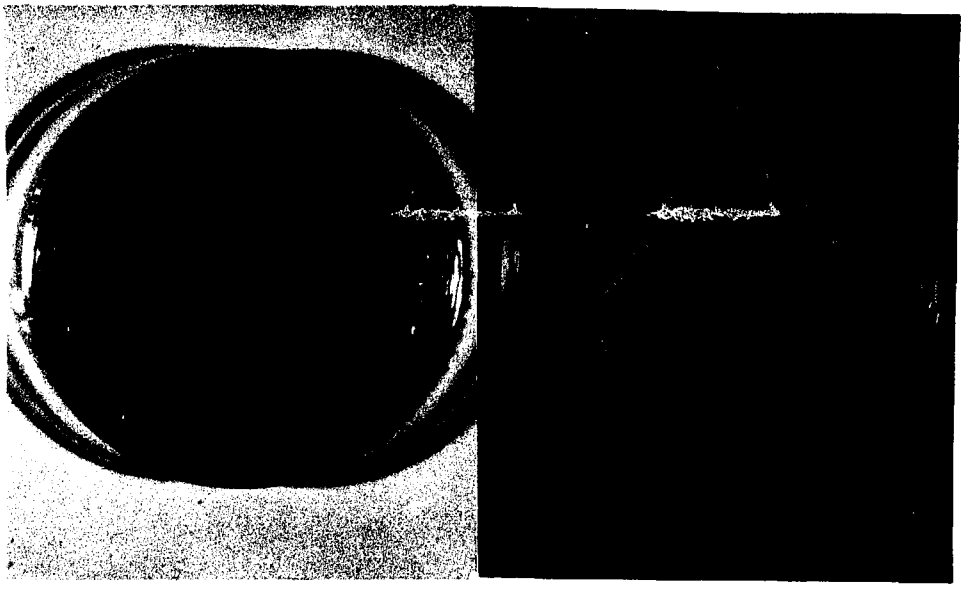

Fig. 3. Two-week old cultures of Tuberculina persicina.--Culture on the left is growing on PDA and producing masses of conidia which appear as dark areas. Culture on the right is growing on cornmeal agar and has produced no conidia (X 0.6).

Fig. 4. Tuberculina persicina cultures grown for 2 weeks on PDA.--Masses of conidia appear as dark brown areas (Left - 2X, Right - 4X).

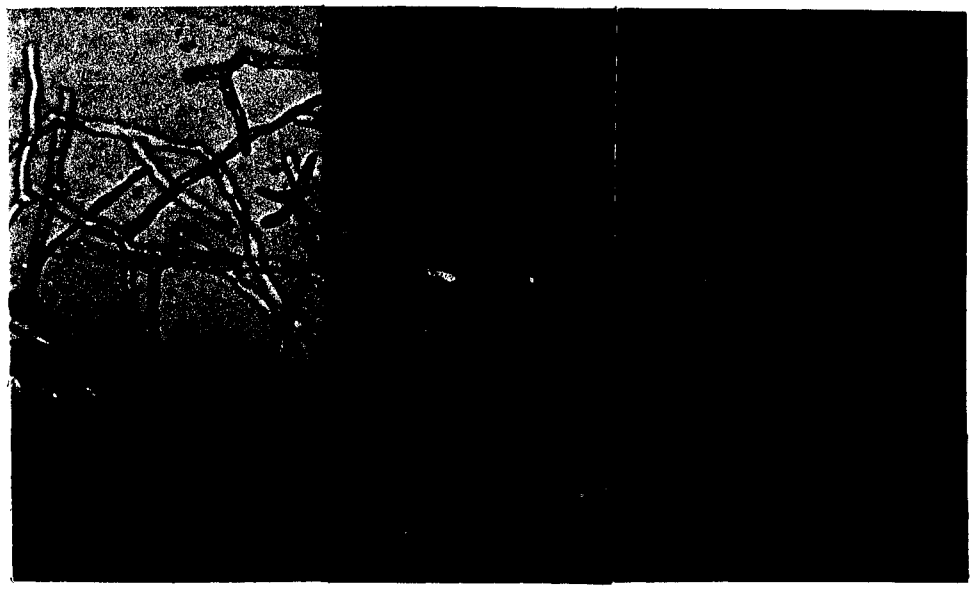

Fig. 5. Hyphal characteristics of Tuberculina persicina on PDA.-- Left: typical vegetative hyphae (X 225), unstained; center: hyphae with and without oil-like globules (og) (X 1000), unstained; right: swollen hyphae, stained with orseillin BB (X 1000).

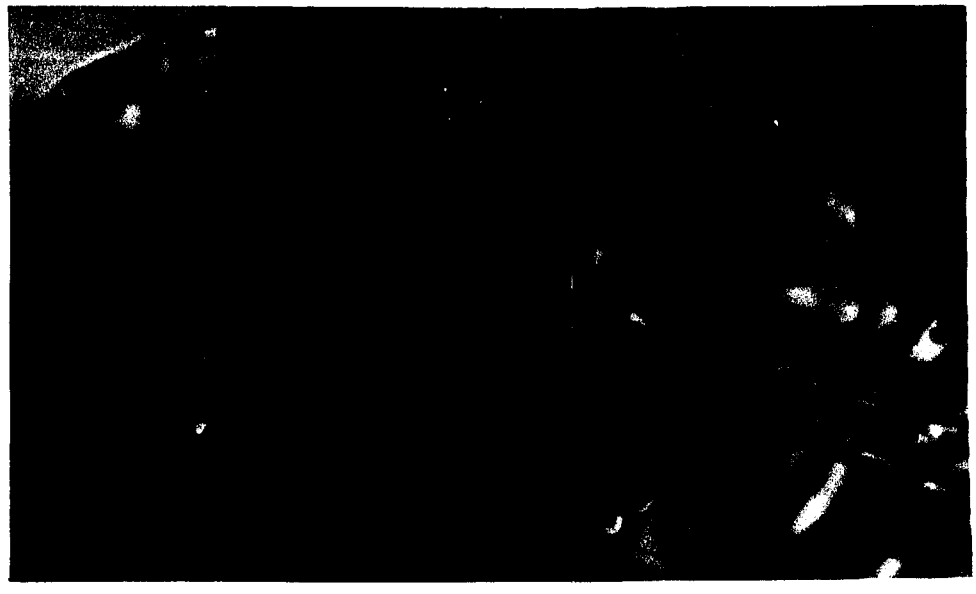

Fig. 6. Hyphal swellings of Tuberculina persicina grown on PDA.-- Left: typical hyphal swelling (hs) (X 1000), unstained; right: brown to red hyphal swelling (X 1000), unstained.

Fig. 7. Conidia of Tuberculina persicina grown on PDA.--Left: conidia (c) formed on conidiophore (cp) as the result of blown-out cells (X 1000); center: newly formed conidium (c) at the end of a conidiophore (X 1000); right: germinating conidia on PDA at 18 hr, stained with orseillin BB (X 225).

