STUDIES OF SOUTHWESTERN COTTON RUST

(PUCCINIA CACABATA ARTH. & HOLW.)

by

Bill B. Berkenkamp

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SIGNED: Bill B Beckeahamp

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This thesis has been approved on the date shown below:

R B Streets — May 20 1958

R. B. Streets — Date

Professor of Plant Pathology

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INTRODUCTION AND HISTORY

Southwestern cotton rust is becoming increasingly important in the southwestern United States and Mexico. This is due to the increased acreages planted in cotton especially in new areas adjacent to range land, and the existing prevalence of inoculum on alternate host grasses.

The rust attacking cotton is a typical macrocyclic beteroecious species. It overwinters as heavy walled, two-celled teliospores on various species of Bouteloua. After these spores have been subjected to winter weather and are soaked by summer rains, they germinate to produce a promycelium, or basidium, which bears four basidiospores, or sporidia, on sterigmata. (Fig. 1) The basidiospores are forcibly discharged from the sterigmata and are airborne to the alternate host, i.e., Gossypium spp. The sporidia germinate and infect cotton to produce pycnia, shown in Fig. 2. The pycnia produce pycniospores and go through dicaryotization to produce aecia (Fig. 3) containing aeciospores. The aeciospores are released and cannot reinfect cotton, but must infect species of Bouteloua. These infections give rise to uredinia

and uredospores (Fig. 4) which is a repeating stage on the grass host. Later in the season, telia and teliospores are produced which must overwinter to break their dormancy.

A rust attacking cotton was described as Aecidium gossypii Ell. & Ev. in San Jose del Cabo, Lower California, in 1897 (9).[a] The specimen was collected by K. Brandegee (No. 8) in 1893. No alternate host was found. In Texas, Taubenhaus (14) reported a sudden outbreak of rust which he diagnosed as Aecidium gossypii. Muhlenbergia spp. or Sporobolus spp. were suggested as possible alternate hosts. Brown and Gibson (2) reported cotton rust, probably for the first time in Arizona in the Postvale district in 1922. Brown and Streets (3) determined the rust to be Puccinia hibiscata, a perfect stage of Aecidium, and they disclosed its erratic nature. Heavy losses, up to seventy-five percent in yield, occured in 1922 and in 1930. The rust seems to be mild in some years and may remain unnoticed until it suddenly becomes severe. In 1942 Presley (11) established by inoculation that a grama grass, Bouteloua, is the alternate host. Several species were inoculated and found to be susceptible. This stage was tentatively identified as Puccinia boutelouae (Jennings) Holw., which closely resembles Puccinia vexans Farl. It was suspected they were synonymous. The next year (1943) Presley and King (12) described the rust on

[a]Numbers in parentheses refer to literature cited.

cotton as a new species, Puccinia stakmanii Presley and surveyed its host range. They found that many varieties of cotton and various species of Bouteloua were susceptible. This rust was described as different from other rusts on Bouteloua by its usual possession of three equatorial pores in the wall of the uredospores. In similar species the pores are scattered. In 1956 Hennen and Cummins (10) showed that Puccinia stakmanii is synonymous with Puccinia cacabata Arth. & Holw. They surveyed South American literature and reported various species of Chloris as alternate hosts and also a greater geographical distribution. Puccinia cacabata was first described by Arthur (1), based on the Holway collections in South America in 1925. This designation seems to have been accepted (8). The binomial Puccinia cacabata, takes precedence because of prior publication.

In order to predict accurately outbreaks of cotton rust, more information in the life history of the fungus and factors influencing infection is essential. Teliospores are of prime importance, being the overwintering stage and the source of basidiospores which infect cotton.

MATERIAL AND METHODS

The rust was identified using as criteria, spore morphology and size, as well as hosts attacked. These for the most part agreed with descriptions of Puccinia cacabata (1). Inoculations and various tests on spores were made by utilizing infected plant material from the field or produced in the greenhouse. Inoculations were made in several ways: infected grass was suspended on a screen above cotton plants in pots in a cloth covered moist chamber in the greenhouse. Bell jars were used when inoculating small numbers of plants. These two methods and a sheet metal box were used in grass inoculations.

