AN INVESTIGATION OF PUCCINIA CYNODONTIS LACROIX,

THE PATHOGEN OF THE RUST OF BERMUDAGRASS

By

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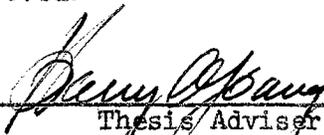
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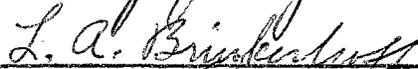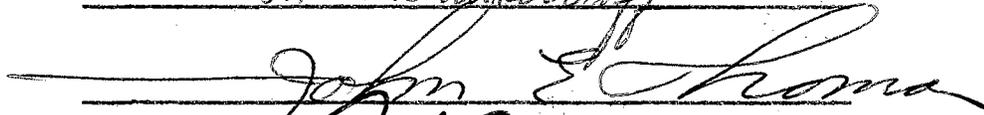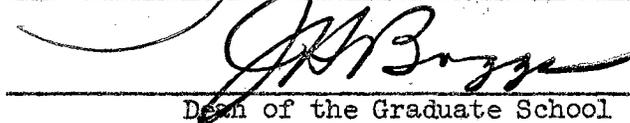
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Dean of the Graduate School

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

The leaf rust of bermudagrass (Cynodon spp.) is caused by the fungus Puccinia cynodontis Lacroix. The uredial-telial stages are not known to occur in the United States on commercially grown strains of Cynodon dactylon, although they have been reported to occur on strains of this species in Europe (3, 5). On the other hand, a variety of Cynodon magennisii (Sunturf) grown principally as a turfgrass in Oklahoma and adjacent states, has been found to be very susceptible. The disease seldom, if ever, becomes severe enough to kill the plant, but it does produce an objectionable brown discoloration and dusty residue.

The pathogen of the leaf rust of bermudagrass has been established and described, and an alternate host, Plantago, which will support the pynical-aecial stages has been determined. However, no other studies concerning this disease or its pathogen have been reported. Information which would be useful in determining the reaction of new or introduced strains of bermudagrass, such as the effect of temperature upon urediospore germination and infection, the incubation period, longevity of urediospores in storage, and physiologic specialization is lacking. Therefore, the purpose of the study reported here was to determine: (1) the optimum conditions for urediospore germination and infection; (2) the incubation period of P. cynodontis within the host; (3) the longevity of urediospores in storage; (4) the presence or absence of

1

physiologic specialization; and (5) the reaction of certain native species of the alternate host, <u>Plantago</u>.

LITERATURE REVIEW

According to Arthur (2) the initial description of P. cynodontis

occurred in Desmaziere's "Plant Crytogrames of Northern France" publish-

ed in 1859 (4). This publication was not available for perusual, but

Arthur cites the fungus as P. cynodontis Lacroix, which would indicate

that Desmaziere had given credit to Lacroix for the initial description.

Fuckel (6) lated described the urediospores as being irregularly spear-

like or elliptical, 19-28µ in diameter, while Arthur (2) considered the

spores to be globoid and 19-24µ by 20-26µ in size. It appears that

Fuckel's description was based upon dried spores, while Arthur's de-

scription was based upon spores suspended in water, which may account

for the difference in shape. Arthur (2) described the uredial lesion as

chiefly hypopylous and cinnamon-brown in color. Both authors state that

the cell wall is finely verrucose, but the spore wall in Arthur's spe-

cimen was cinnamon-brown, while Fuckel described his spores as light

brown in color. Arthur (2) described the spore cell wall as 1.5-3µ

thick and having two or sometimes three equatorial pores.

Fuckel (5, 6) found that the teliospores were formed as long rigid

brownish stems, were mostly elliptical or oblong with the upper cell al-

most as long as the lower cell, but becoming strongly thicker at the

head or crown and normally ending in a more or less elongated conical

tip. Frequently, however, the conical tip was absent. The lower cell

was rounded at the bottom or else was somewhat more narrow at the stem

end. The spores were a little constricted at the septum with smooth brown walls, 30-60μ long and 14-26μ thick.

Arthur (2) found the telial lesion to be chiefly hypopylous and blackish-brown in color. He described the teliospores as ellipsoid or oblong, 16-22μ by 28-42μ, and obtuse or attenuate at each end. The spore walls were 1.5-2.5μ thick at the side and 6-12μ at the top of the upper cell. The pedicle was almost colorless and about 1 1/2 times the length of the spore or less.