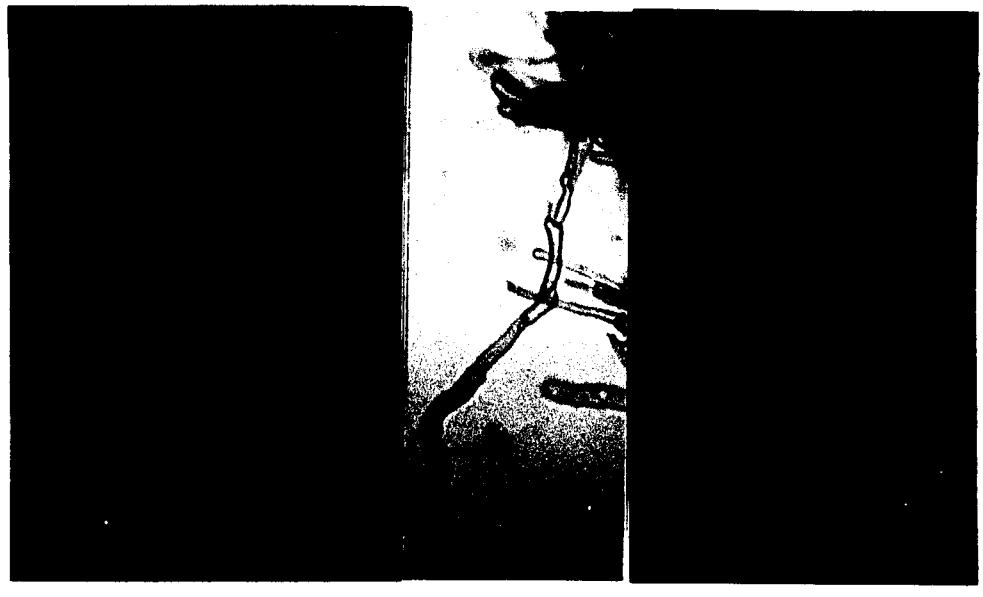

Fig. 8. Sporodochium characteristics of Tuberculina persicina grown on PDA.--Left: sporodochium with conidia (c) and individual hyphae (h) (X 90); center: single hypha (h) of sporodochium (X 225); right: individual hypha (h) of sporodochium with conidia (c) and conidiophores (cp) (X 225).

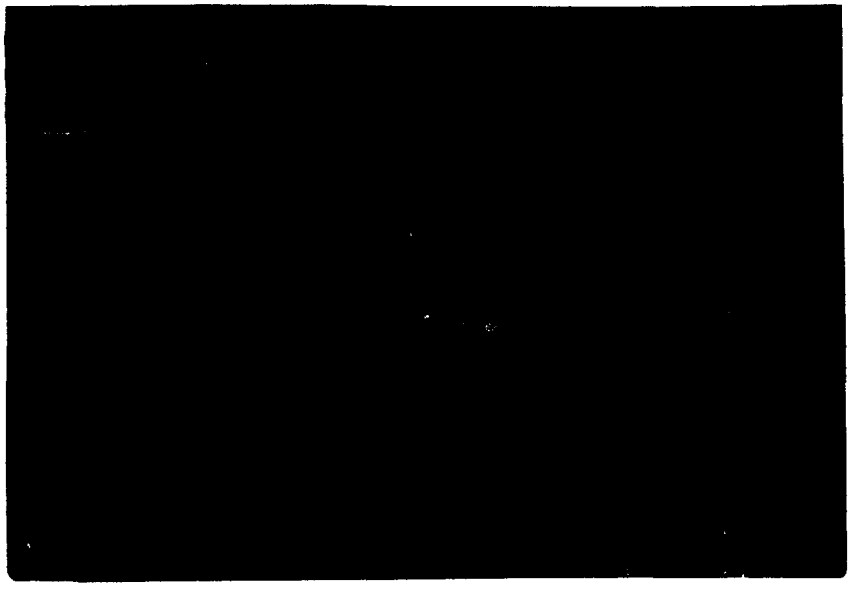

Fig. 9. Dikaryotic hypha of Tuberculina persicina from a culture grown on PDA and stained with orseillin BB and cotton blue (X 1000).

Growth of T. persicina at Various Carbon Levels

There were no measurable differences between treatments in total growth or cultural characteristics of T. persicina after an incubation period of 2 weeks. Differences in total colony diameter at sixty-five days are shown in Table 4.

Growth in Liquid Media

Carbon Source Test: Tuberculina persicina grew on all of the carbon sources tested except L sorbose. Overall growth on all media was poor. Since differences of growth between treatments were so slight, weight measurements were not taken (Table 7). Tuberculina persicina grew as small, individual colonies measuring 5 to 10 mm in diameter. The mycelia at the edges of the colonies were hyaline to brown in color while the centers of the colonies were orchid to lavender. Conidia were not observed.

pH Test: Growth of T. persicina was influenced by the hydrogen ion content of the liquid basal medium. Best growth occurred in media with a pH of 5.0, 6.0, or 6.5. Poor growth resulted at a pH of 7.0, only traces of growth resulted at 7.5, and no growth occurred in the medium at 8.0 (Table 8). Conidia were not observed in any of the cultures.

Comparison of Conidia and Hyphae for Subculturing

Only slight differences in lateral growth of T. persicina colonies resulted in subcultures when hyphae were used rather than conidia. There were no visible differences in the type of growth, color, or number of conidia produced between treatments. The mean lateral diameters of colonies grown on PDA in Petri dishes for 44 days are as

TABLE 7. Growth of Tuberculina persicina on basal liquid media plus various carbon sources[a].

Carbon Source[b]	Visual growth of T. persicina[c]		
	10 days	17 days	24 days
D+ maltose hydrate	+	+	+
D⁻ mannose	+	+	+
galactose	+	+	+
sucrose	+	+	+
lactose	+	+	+
L sorbose	−	−	−
D⁻ levulose	+	+	+
starch	−	+	+
cellulose	+	+	+
check[d]	−	−	+

[a] Tuberculina persicina conidia were added to 250 ml Erlenmeyer flasks containing 25 ml of the liquid basal medium and incubated at 26 C.

[b] One of the carbon sources listed was added to the liquid basal medium for each treatment at the rate of 10 g per liter. Four flasks were used for each treatment.

[c] Each culture was examined at 10, 17, and 24 days after conidia were added. + indicates visual growth of T. persicina; − indicates no obvious growth.

[d] Asparagine used as a source of nitrogen in the basal liquid medium was the primary source of carbon in this treatment.

TABLE 8. Growth of Tuberculina persicina on basal liquid media adjusted to various pH levels[a].

pH[b]	Growth of T. persicina[c]			
	2 weeks	6 weeks	8 weeks	25 weeks (g)
5.0	−	++	++	0.299
6.0	+	++	++	0.212
6.5	+	++	++	0.123
7.0	−	+	+	0.045
7.5	−	−	+	0.028
8.0	−	−	−	0.0

[a] Tuberculina persicina conidia were added to 250 ml Erlenmeyer flasks containing 100 ml of the liquid medium and incubated at 26 C.