In the attempts to break dormancy, pieces of infected grass, which had not overwintered, were attached to the narrowed bottoms of corks that had been dipped in paraffin. The pieces of grass were arranged so that they would extend into a vial when the corks were inserted in the usual manner. The rusted pieces of grass attached to corks were then subjected to various conditions of moisture and temperature. When being subjected to different temperatures the corks bearing the infected grass were placed in vials so that the grass projected inside. During the moisture tests (soaking) the corks were floated in beakers of tap water with the grass

submerged for various periods of time. After the treatments, germination was tested in hanging-drop mounts in Van Tieghem cells. The cover-slips were then placed over a drop of lactophenol and cotton blue on a slide and the germinated teliospores counted.

To establish the humidity and/or water requirements for germination of teliospores, a modification of Clayton's method (4) was used. Corks fitting jars of about two hundred cc capacity were dipped in paraffin and a small glass rod about 2.5 cm long was attached so that it extended into the jar when the corks were in place. Cover-slips were attached with paraffin to the lower end of the rods in a horizontal position and were used as carriers for spores, both in drops of water and dry. Solutions which control the humicity when kept at 20°C were placed in the bottom of the jars. The solutions used were taken from data by Clayton (4) and Spencer (13). The compounds and concentration at 20°C give the humidities shown in Table 1. After treatment the cover-slips were removed and inverted over drops of lactophenol and cotton blue on slides. Counts were made to determine the percentage of germination.

TABLE 1

MATERIALS USED IN CONTROLLING HUMIDITY AT 20°C.

Material and Concentration	Percent Relative Humidity
Distilled water	100
Sucrose solutions (Molal Concentrations)	
0.2	99.62
0.5	99.04
0.7	98.65
1.0	98.03
Saturated solution with excess solid of the following:	
Potassium sulfate	97.0
Potassium nitrate	95.0
Zinc sulfate	90.0
Potassium chloride	86.0
Ammonium sulfate	81.0
Ammonium chloride	79.0

FUNGICIDE TESTS

IN GREENHOUSE

Several fungicides were tested against cotton rust in the greenhouse and in the field (Table 2). In the greenhouse plants were grown in eight inch pots in a manure, sand and soil mixture; ratio of 1:1:3. When the plants reached the five leaf stage they were placed in the moist chamber and inoculated. The fungicides were applied after inoculation until the leaves were covered and plants were dripping. Readings were made when pycnia appeared. A card with a square hole 2 cm square was placed at random once on each leaf and the number of pycnia showing within the hole was counted.

IN FIELD[b]

In the field the fungicides were sprayed on from a three gallon sprayer. Each fungicide was applied at approximately weekly intervals to the same rows. Forty-six feet of row was sprayed each time so that applications overlapped twenty-three feet. All but the first and last twenty-three foot strips received two applications one week apart.

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[b]These tests were conducted in cooperation with Dr. Lester M. Blank, Pathologist, Agricultural Research Service, Cotton Research Center, Tempe, Arizona.

The treated plants were separated by one row of untreated cotton. All of the fungicides were applied at the rate of 505 cc to forty six feet or forty gallons per acre. The fungicides used are shown in Table 2.

TABLE 2

FUNGICIDES USED IN GREENHOUSE AND FIELD TESTS

Fungicide	Concentration Used	
	Field	Greenhouse
Lithium nitrate[a]	3 lbs./A.	2,000 ppm
Lithium carbonate[a]	5 lbs./A.	
Experimental chemical #8525[b]		5,000 ppm
Actidione derivatives[c]		
Cyclohexamide tablets (7.7%)		25 ppm
Cyclohexamide (#68: 0.6%)	30 ppm	
Oxime (#83: 8%)	200 ppm	
Acetate (#84: 8%)	150 ppm	
Semicarbazone (#85: 8%)	200 ppm	
Actidione M (#86: 8%)	150 ppm	
Actidione T (#87: 8%)	200 ppm	
Sulfur[d]		10,000 ppm
C-O-C-S (Copper oxychloride sulfate: metalic copper 50%)	4 lbs./A.	
Kolofog (Bentonite sulfur: sulfur 30%)[e]	4 lbs./A.	
Kolo 100 (Bentonite sulfur 74.4%: Dichlone 3.5%)[e]	4 lbs./A.	
Karathane (Dinitro (1-methyl heptyl) phenyl crotonate: 25%)[f]	2 lbs./A.	
Zineb (Zinc ethylene bisdithiocarbamate: 65%)[f]	2 lbs./A.	
Fermate (Ferric dimethyldithiocarbamate: 76%)	2 lbs./A.	