Transchel (9) in 1906 was able to show that the pycnial-aecial stage of P. cynodontis occurred on Plantago lanceolata, thus invalidating the name Aecidium plantaginis. Bubak (3) in 1907 verified Transchel's results by carrying P. cynodontis through its entire life cycle using Cynodon dactylon and Plantago lanceolata. Arthur (2) stated that Bubak was able to extend the alternate host range to include Plantago major and Plantago media. However, Bubak's original paper (3) was examined and he definitely stated that he could not extend the alternate host range to either Plantago major or Plantago media. He (2) described the pycnia as being 90-115μ wide and honey yellow in color. The aecia occurred on the upper surface of the leaves of Plantago lanceolata. These lesions were roundish and 1-4 mm. in diameter. The aecia were at first light green, later becoming leathery and brown as the lesion collapsed. The pseudoperidia were circular and irregularly cup shaped, 300-350μ wide, and white with irregularly recurved edges. The pseudoperidial cells were in fixed rows and were polygonal roundish or polygonal elliptical in shape. He also described the aeciospores as 20-28.5μ long and 20-24μ wide, either roundish or oblong, in distinct rows, yellow and with walls 2-2.5μ thick.

MATERIAL AND METHODS

The initial collection of urediospores of P. cynodontis used in the
study were obtained from four separate locations in the vicinity of
Stillwater, Oklahoma. The collections were obtained from infection on
Cynodon magennisii, variety Sunturf. The collection sites were: (1)
the turf disease plots on the Plant Pathology Farm; (2) the turf plots
on the west Agronomy Farm; (3) a lawn in the north part of town; and (4)
the original entry plots of this variety on the Lake Carl Blackwell
Agronomy Farm.

The urediospore germination studies were carried out by placing two
spore masses on each slide and adding a drop or two of tap water to the
spores. Adding the water to the spores caused them to be dispersed to
the edged of the droplet where the best germination took place. The
slides were then placed in a petri dish on top of two glass rods. The
humidity was maintained with moist paper toweling placed in the bottom
of the dish.

Four sprigs of Cynodon magennisii variety Sunturf were planted in
four inch pots containing a mixture of sand, soil and peat. With sub-
sequent growth and cutting, an average of 8-12 stems per pot was ob-
tained. These pots of plants were used for the minimum wetting period
and incubation period studies. The pots were kept in a separate rust-
free room until needed.

Four methods were used to inoculate leaves of bermudagrass plants.

5

In the first method the urediospores were dispersed in a light oil (Mo-isol 100) and then sprayed on the leaves with a hand atomizer (De Vilbiss No. 15). A cotton swab was used in the second method. This involved wrapping a small piece of cotton around the end of a toothpick and then dipping the swab in a surface tension depressant or surfactant (Tween 20). The excess surfactant was then washed out of the cotton swab to prevent water soaking of the leaf blades which was otherwise found to occur. The wet cotton swab was dipped into a vial of urediospores and gently brushed on the grass blades. The third method involved simply dipping the plants into a beaker of water upon which urediospores had been dispersed. When this method was used, the plants were fist dipped in a beaker containing a dilute solution of surfactant (Tween 20, two drops in each 1000 ml.). In the fourth method, heavily rusted plants were used to brush the plants that were to be inoculated. Again, the plants to be inoculated were sprayed with a dilute surfactant solution before they were brushed with the spores.

In all cases, once the plants had been inoculated they were sprayed with a dilute solution of surfactant and placed in a moist chamber to maintain a high relative humidity.

The plants used for inoculation with individual spores were sprigs of Sunturf placed inside hollow glass rods which in turn were placed in 50 ml. flasks. The flasks contained a small amount of sand and approximately 20 ml. of a 40 ppm. solution of benzamidazole. A droplet of water containing a surfactant was then placed on the grass blade. Single spores were picked off a glass slide under the microscope using a glass needle which had been dipped in water containing a surfactant. The spores were then placed at the edge of the droplets on the leaf blades.

The flasks containing the inoculated plants were then placed in 1000 ml.

beakers which had water covering the bottom. The beakers were sealed

with aluminum foil to act as a moist chamber.

RESULTS

Description of the Pathogen. Observations and measurements of the ure-
dial and telial sori and their associated spores were made on the cul-
tures used in these studies. The description which follows corresponds
rather closely with that given by Arthur (2).