[b] The liquid medium in each Erlenmeyer flask was adjusted with NaOH or HCl for each pH level studied. Four Erlenmeyer flasks of media were used for each treatment.

[c] Each culture was examined at 2, 6, and 8 weeks after conidia were added and estimates were made of growth. ++ indicates good growth, + indicates poor growth, and − indicates no growth. Final weights were taken at 25 weeks; each value is the mean weight of growth per flask in four Erlenmeyer flasks.

follows: conidia only, 23.1 mm; conidia + hyphae, 21.2 mm; and hyphae only, 19.8 mm.

Influence of Temperature upon Growth of T. persicina

Tuberculina persicina grew on PDA dishes at 17 C, 23 C, 27 C, 30 C, and 34 C; but failed to grow at 9 C or 37 C by the end of the 40-day growth-period (Table 9). Optimum growth occurred between 27 - 30 C.

Conidial Germination Affected by Water

The number of T. persicina conidia germinating was reduced when they were soaked in water for more than 15 min. Germination dropped to 1% when conidia were soaked in water for 24 hr or longer and then tested on 2% water agar. Germination of conidia was higher on 2% water agar than on PDA (Table 10).

Survival of T. persicina in Culture

Germination of T. persicina conidia from a 1½ month-old culture grown on PDA was near 100% when tested on 2% water agar and on PDA; however, it dropped to 1% at the end of a 6 month test period after the cultures were allowed to dry. Conidia from 8-month-old cultures and older failed to germinate and the fungus did not grow after PDA was poured onto the cultures (Table 11).

Survival of T. persicina on Plant Tissue

Tuberculina persicina conidia from naturally infected aecia of P. cacabata on cotton leaves stored for 2½ years in a Petri dish at laboratory temperatures failed to germinate when tested on 2% water agar and also failed to germinate and grow on PDA (Table 12).

TABLE 9. Growth of Tuberculina persicina on PDA after 34 days at various temperatures[a].

Temperature (C)	Diameter of Colony (mm)[a]
9	0.0
17	15.8
23	19.8
27	27.1
30	26.5
34	10.4
37	0.0

[a] Each figure is the mean diameter of 12 T. persicina colonies grown on PDA in three Petri dishes.

TABLE 10. Germination of Tuberculina persicina conidia as affected by various soaking periods in water[a].

Soaking Time	Mean Germination Percent			
	Water Agar[b]		PDA[c]	
	24 hr	6 Days	24 hr	6 Days
15 min	28	39	17	25
2 hr	9	34	8	19
6 hr	17	25	8	19
24 hr	1	4	1	3
48 hr	1	1	1	2
72 hr	1	1	1	1

[a] A 0.5 ml spore suspension containing approximately 200 T. persicina conidia were transferred to Petri dishes containing 2% water agar and to Petri dishes containing PDA. Dishes were incubated at 26 C and germination counts were made at 24 hr and again at 6 days. Germination counts were made by counting 100 conidia in each dish and recording the no. of conidia with germ tubes.

[b] Each figure represents the mean percentage of conidia germinating on two water agar dishes.

[c] Each value represents the mean percentage of conidia germinating on four dishes of PDA.

TABLE 11. Survival of Tuberculina persicina cultures grown on PDA at 23 C.

Age of Cultures (months)	Mean Percentages[a] of Conidia Germinating	Viable Growth on PDA at End of Storage Period[b]
1.5	99+	Yes
6.0	1	Yes
8.0	0	No
10.5	0	No
12.5	0	No

[a] Conidia from each of four Petri dishes in each age group were suspended in SDW; then, 0.2 ml (approximately 250 conidia) was transferred to each of four Petri dishes of PDA and four Petri dishes of 2% water agar and incubated at 23 C for 6 days. Determinations were made by counting 100 conidia in each dish and recording the no. with germ tubes. Each figure represents the mean percentage germinating in four dishes of 2% water agar and four dishes of PDA.

[b] Cooled PDA was poured on the cultures in four Petri dishes selected from each time period. The cultures were incubated for 14 days and then checked for growth.

TABLE 12. Mean percentage germination of *Tuberculina persicina* conidia stored on plant tissues under various conditions[a].

Collection Site	Stored Material	Treatment	Conidial Germination Percent
Deming, N.M.	Dry cotton leaves	2½ yrs on lab shelf	0
Bowie, Ariz.			
Test No. 1	Green cotton leaves	Refrigerate at 5 C for 7 days and 41 days on lab shelf	0
Test No. 2	Green cotton leaves	Refrigerate at 5 C for 8 days	84
Test No. 3	Green cotton leaves	Refrigerate at 5 C for 14 days (Streptomycin sulfate)[b]	98
Test No. 4	Dried cotton leaves	Lab shelf for 23 days	10
Robles Junction, Ariz.			
Test No. 1	Green cotton bolls	Refrigerate at 5 C for 1 day	64
Test No. 2	Dry conidia	-16 C for 30 days	1
Test No. 2	Dry conidia	Desiccator at 25 C for 30 days	0
Test No. 2	Dry conidia	Incubator at 17 C for 30 days	0
Test No. 2	Dry conidia	Lab table for 30 days	0
Test No. 3	Green cotton bolls	Refrigerate at 5 C for 4 days (Streptomycin sulfate)[b]	70
Test No. 4	Green cotton bolls	-16 C for 30 days	1

[a] *Tuberculina persicina* conidia were tested for germination on 2% water agar after various periods of storage under the listed conditions. Determinations were made after 3 days by counting 100 conidia in each dish and recording the no. with germ tubes.

TABLE 12 (Continued)

[b] Aecial material containing T. persicina were placed in an aqueous solution of 100 ppm streptomycin sulfate for 5 min before removing the T. persicina conidia.

Conidial germination at the end of an 8-day storage period at 5 C was 84% when tested on 2% water agar. However, 98% of the conidia from the same collection germinated when leaves stored for 14 days at 5 C were treated with streptomycin sulfate (100 ppm) before the conidia were removed for the germination tests. Further testing of conidial germination was discontinued after 14 days storage at 5 C because of excessive growth of other organisms on the green cotton leaves and aecia (Table 12).

Tuberculina persicina conidia from aecia on green cotton bolls also were tested for germination on 2% water agar. Germination was 70% when stored for 4 days at 5 C but dropped to 1% when conidia from the same collection were held for 30 days at -16 C. Conidia failed to germinate when stored at 25 C for 30 days in a desiccator, in an incubator at 17 C, or on a lab table at normal lab temperatures (Table 12).