[a]Foote Mineral Co.
[b]Monsanto Chemical Co.
[c]The Upjohn Co.
[d]Southern Acid and Sulfur Co.
[e]Niagara Spray Corporation
[f]Rohm & Haas
[g]E. I. du Pont de Nemours & Co.

RESULTS

BREAKING DORMANCY

Freshly collected teliospores that had not been subjected to winter weather showed very low germination, 0.2 to 6 percent and seemed to be dormant. Attempts to break dormancy were made. Pieces of grass bearing telia were attached to corks with paraffin and kept at the following temperatures: 19 to 20, 0 to 5, -12 to -18°C and at room conditions. All the samples while kept at room temperature were soaked for two to three minutes in tap water three times a week.

At weekly intervals samples were removed from the lower temperatures and maintained at room temperature. The samples were subjected alternately to soaking and drying. After the first two weeks treatment and weekly thereafter, the germination of the spores was tested. The germination was low and erratic. Thus dormancy seemed only slightly affected. Spores kept at -12 and -18°C germinated and did not seem to be adversely affected by low temperatures. No germination was found in any of the samples that were not kept at, or removed to room temperature and soaked. Tests were then begun to find the effect of different periods of

soaking. The same method of alternate soaking and drying was used with a different grass sample. The treatments were ten minutes, thirty minutes, one hour, two hours and four hours. Unsoaked control tests were also observed for comparison. The results are shown in Table 3.

These results suggest that soaking plays a part in breaking dormancy of teliospores. After three treatments (soaking) for one hour the spores are activated. After six treatments a smaller increase is obtained for the one hour treatments, but a larger increase for the spores exposed for shorter and longer periods.

The overwintered spores were checked to determine the minimum time for germination. It was found that the spores begin germinating in slightly less than seven hours. Spores exposed to air produced promycelia about five times the length of the spore and formed basidiospores. These are illustrated in Fig. 1. The spores covered with water produced extremely long promycelia and formed basidiospores only after reaching air.

EFFECTS OF HUMIDITY

Studies to determine the effect of humidity on germination of teliospores were undertaken. All of these tests were run at 20°C. Dry spores were scraped from the grass to cover-slip carriers and placed in humidity chambers. In this test only the spores exposed to one hundred percent humidity

germinated. A similar test with aeciospores gave the same results. A second test was set up and the teliospores were placed on the cover-slips in drops of distilled water. The water was allowed to evaporate until the spores appeared dry and then were placed in the humidity chambers. The spores germinated at all humidities tested and the results are shown in Table 4.

INOCULATION OF COTTON

In the inoculation tests on cotton, infected grass bearing teliospores was soaked and then placed on a screen above the plants in a moist chamber. By introducing and removing pots daily it was found that infection would occur in twenty-four hours and daily thereafter. Larger numbers of infections occurred on plants after four days continuous exposure. Pycnia appeared on cotton five days after inoculation. Aecia appeared after thirteen to seventeen days. Generally infected cotton plants placed in insect-proof cages did not produce aecia, unless two or more pycnia were in close proximity. Isolated lesions enlarged, produced more pycnia and reached a diameter of sixteen mm after forty-five days. Exudates with pycniospores from different pycnia were crossed using a glass rod to effect the transfers. These findings are in agreement with the discoveries of Craigie (5,6,7).