The uredia were for the most part, hypopylous and mummy brown (7)
in color. Secondary uredial formation occurred adjacent to the original
pustule in the same interveinal line causing a striping effect. The
urediospores were globoid, 17-26μ, with very finely verrucose cinnamon-
brown walls 1.5-3μ thick with two or sometimes three equatorial pores.

The telial lesions also were chiefly hypopylous and appeared
blackish-brown (7). The teliospores were produced in the same sorus as
the urediospores and could be observed beginning about 10 days after
inoculation. The teliospores were ellipsoid or oblong, 14-23μ by 32-
54μ, obtuse or acute above and rounded below. They were only slightly
constricted at the septum. The cell walls were dark chestnut-brown (7),
1.5-2.5μ thick at the sides and 4-11μ thick above. The almost hayline
pedicles were 1 1/2 times the length of the spore or less.

The teliospores described above were considered to be mature spores.
However, in any given telial sorus a tremendous amount of variability
could occur. For the most part, the spores could be placed in three
groups: (1) long, slender, light brown spores with comparatively thin
walls; (2) spores that were rounded at both ends and dark chestnut-brown

8

in color; (3) those that were considered to be mature spores that varied
from light brown to chestnut-brown in color and were obtuse to acute at
the top of the spore and rounded at the bottom (Figure 1).

Urediospore Germination. The germination studies were carried out in
growth chambers, either the Sherer-Gillet Company model CEL 37-14 or the
Percival Refrigeration Company model PCG-78. The temperature within the
boxes varied no more than $\pm$ 1°C. The lights were so adjusted that ap-
proximately 30 percent of the illumination was from an incandescent
source and 70 percent from a florescent source. The study that included
temperatures of 24°C, 30°C and 34°C was replicated five times with six
separate spore masses at each light intensity. The study made at 6°C,
12°C and 18°C was replicated only twice, but 10 spore masses were used
at each light intensity.

Ten spores were counted at each of five sites taken at random in
each spore mass. The counts for each temperature and light intensity
were averaged and a percent calculated.

The first study was made in total darkness and the results are
given in Table I and illustrated graphically in Figure 2. Under these
conditions there were a few spores that germinated at 1.5°C. The per-
cent of germination increased as the temperature increased to a maximum
of 95 percent at 20°C. Between the temperatures of 20°C and 30°C there
was little or no difference in the percent of germination, but at 34°C
germination was almost completely inhibited.
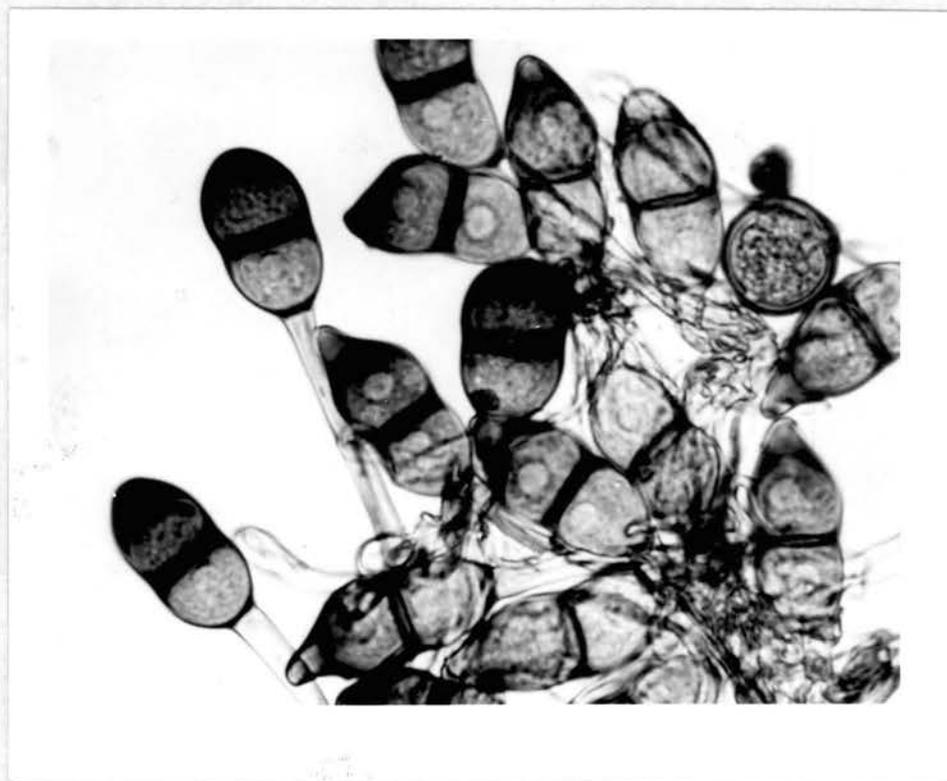
Figure 1