Inoculation of Cotton Rust with T. persicina

Typical masses of violet-to-lavender-colored conidia of T. persicina were observed on aecia and occasionally on spermagonia 6 days following inoculation with T. persicina conidia (Fig. 10). Tables 5 and 6 list the incidence of T. persicina found colonizing rust pustules at various time periods after inoculation. The masses of conidia were easily removed from the sporodochia by running tap water on the colony. The sporodochia beneath the conidia were dark blue to purple in color and appeared as hard, sclerotial-like bodies (Fig. 11). Sporodochia appeared to restrict the escape of any aeciospores that may have formed within colonized aecia.

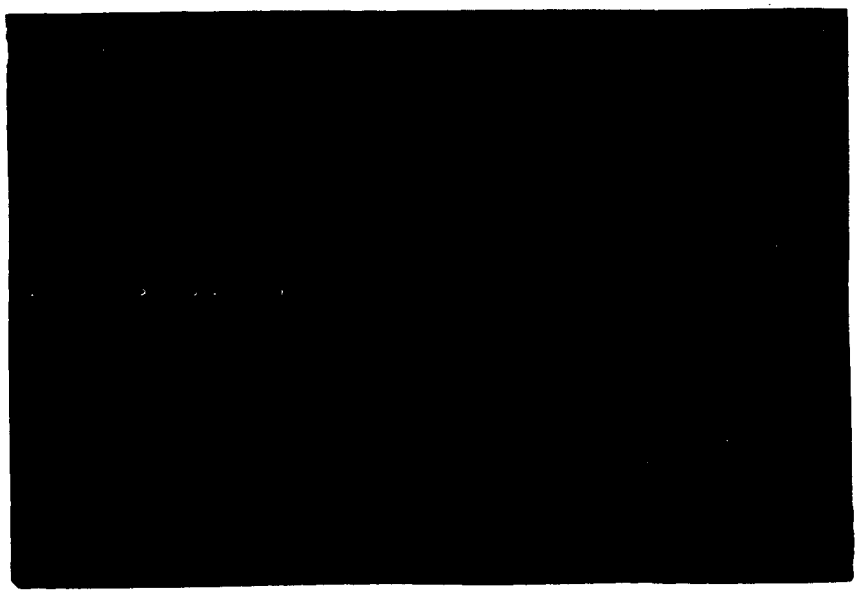

Fig. 10. Lower cotton leaf surface with aecial pustules (ap) of Puccinia cacabata and aecial pustules colonized by Tuberculina persicina (tp).--Cotton leaf was inoculated with T. persicina conidia when spermagonia were evident.

Fig. 11. Aecial pustules of Puccinia cacabata on cotton boll colonized by Tuberculina persicina under natural conditions.--Tuberculina persicina conidia were removed from the center of the pustule to expose the deep purple sporodochium (S).

Tuberculina persicina did not colonize spermagonia or aecia on cotton leaves used as uninoculated checks. Visual evidence of T. persicina, i.e.,conidia and sporodochia, was restricted to P. cacabata spermagonia and aecia (Fig. 10). Tuberculina persicina did not colonize cotton leaf areas between rust pustules and failed to colonize cotton leaves free of spermagonia and aecia.

Aecia on the uninoculated check plants and aecia that appeared to be free of T. persicina on inoculated plants produced aeciospores in 12-15 days following inoculation with basidiospores; however, there was no visual evidence of aeciospore production on aecia colonized by T. persicina.

Square pieces of Scotch Brand transparent tape placed on either cotton leaf surface was not a reliable method of excluding T. persicina conidia as the tape wrinkled when wet and allowed water to seep under the tape. However, two layers of tape applied to either leaf surface appeared to be a satisfactory method of excluding T. persicina conidia. Cotton rust pustules were colonized by T. persicina when either the upper or lower leaf surface was inoculated with a conidial suspension of T. persicina (Table 6).

Histological Studies

Fruiting structures of rust and T. persicina were easily distinguished from cotton leaf tissue when any of the staining methods, previously described, were used (Fig. 12, 13, 14).

Orseillin BB + fast green stained cotton leaf tissues blue to green and the two fungi pink or red (Fig. 14, 15). These two stains were very useful in distinguishing between the mycelium of P.

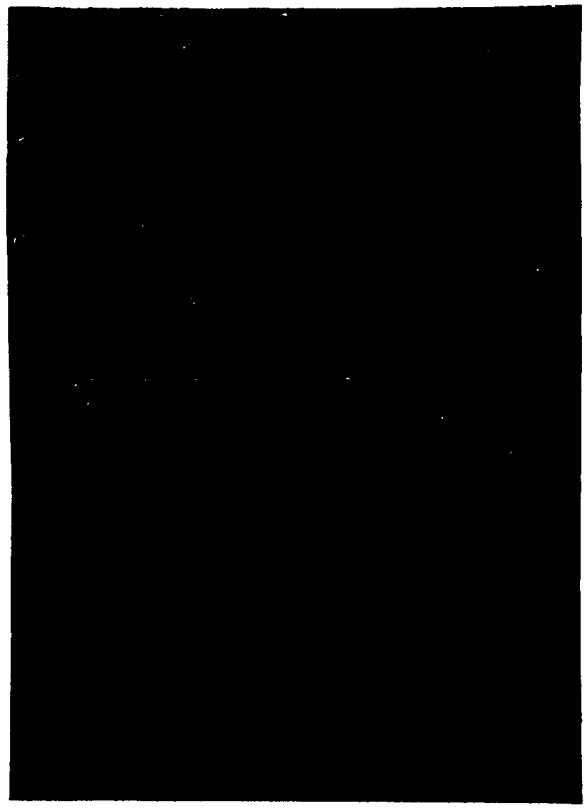

Fig. 12. Single spermagonium of Puccinia cacabata on lower surface of cotton leaf 15 days after exposure to basidiospores.--Stained with pianeze and cotton blue (X 590).

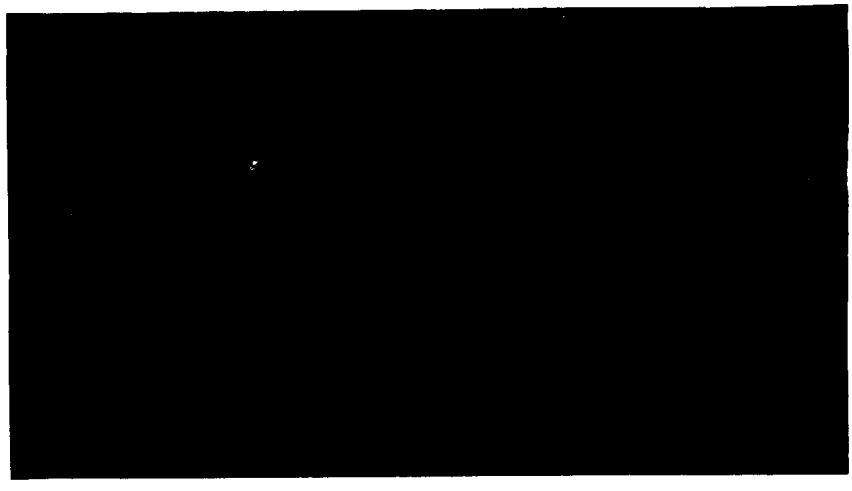

Fig. 13. Spermagonia of Puccinia cacabata on lower cotton leaf surface.--Note "build-up" of spermatial ooze (SO) below the spermagonia (S). Stained with orseillin BB and cotton blue (X 335).