TABLE 3

EFFECT OF SOAKING ON TELIOSPORE DORMANCY

Time of soaking	Germination percent after three treatments	Germination percent after six treatments
10 minutes	12.5	23
30 minutes	26.5	33.5
1 hour	31	32.5
2 hours	25	35
4 hours	23	41
Control (no soaking)	0	2

TABLE 4

HUMIDITY EFFECTS ON SPORE GERMINATION

Humidity	Germination /100 Spores			
	1st Test	2nd Test	3rd Test	Average
100 %	26	22	25	24.33
99.62	22	28	-	25.00
99.04	28	23	26	25.66
98.65	25	21	-	23.00
98.03	19	25	24	22.66
97.	27	25	31	27.66
95.	24	22	26	24.00
90.	20	24	27	23.66
86.	21	26	20	23.33
81.	22	23	19	21.33
79.	17	20	14	17.00

FUNGICIDE TESTS

IN GREENHOUSE

In the greenhouse rust inoculations were made in the moist chamber. Plants were maintained here for three days. The plants were then removed and sprayed with the various fungicides (Table 2). Counts were made when pycnial lesions became prominent. No practical method for obtaining an even distribution of basidiospores was found and the infections were variable. Pots were set up in a Latin square using five replications and five treatments. The analysis of variance was non-significant; however the averages of the treatments showed some effect of fungicides. The averages for the treatments given as number of pycnia per four square centimeters were: control (no treatment) 12.0, Ethionine (Monsanto #8525) 6.4, Sulfur 4.6, Lithium nitrate 4.4, Actispray (Actidione) 2.6.

IN FIELD

In the field no conclusions could be drawn due to the lack of a readable amount of naturally occuring rust on cotton in 1957. Some of the fungicides showed phytotoxicity to cotton in the field. Lithium nitrate and Upjohn

Experimental Chemicals numbers 84 and 86 burned leaves after the first spraying. Subsequent concentrations were reduced from five pounds to three pounds per acre and from 200 to 150 ppm, respectively.

GRASS INOCULATIONS

Seed of grasses grown for inoculation was obtained through the kindness of Dr. Neal Wright, Research Agronomist at the University of Arizona, Plant Materials Center. The seed was planted in six or eight inch pots containing the soil, sand, manure mixture.

Inoculations were made both in the cloth moist chamber and in a sheet metal box. Aeciospores were dusted over the grass or infected leaves were suspended above the grass on a screen. Pustules appeared in nine to thirteen days after inoculation and produced uredospores (Fig. 4) and later teliospores. Presley (12) surveyed the host range and found the following species of Bouteloua susceptible: B. aristidoides (H.B.K.) Griseb., B. barbata Leg., B. parry (Fourn.) Griff., B. gracilia (H.B.K.) Lag., B. rothrockii Vasey., and B. curtipendula (Michx) Torr. Hennen and Cummins (10) state that Chloris berroi Arech., C. ciliata Sw., and C. polydactyla (L.) Sw. are susceptible. In these greenhouse tests, C. gayana Kunth., C. virgata Swarts., and C. berroi were not infected. Of the various species of Bouteloua, B. aristidoides seemed to be the most susceptible, both in

the greenhouse and in the field. B. eriopoda Torr., a previously unreported host, was found to be very susceptible in greenhouse tests. B. barbata was not tested in the greenhouse but was very susceptible in the field. Chloris virgata growing in the field in close association with infected species of Bouteloua was never found to be attacked by the rust.

DISCUSSION AND CONCLUSIONS

Relatively little work has been done on Puccinia cacabata and it seems to be somewhat different from other rusts in its dormancy breaking requirements. The tests show that moisture and not low temperature is the main factor in breaking dormancy in teliospores. Further studies with a range of closely controlled temperatures would be valuable. A period of soaking seems necessary for the germination of teliospores. The spores germinate readily at higher humidities after being wet, but germination occurs only at one hundred percent relative humidity unless the spores are previously dampened. This may be due to water condensing on the surface of the spore in a saturated atmosphere. The requirement of liquid moisture may be a biological adaptation to insure sufficient humidity for the infection of cotton after germination. Both germination and infection are relatively rapid. The teliospores begin germinating in less than seven hours and cotton is infected in less than twenty four hours of high humidity after the soaking of teliospores. In the greenhouse pycnia appear in five and aecia in about fifteen days after inoculation.

In greenhouse tests several fungicides which seem to reduce the amount of rust were found. Field checks were

impossible due to the lack of sufficient natural infection in 1957.