VARIATION IN THE CELL WALL THICKNESS AND THE CAP
OR CROWN THICKNESS IN TELIOSPORES
OF PUCCINIA CYNODONTIS

TABLE I

THE PERCENT OF GERMINATION OF UREDIOSPORES OF PUCCINIA CYNODONTIS
AT VARIOUS TEMPERATURES IN TOTAL DARKNESS

Temperature	$1.5°C$	$5°C$	$7°C$	$12°C$	$14°C$	$20°C$	$24°C$	$30°C$	$34°C$
Percent of Germination	5	25	75	80	85	95	95	95	1

In the second study, both temperature and light conditions were
varied. The initiation of germination took place more rapidly in dark-
ness occurring within one hour at temperatures between $19°C$ and $30°C$,
two and one-half hours at $7°C$, and five hours at $1.5°C$. At any given
temperature the greatest percent of germination of urediospores of P.
cynodontis occurred in total darkness (Table II). In darkness the opti-
mum temperature for urediospore germination again fell between $20°C$ and
$30°C$. Light inhibited germination at all temperatures and this inhi-
bition increased as the light intensity increased. The effect of light
intensity was approximately the same at all temperatures up to and in-
cluding $24°C$ but was much more pronounced at $30°C$ (Figure 3). Complete
inhibition of germination, for all practical purposes, occurred at
light intensities above 3000 ft-c and at temperatures above $30°C$. The
inhibition of germination induced by either light or temperature, how-
ever, can be reversed by placing the spores under proper conditions for
germination. At all temperatures the germ tube length at 12 to 14 hours
was greatest in darkness and decreased as the light intensity increased.
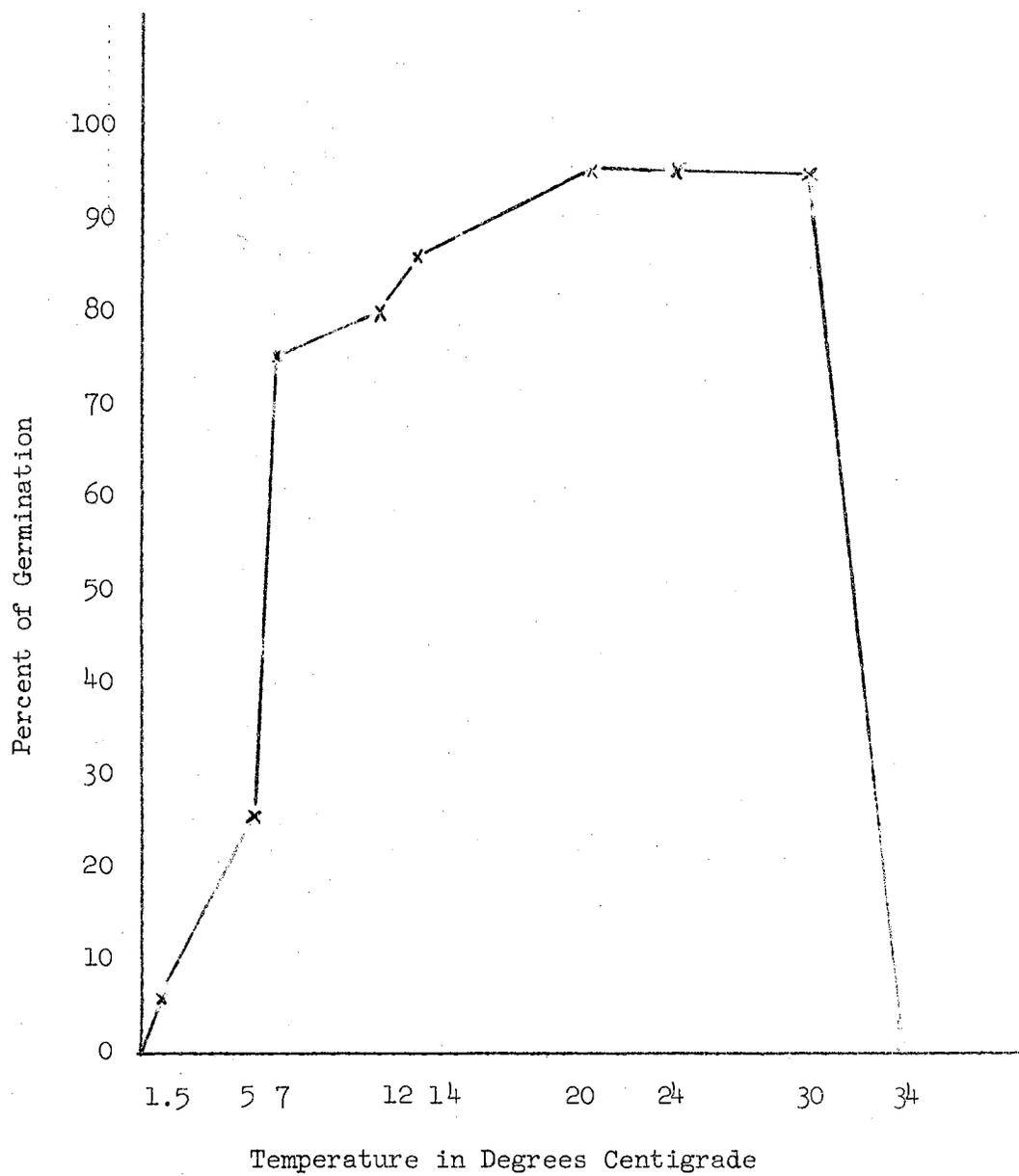
FIGURE 2