Fig. 14. Two spermagonia of Puccinia cacabata "overrun" with Tuberculina persicina.--Conidia (C) of T. persicina appear above the conidiophores (CP). Note dikaryotic hyphae (TH) of T. persicina below the conidiophores. Stained with orseillin BB and fast green (X 590).

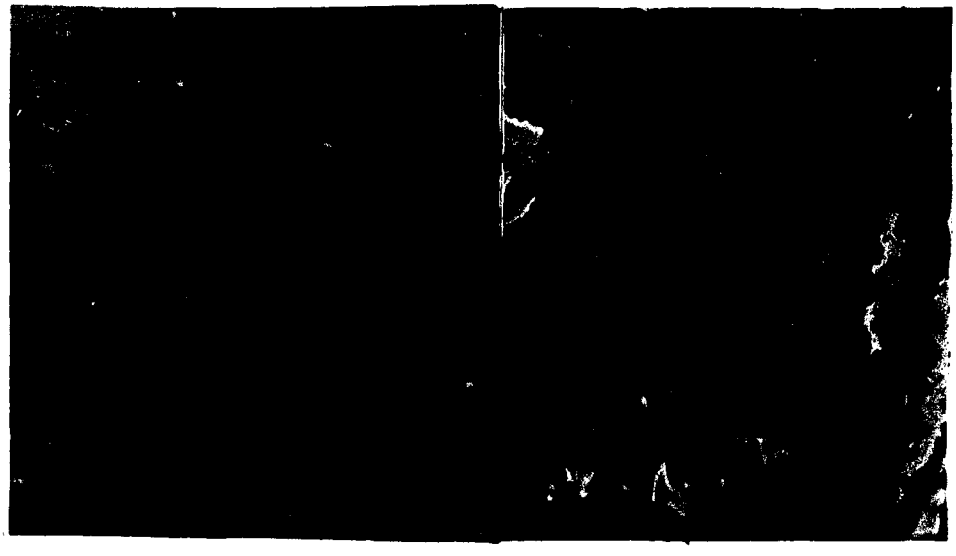

Fig. 15. Hyphae of Puccinia cacabata and Tuberculina persicina within a cotton leaf.--Left: hyphae of P. cacabata with large, single nucleus (PH); right: T. persicina with mottled, dikaryotic hyphae (TH) (X 590).

cacabata and T. persicina as the dikaryotic hyphae of T. persicina could be detected in a few properly stained slides. The nuclei of T. persicina were smaller than the rust nuclei and the cell contents of P. cacabata were generally clear, while T. persicina cells were mottled or speckled (Fig. 14, 15).

Orseillin BB + cotton blue stained the cotton cells tan to brown while the two fungi stained pink to red (Fig. 13, 16, 17). This stain combination also was helpful in distinguishing between P. cacabata from T. persicina, as T. persicina hyphae stained a deeper red than that of P. cacabata. Further, the mottled appearance of the cell contents of T. persicina was easily detected. Conidiophores of T. persicina were easily distinguished from spermagonia and aecia by their morphological differences and by differences in color; T. persicina stained deep red or brown while spermagonia and aecia stained pink or red (Fig. 16, 21 (p. 64), 24 (p. 66).

Pianeze + cotton blue stained the cotton cells blue to purple while the two fungi stained green or blue-green (Fig. 12). However, this combination was inferior to the other stains because P. cacabata and T. persicina could not be easily distinguished from each other.

Tuberculina persicina germ tubes could be detected within spermagonia and spermatial ooze 24 hr after inoculating cotton leaves with T. persicina conidia (Fig. 18, 19). A few germ tubes of T. persicina found within spermagonia measured 50 μ in length and were approximately 1.0 μ in diameter 24 hr after inoculation.

Hyphae of T. persicina could be detected in cotton leaf tissue infected by P. cacabata 2 days after inoculating with T. persicina

Fig. 16. Sporodochium of Tuberculina persicina has replaced spermagonium of Puccinia cacabata.--Conidiophores (CP), conidia (C), and spermatial ooze (SO) appear above the sporodochium (SP) of T. persicina. Three spermagonia (S) are apparently free of T. persicina. Stained with orseillin BB and cotton blue (X 150).

Fig. 17. Tuberculina persicina (TP) has colonized the spermatial ooze of Puccinia cacabata on the lower cotton leaf surface.--Stained with orseillin BB and cotton blue (X 150).

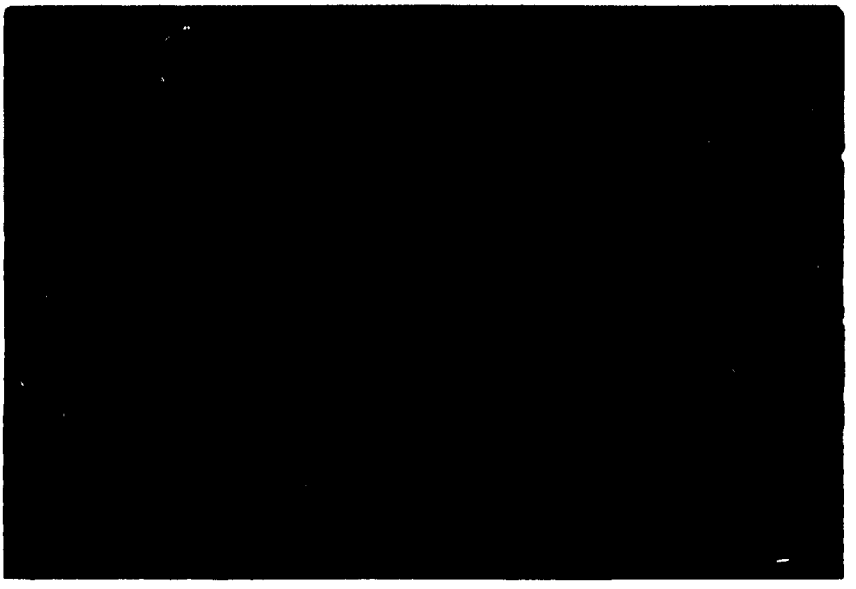

Fig. 18. Tuberculina persicina conidia adhering to spermatial ooze 24 hr after inoculation.--Spermagonia (S) of Puccinia cacabata were inoculated with conidia (C) of T. persicina and are adhering to spermatial ooze (SO). Stained with orseillin BB and cotton blue (X 335).