Grasses were inoculated in the greenhouse and an unreported species, Bouteloua eriopoda, was found to be susceptible. In South America Chloris berroi, C. ciliata, and C. polydactyla are reported susceptible. In the greenhouse inoculation tests Chloris berroi, C. virgata, and C. gayana were not infected. This may be due to inadequate testing in the greenhouse or existence of two or more physiological races which may or may not be restricted to certain areas of the geographical distribution of the rust.

SUMMARY

Soaking teliospores periodically is necessary in breaking dormancy in Southwestern cotton rust, Puccinia cacabata Arth. & Holw. After breaking dormancy, teliospores will germinate after moistening and maintaining at high humidities. However, wetting is not necessary under conditions of one hundred percent humidity. After wetting, spores germinate about equally from one hundred down to eighty one percent relative humidity. Teliospores begin germinating after seven hours high humidity and produce basidiospores, which can infect cotton in less than twenty four hours at high humidity. Pycnia appear five days after inoculation. Aecia appear on cotton about fifteen days after inoculation, provided exudates from different pycnia are mixed.

In greenhouse fungicide tests actidione, Ethionine (Monsanto #8525), Sulfur and Lithium nitrate reduced the amount of rust when sprayed on cotton after inoculation. The field fungicide tests could not be evaluated due to lack of rust on cotton in 1957.

Grasses were inoculated in the greenhouse and an unreported species, Bouteloua eriopoda was found susceptible. Chloris berroi, reported susceptible in South America, could not be infected in greenhouse tests. Other species of Chloris were inoculated and seemed not to be susceptible.