THE EFFECT OF TEMPERATURE ON GERMINATION OF UREDIOSPORES OF
PUCCINIA CYNODONTIS IN TOTAL DARKNESS

TABLE II

THE PERCENT GERMINATION OF UREDIOSPORES OF PUCCINIA CYNODONTIS
AT DIFFERENT TEMPERATURES AND LIGHT
AFTER 14 HOURS

Temperature	Darkness	Continuous Light Intensity in Foot-Candles			
		500	1500	3000	3500
5°C	60	50	50	40	<1
12°C	80	60	60	25	<1
14°C	95	70	50	25	<1
24°C	95	70	45	25	<1
30°C	95	15	10	<1	<1
34°C	<1	<1	<1	<1	<1
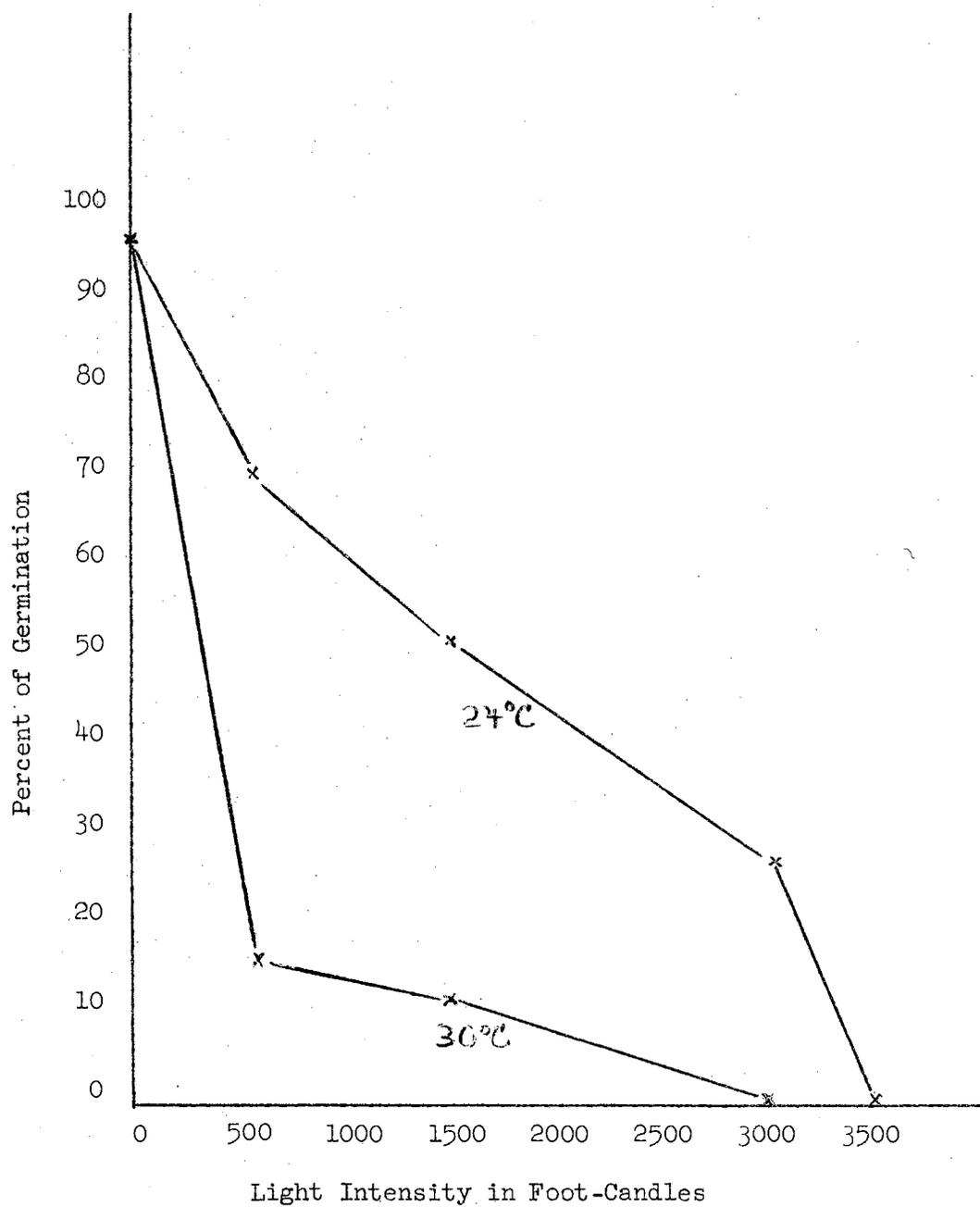
FIGURE 3