Fig. 19. Germinating conidium of Tuberculina persicina on cotton leaf 24 hr after inoculation.--Conidium (C) has a germ tube near a cotton leaf stoma (ST). Stained with orseillin BB and cotton blue (X 335).

conidia. Tuberculina persicina mycelia appeared to follow the mycelia of P. cacabata through the intercellular spaces of the cotton leaf.

Haustoria, or other specialized structures, of T. persicina coult not be detected within hyphae of P. cacabata. The hyphae and conidiophores of T. persicina do, however, grow very rapidly within spermagonia and aecia and completely overran these structures within 6 days after inoculation.

Conidiophores of T. persicina could be found erupting from cotton leaf tissue and spermagonia of P. cacabata 6 days after inoculating with T. persicina (Fig. 14, 16). Conidiophores were frequently found growing on spermatial ooze adjacent to spermagonia (Fig. 17). Tuberculina persicina sporodochia were frequently detected growing beneath the surface of leaves where spermagonia or aecia had started to develop. However, subsequent to infection by T. persicina, the walls of spermagonia and aecia appeared to break down (Fig. 14, 16, 20, 21, 22, 23, 24).

Hyphae of T. persicina were found penetrating the walls of aecia. In some instances, the hyphae could be traced to an infected spermagonium on the upper leaf surface. After entering the aecium, conidiophores arise from the walls of the aecium and from the spore mother cells (Fig. 22, 23). Further development of aecia and aeciospores is halted once aecia have been invaded by T. persicina (Fig. 22, 24). Occasionally, aecia escape T. persicina infection and produce normal aeciospores (Fig. 25).

In some instances, the sporodochium of T. persicina appeared to block the exit of aeciospores from an aecium (Fig. 24).

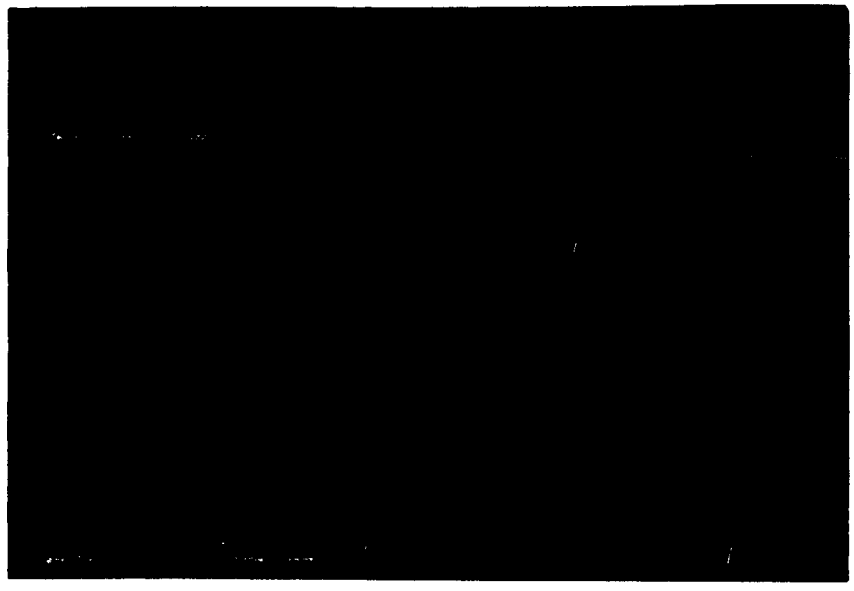

Fig. 20. Tuberculina persicina colonizing Puccinia cacabata spermagonia on cotton leaf.--Conidia (C) and conidiophores (CP) are pushing through the lower epidermal cells 7 days after inoculating the leaves with T. persicina conidia. Stained with orseillin BB and cotton blue (X 150).

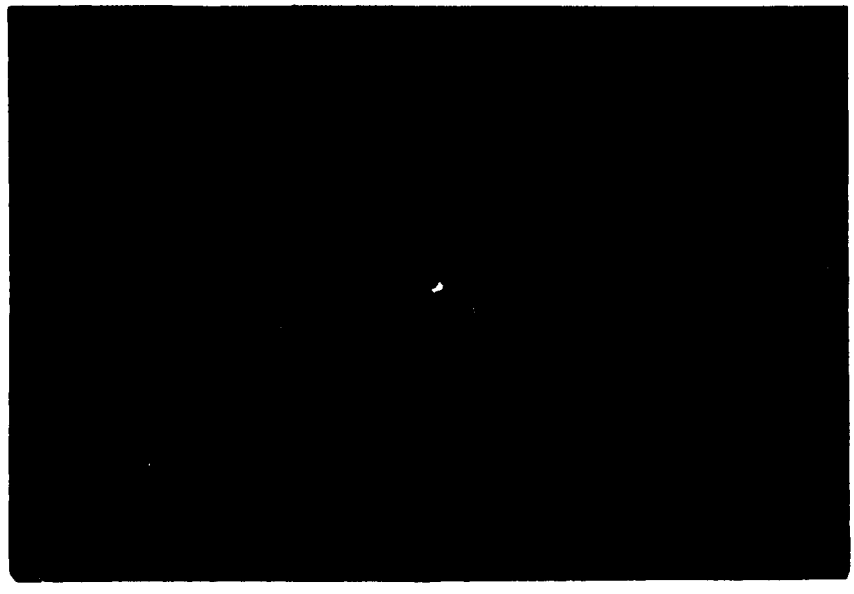

Fig. 21. Tuberculina persicina colonizing both leaf surfaces of cotton.--Conidiophores (CP) ruptured the epidermal cells 7 days after inoculating leaves with conidia. Puccinia cacabata spermagonia (S) are barely visible. Stained with orseillin BB and cotton blue (X 150).

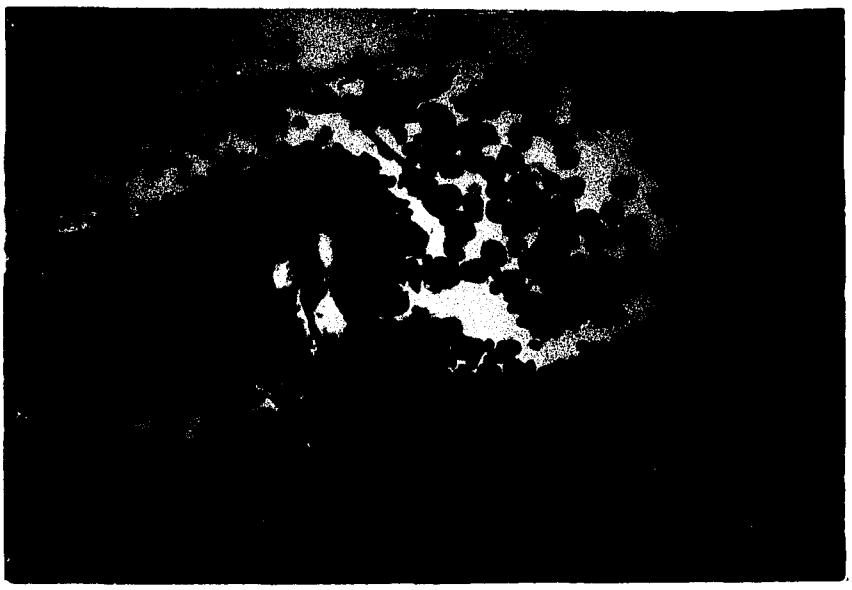

Fig. 22. Two Puccinia cacabata aecia colonized by Tuberculina persicina.--Conidia (C) and conidiophores are within the aecium on the right. A few aeciospores (AS) have developed. Stained with orseillin BB and fast green (X 235).