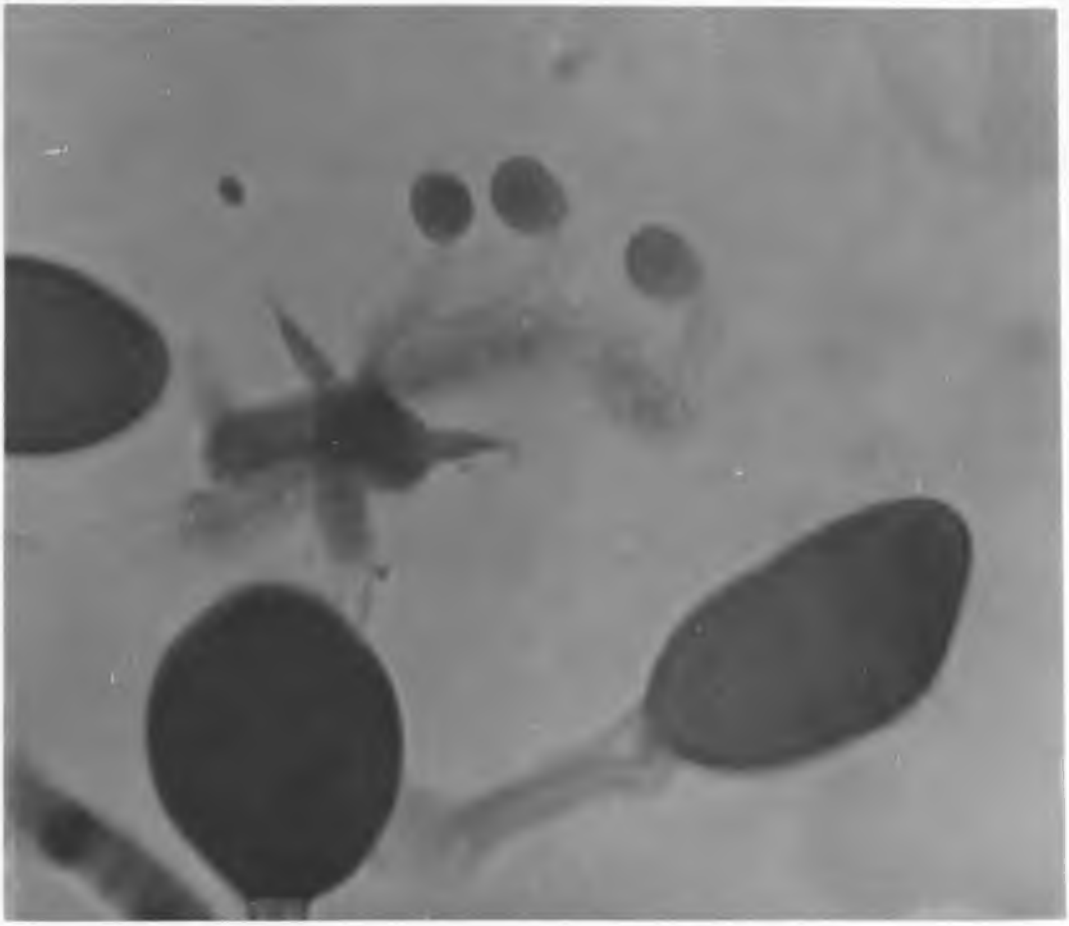

Fig. 1 Photomicrograph showing germinated teliospores with basidia, sterigmata and basidiospores of Puccinia cacabata. (Approximately 1500x)

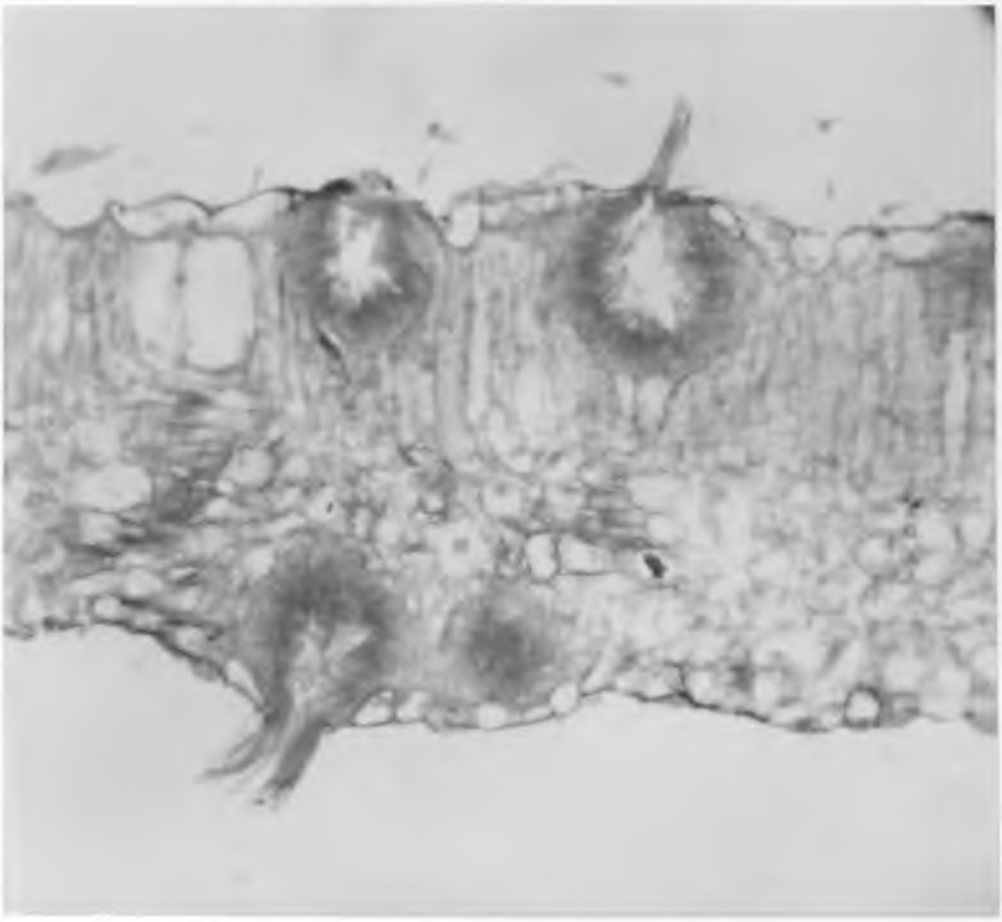

Fig. 2 Photomicrograph of a section of a cotton leaf showing pycnia of Puccinia cacabata. (Approximately 300x)