THE EFFECT OF LIGHT ON PUCCINIA CYNODONTIS UREDIOSPORE
GERMINATION AT TWO DIFFERENT TEMPERATURES

Infection Process. Following the germination studies an experiment was made to determine the minimum wetting period for infection. The test was repeated 11 times. Two to four pots of plants were removed from the moist chamber for each wetting period for as long as the particular test was carried out. In most of the tests, plants were held at 20°C, 22°C, and 24°C during the wetting period. However, in a few tests temperatures of 17°C, 27°C, and 30°C were also used. Inoculation was accomplished by one of the four methods previously described. In each test, regardless of temperature or method of inoculation, the results were essentially the same. The minimum wetting period required for germination and infection in this study was found to be three hours at 20°C. This minimum wetting period requirement was obtained only once. In all of the other trials a minimum wetting period of four hours was required. A wetting period of four hours produced less than 1 percent severity (modified Cobb scale), a six hour wetting period produced a 10-25 percent severity, and 10 or more hours were required to produce a severity of infection of 50 percent or more. Data from a representative test are presented in Table III.

TABLE III

THE PERCENT SEVERITY (MODIFIED COBB SCALE) OR INFECTION OF
PUCCINIA CYNODONTIS ON CYNODON MAGENNISII FOLLOWING
DIFFERENT LENGTHS OF POST-INOCULATION WETTING
PERIODS AT 20°C, 22°C, and 24°C

Duration of Wetting Period in Hours	Temperature		
	20°C	22°C	24°C
2	O	O	O
4	1	1	1
6	10-25	10-25	10-25
10	50	50	50
24	75-100	75-100	75-100

Incubation Period. The plants used in the determination of the incuba-

tion period of the fungus within the host were inoculated with one of

the methods previously described and subjected to a wetting period of 20

to 26 hours at a temperature between $20°C$ and $25°C$ in darkness or with a

light intensity not exceeding 200 ft-c. Following inoculation and post-

inoculation wetting period, several pots of plants were held at each of

six different combinations of temperature and light intensity. In all

cases a 12 hour day was used. These results are given in Table IV. The

shortest period from incubation to initial pustule formation was found

to be five days at a constant post-inoculation temperature of $30°C$ and a

light intensity of 3000 ft-c, although the average incubation period

for these conditions was five and one-half days. The average length of

time for pustule formation at a constant temperature of $24°C$ with

TABLE IV

THE EFFECT OF LIGHT INTENSITY AND TEMPERATURE ON THE TIME REQUIRED FROM INOCULATION TO INITIAL
PUSTULE FORMATION BY PUCCINIA CYNODONTIS ON CYNODON MAGENNISII

Temperature in Degrees Centigrade		Light Intensity in Foot-Candles With a 12 Hour Day	Number of Days from Infection to Pustule Eruption		Reaction Types[a]
Day	Night		Average	Range	
5	5	1000	9	9	4[b]
18	18	3000	9	8-9	4
24	24	3000	7	6-8	4
30	30	3000	5.5	5-6	4
34	34	3500	9	8-9	1[b]
34	18	3500	7	6-7	4

[a] Reaction rated on a scale from 0 = immune to 4 = susceptible.