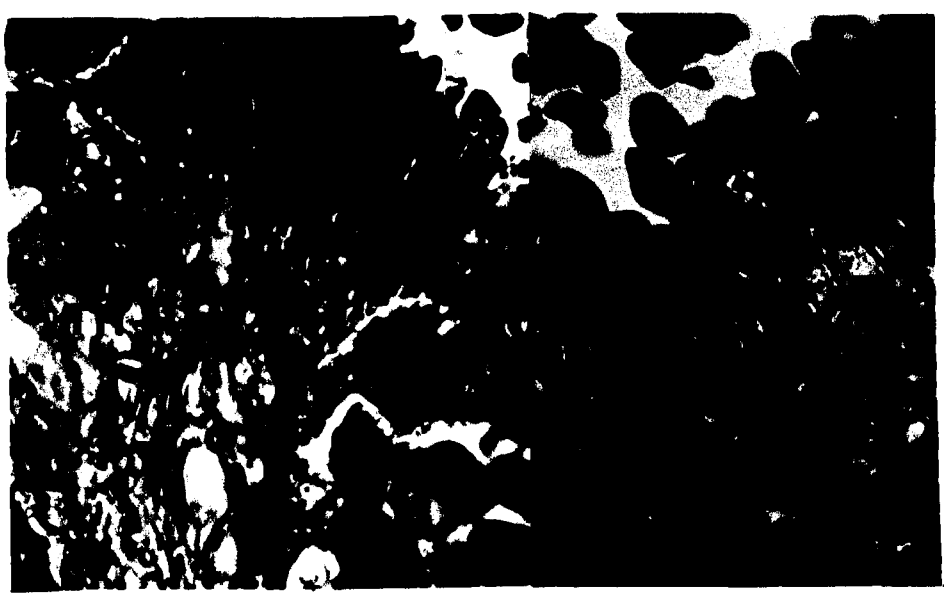

Fig. 23. Hyphae of Tuberculina persicina growing near Puccinia cacabata aecia on cotton leaves.--Left: hyphae of T. persicina (TH) within leaf tissues. Conidiophores (CP) of T. persicina are growing on peridium of aecium. Right: hyphae of T. persicina (TH) growing between aeciospore mother cells (X 590).

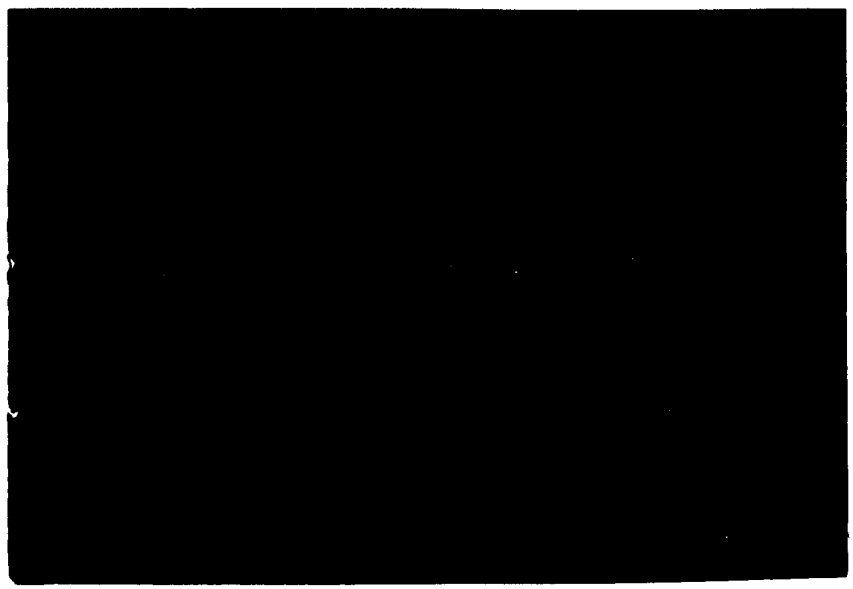

Fig. 24. Tuberculina persicina colonizing a developing aecium of Puccinia cacabata.--Cotton leaves were inoculated 18 days previously with T. persicina conidia. Aeciospores (AS) are poorly developed and restricted from exiting by the conidiophores of T. persicina. Stained with orseillin BB and cotton blue (X 235).

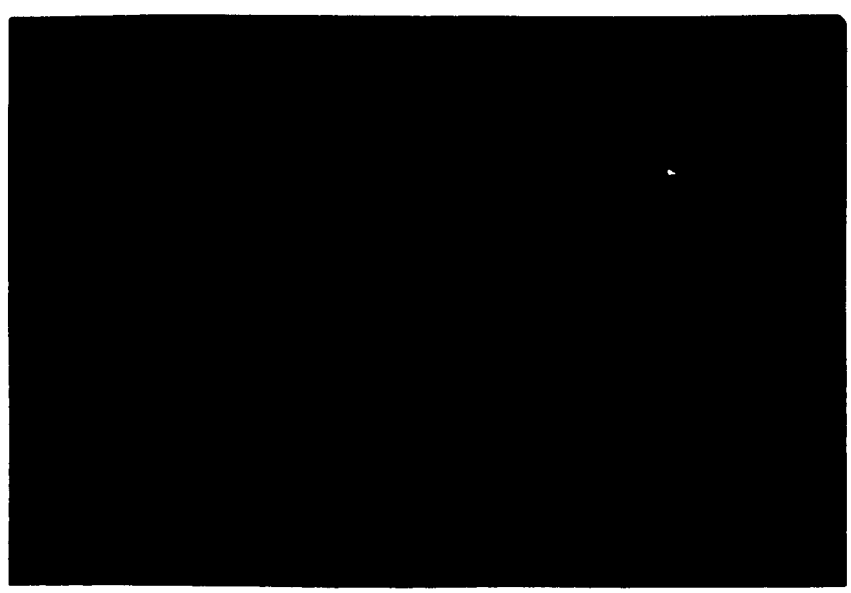

Fig. 25. Puccinia cacabata aecium free of Tuberculina persicina.--Aeciospores (AS) are escaping from the developing aecium (A). Stained with orseillin BB and cotton blue (X 150).