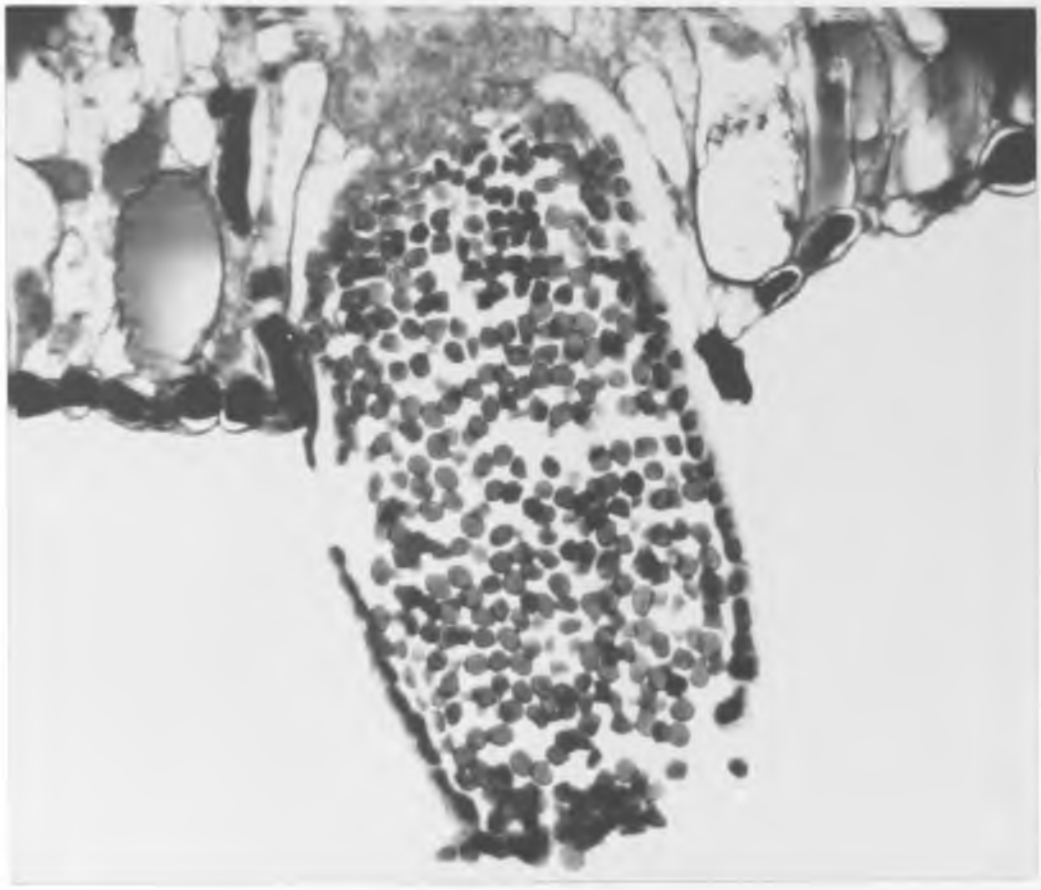

Fig. 3 Photomicrograph of a section of a cotton leaf showing an aecium containing aeciospores of Puccinia cacabata. (Approximately 250x)