[b] Extremely sparse infection.

17

3000 ft-c of light was found to be seven days. The same was true for the diurnal cycle of 34°C at light intensity of 3500 ft-c and 18°C during the dark cycle. At the other three temperature and light combinations, the incubation period was extended to nine days. At a constant post-inoculation temperature of 34°C with 3500 ft-c of light or a temperature of 5°C with 1000 ft-c, the amount of infection was greatly reduced. From 50 to 100 percent severity was observed on plants held at the other five temperature and light combinations used, but only an average of three pustules per plant was found at these minimum and maximum temperature and light combinations. Also, the pustules formed at 34°C were a type "1," or resistant reaction, instead of the susceptible type "4" reaction observed at the other temperature and light combinations. If the plants were removed from these minimal and maximal conditions within 48 hours after inoculation and placed under more optimal conditions for pustule formation, the usual 50 to 100 percent severity developed. If the plants at the 34°C - 3500 ft-c combination were removed and placed under more optimum conditions for pustule formation within 72 hours after the pustules had erupted, the susceptible type "4" reaction developed. However, if the plants were left at 34°C or removed later than three days after pustule eruption, the reaction remained type "1."

Urediospore Storage. Two small test tubes containing urediospores of P. cynodontis were stored at 0°C. Tube A, 1 cm. inside diameter and 10 cm. tall containing approximately 2 cm. of spores, was stored on March 7, 1964, and tube B, 0.8 cm. inside diameter and 7 cm. tall containing 0.5 cm. of spores, was stored on March 19, 1964. The spores

in both tubes had been air dried for three days prior to storage. On

March 20, 1964, the germination of the spores in both tubes was tested

and found to be 95 percent. This was accomplished by placing a glass

slide in a Petri dish which contained moist paper to maintain the humid-

ity. A small quantity of spores was placed at each end of the slide

and a drop of tap water was added to the spores. Two such Petri dishes

were used to test the spores in each tube. The dishes were then placed

in the dark for 24 hours and the observed for germination. The germi-

nation was checked in this manner periodically for 14 months. The re-

sults are given in Table IV. Even after 14 months, 60 percent of the

spores in tube A and 70 percent of the spores in tube B were viable.

TABLE V

THE PERCENT OF VIABILITY OF UREDIOSPORES OF PUCCINIA CYNODONTIS
STORED AT 0°C FOR VARIOUS LENGTHS OF TIME

	3/20/64	6/2/64	10/5/64	10/17/64	1/20/65	3/30/65	5/29/65
Tube A[a]	95	80	75	50	50	25	60[b]
Tube B	95	95	65	85[b]	80	80	70

[a] Tube A spores harvested, March 4, 1964, and stored March 7, 1965.
    Tube B spores harvested March 16, 1964, and stored March 19, 1964.

[b] The apparent increase in viability may have been caused by self-
    inhibitors in previous tests, since no effort was made to control
    the number of spores or the size of the water drop.

Physiologic Specialization. Urediospores from a mass field collection

from the variety Sunturf, C. magennisii, were used to inoculate six

selections in three Cynodon species. The selection of C. magennisii (variety Sunturf) was obtained from the turf disease plots of the Department of Botany and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma. The remaining selections, two of C. transvaalensis (Accession numbers 220 and 292) and three of C. dactylon (Accession numbers 491, 497, and 700) were obtained from Dr. W. W. Huffine, Department of Agronomy, Oklahoma State University, Stillwater, Oklahoma. The later five selections were from a portion of a world-wide collection of Cynodon species made by Dr. Huffine. Accession numbers 220 and 497 came from South Africa, Accession numbers 491 and 700 from Kenya and Accession number 292 from Rhodesia.

A description of the infection types used in this study is presented in Table VI. These were based on the infection types used by Stakman and Harrar (8) for Puccinia graminis var. tritici. Since the collections of urediospores came from C. magennisii variety Sunturf, it was supposed that all spores would be virulent on this selection. Therefore, whenever an increase of urediospores was required, it was made on this selection.