DISCUSSION

Despite attempts to culture T. persicina for the past several years (34, 35), only one obscure report (34) described successful procedures for growing this fungus on agar and in liquid media. In the present investigation, success in isolating and growing the fungus on a variety of artificial media was achieved. This is the first report of successful isolations of T. persicina from P. cacabata. The key to successful culture lay in understanding the extremely slow growth rate of T. persicina on artificial media.

Best cultural growth of T. persicina was obtained on media containing natural plant extracts. Poor growth, however, resulted when defined media were used for culturing, suggesting that essential nutrients were not present in these media.

Various amounts of glucose added to PDA increased the total colony growth, but did not influence cultural characteristics of T. persicina. Vladimirskaya (35) found that media high in sugars and low in protein were most favorable for cultivation of his isolate of T. persicina.

Differences in cultural characteristics or conidial production were not observed when either hyphae or conidia were used as the fungus source added to media in Petri dishes; however, hyphae were not used as inoculum as it was much easier to obtain a uniform amount of the fungus for each treatment by using conidia.

Tuberculina persicina utilized several carbon sources when grown in liquid cultures. The fungus, however, failed to grow in a medium when L sorbose was used as the carbon source. L sorbose has previously been reported as being detrimental to the growth of numerous fungi (3, 4). Villanueva (34) also found that his isolate of T. persicina could utilize several carbon sources in liquid cultures.

Further tests with T. persicina in liquid cultures revealed that T. persicina growth is greatly influenced by the hydrogen ion content. The fungus failed to grow at pH 8.0 and made poor growth at pH 7.5. Since T. persicina grew poorly at a high pH, this may be one explanation for the poor growth rate recorded for T. persicina on Czapek's agar medium as the pH was 7.3. If it is desirable to grow T. persicina on a defined medium, such as Czapek's agar in future studies, adjustment in pH to 6.0 might improve the growth rate.

One of the primary reasons for attempting to grow T. persicina in liquid culture was to provide conidia as a source of the fungus for the tests. However, conidia were not obtained in any of the liquid media. Hyphae grown in liquid media could be used as a source of the fungus, but it was difficult to measure the exact amount of the fungus used in each treatment when hyphae were used. Conidia, therefore, grown on agar media were used as a source of the fungus for treatments and further use of hyphae from liquid cultures was discontinued.

Tuberculina persicina grew over a temperature range from 17 C to 34 C but no growth resulted at 9 C or 37 C. The most favorable range was from 27 C to 30 C. These results differ slightly from those of Barkai-Golan (2) who found that his isolate of T. persicina from

Israel grew best at 23-25 C. Results do, however, differ considerably from those of Vladimirskaya (35). His isolate of T. persicina from Russia grew at temperatures from 9-28 C but most rapidly at 15 to 25 C.

Germination of T. persicina conidia was reduced when soaked in water for more than 15 min. Only 1% germination resulted when they were soaked in water for 24 hr. Delp (12) found that exposure to water for long periods inhibited germination and caused death of conidia. These results suggest that conidial suspensions should not be made far in advance of inoculation or poor germination might occur. Results also suggest that prolonged rainfall might cause poor germination.

Tuberculina persicina did not survive for long periods on agar media when stored at 23 C. Germination of conidia was near 100% in a 1½ month old culture grown on PDA, while a 6 month old culture had but 1% germination. Conidia from cultures 8 months old or older failed to germinate, and the cultures could not be revived by pouring PDA onto the old cultures. Failure of conidia to germinate in older, dried cultures is not surprising as the conidia are extremely small and lack a thick wall. Isolates, therefore, should be transferred at short intervals if they are to be maintained in culture.

Illustrations or descriptions of T. persicina grown on artificial media have not been given in the literature. Rather, T. persicina (2, 14) always has been shown growing in association with spermagonia and aecia of various rust fungi. These illustrations show a single terminal conidium at the ends of conidiophores. The observations made in this study on artificial media show clearly that masses of conidia are produced laterally, as well as at the ends of conidiophores, as the result

of "blown-out" cells. Illustrations used in this dissertation depict several other characteristics of T. persicina in culture. Hyphae at the base of sporodochia were found to be much wider than vegetative hyphae and to have thicker walls. Sporodochial hyphae and vegetative hyphae are very thickly woven in culture. Hyphal swellings were found at the base of sporodochia and have the appearance of chlamydospores. The enlarged sporodochial hyphae and chlamydospore-like swellings were not observed in histological tissue when the fungus was studied in relationship with P. cacabata. If the thickened, tightly woven hyphae, and hyphal swellings are formed in nature, they very likely serve as a survival mechanism.

The relationship between T. persicina and P. cacabata might be more fully explained in future studies if the two fungi could be grown together in pure culture. Several attempts, not reported in the results of this dissertation, were made to culture P. cacabata on artificial media but all attempts failed. In other tests, aeciospores of P. cacabata were placed on agar media with T. persicina conidia to determine the affect of one fungus upon the germination and growth of the other. Since 1% or less of the aeciospores germinated with or without the presence of T. persicina conidia, this line of investigation was discontinued.

Various interpretations have been made over the past several years concerning the relationship of T. persicina and other Tuberculina species with several rust "hosts." To describe this relationship, several terms have been used in an attempt to categorize their relationship. These terms include: "commensalism" (14), "hyperparasitism" (8),

"antagonism" (18), and "mycoparasitism" (9). After careful study of the relationship of T. persicina and P. cacabata in cotton, none of these terms adequately, in the author's view, describe the relationship involved. The terms just listed ignore the most obvious feature of their relationship, namely that T. persicina initiates a severe disease of P. cacabata. This disease is manifested by an obvious reduction in the aeciospores produced on cotton. Whether T. persicina is a parasite or not of P. cacabata is of secondary importance. A term that might be coined to describe the relationship of T. persicina with P. cacabata is "mycopathogen." The author feels that there is evidence from this study to support both the concept of pathogenicity and that of parasitism of T. persicina on P. cacabata.

Evidence supporting the concept of pathogenicity of T. persicina on P. cacabata follows:

A. Gross observations

1. Colonization of aecial pustules by T. persicina.
2. Reduction of aeciospore production when T. persicina is present.

B. Histological observations

1. Penetration of spermagonia and aecia by T. persicina conidial germ tubes and mycelia.
2. Colonization of cotton leaf tissue only in close proximity to rust structures.
3. Production of T. persicina fruiting structures within the walls of spermagonia and aecia, thereby reducing

the effectiveness of spermagonia and reduction in the number of aeciospores produced.

4. Prevention of escape of aeciospores from aecia by the physical presence of T. persicina sporodochia.

Evidence supporting the concept of parasitism of T. persicina on P. cacabata follows:

1. Constant association in nature of T. persicina with P. cacabata.
2. Growth and reproduction on cotton, only on spermagonia and aecia.
3. Close association of hyphae of the two fungi within cotton plants.

In the author's view, the pathogenic nature of T. persicina is well established by the evidence presented. Parasitism is less completely documented but all evidence presented points toward parasitism and none detracts.

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