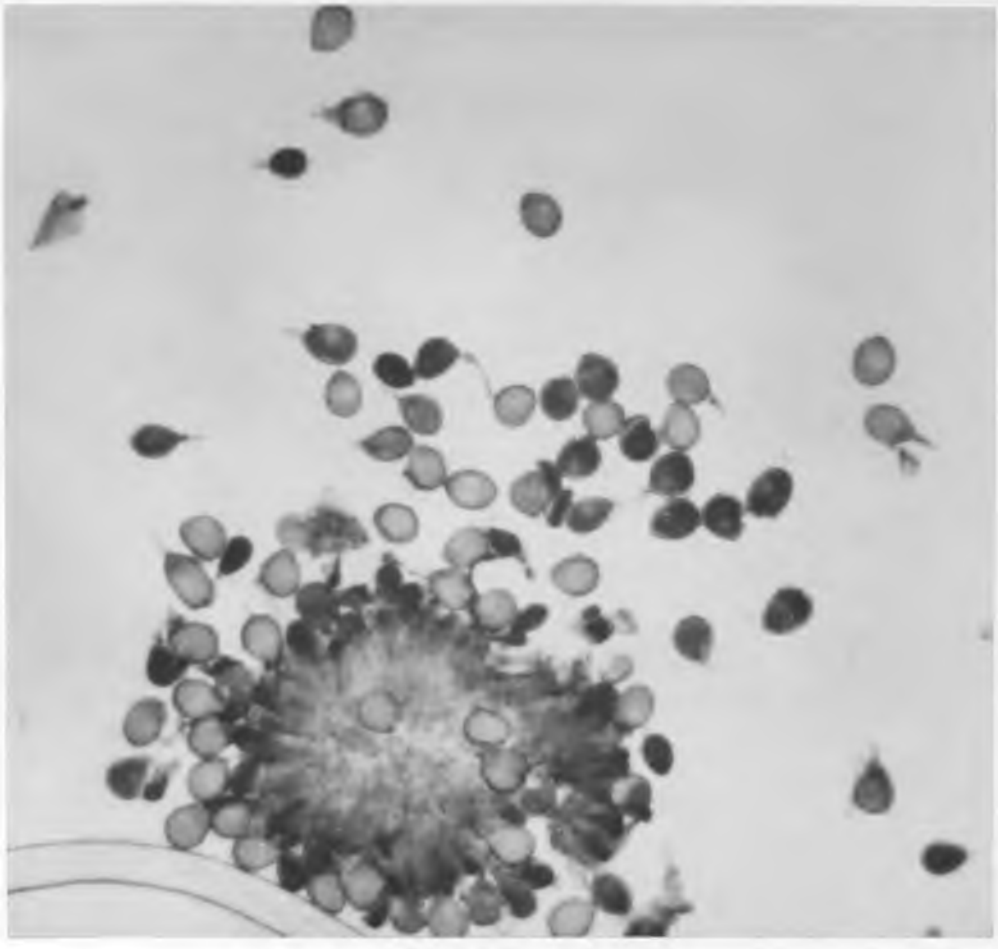

Fig. 4 Photomicrograph of uredospores of Puccinia cacabata. (Approximately 300x)

LITERATURE CITED

1. Arthur, J.C. 1925. The grass rusts of South America; based on the Holway collections. Proc. Amer. Phil. Soc. 64: 131-223.

2. Brown, J.G., and F. Gibson. 1923. Brief summary of plant disease determinations. Ariz. Agr. Exp. Sta. Ann. Rept. 34: 501.

3. Brown, J.G., and R.B. Streets. 1934. Diseases of field crops in Arizona. Ariz. Agr. Exp. Sta. Bull. 148: 157-159.

4. Clayton, C.N. 1942. The germination of fungus spores in relation to controlled humidity. Phytopathology 32: 921-943.

5. Craigie, J.H. 1927. The experiments on sex in Rust fungi. Nature 120: 116-117.

6. Craigie, J.H. 1927. Discovery of the function of the pycnia of the Rust fungi. Nature 120: 765-767.

7. Craigie, J.H. 1931. An experimental investigation of sex in the Rust fungi. Phytopathology 21: 1001-1040.

8. Cummins, G.B., and J.A. Stevenson. 1956. A check list of North American Rust fungi (Uredinales). Plant Dis. Reptr. Suppl. 240: 110-193.

9. Ellis, J.B., and B.M. Everhart. 1897. New West American fungi. III. Erythea 5: 5-7.

10. Hennen, J.F., and G.B. Cummins. 1956. Uredinales parasitizing grasses of the Tribe Chloridae.

Mycologia 48: 126-162.

11. Presley, J.T. 1942. Aecidium gossypii the aecial stage of Puccinia boutelouae. Phytopathology 32: 97-99.

12. Presley, J.T., and C.J. King. 1943. A description of the fungus Puccinia stakmanii n. sp., causing Cotton rust and a preliminary survey of its hosts. Phytopathology 33: 382-389.

13. Spencer, H.M. 1926. Controlling humidity with saturated solutions. International Crit. Tables. 1: 67.

14. Tabenhaus, J.J. 1917. On a sudden outbreak of Cotton rust in Texas. Science. 46: 267-269.