When these six selections were inoculated with the mass culture of urediospores obtained from Sunturf, all of the selections were susceptible except Accession 700, which had a mesothetic reaction ("x"). The pustules with the reaction (type "1") and pustules with a reaction (type "4") were removed separately to Sunturf plants for increase. When the selections were then inoculated with a culture obtained from the resistant (type "1") pustule, Accession 700 and 491 were found to be resistant, Accession 497 was mesothetic, and Sunturf and the

TABLE VI

INFECTION TYPES USED TO CLASSIFY THE REACTION OF CULTURES OF PUCCINIA CYNODONTIS
ON SELECTIONS OF VARIOUS CYNODON SPECIES[a]

Infection Type

0	Immune	- No visible evidence of infection
0;	Very Resistant	- Small flecks of dark brown nucrotic host tissue, but no rust pustules formed
1	Resistant	- Rust pustule extremely small and surrounded by dark brown or straw-colored nucrotic areas
2	Moderately Resistant	- Pustules small to medium in size, usually in green islands of host tissue, surrounded by nucrotic straw-colored areas
3	Moderately Susceptible	- Medium size pustules not surrounded by nucrotic areas although small chlorotic areas may have been present
4	Very Susceptible	- Pustules large and often coalesced longitudinally with no nucrotic or chlorotic areas present
5	Heterogeneous or Mesothetic	- Size of pustule usually including all of the above types and intergradations between them on the same leaf

[a]Adapted from Stakman and Harrar (8).

two C. transvaalensis were found to be susceptible. Later, after a differential series had been established and a key prepared, this culture was labeled race 2 (Tables VII, VIII). The culture obtained from the susceptible type "4" pustule on Accession 700 became contaminated and could not be used. Later, however, a single spore isolate from the mixed culture was obtained and increased. This culture was used to inoculate four of the six selections (Accession 491, 497, and 700 plus Sunturf) and all proved to be susceptible except 491, which was resistant. This culture was later labeled race 3.

Another bulk of urediospores from two of the initial collections mentioned previously was then used to inoculate all six Cynodon selections. This time a mesothetic reaction occurred on Accession 497 as well as Accession 700. When a separation of the resistant and susceptible type reactions was made from Accession 497, it was found that the resistant type reactions yielded a culture which was avirulent on Accessions 497, 491, and 700, and virulent on Sunturf. The two Transvaalensis accessions were not inoculated. This culture was designated race 1. The susceptible reaction on Accession 497 yielded a culture which was virulent on Accession 491, 497, and Sunturf, but produced a mesothetic reaction on Accession 700. Again the two C. transvaalensis accessions were not inoculated. This culture was designated race 4. Separation from the diverse reactions on Accession 700 was not made. The culture designated race 4, however, produced a mesothetic reaction on Accession 700 and the reactions on other selections were also similar to the reactions obtained with the first bulk of urediospores used. When the separation of reactions on Accession 700 was made from the

TABLE VII

THE REACTION OF FOUR CULTURES OF PUCCINIA CYNODONTIS ON SEVERAL SELECTIONS OF CYNODON SPECIES

| Varieties	Race 1	Race 2	Race 3	Race 4
Sunturf	4	4	4	4
Selection 491	0;, 1	0;, 2	0;, 1	3, 4
Selection 700	0;, 2	0;, 2	4	X
Selection 497	0;, 2	X	4	4

TABLE VIII

ANALYTICAL KEY FOR PHYSIOLOGIC RACES OF PUCCINIA CYNODONTIS IDENTIFIED ON SELECTIONS OF CYNODON DACTYLON

Selection Reaction	Race Number
Accession Number 497 Resistant	1
Accession Number 497 Mesothetic	2
Accession Number 497 Susceptible	
Accession Number 491 Resistant	3
Accession Number 491 Susceptible	4

first bulk, races 2 and 3 were obtained. It is therefore possible that
the culture now designated race 1 may actually be a mixture of two or
more of the cultures. This possibility is being investigated.

It was evident that Accessions 491, 497, and 700, all in the species
C. dactylon, would differentiate cultures of P. cynodontis and that spe-
cialization did exist. The reactions of the cultures obtained in these
studies are listed in Table VII and illustrated in Figures 4 and 5. A
key was then prepared for the identification and nomenclature of these
cultures and is given in Table VIII.

Alternate Host. The only known alternate host for P. cynodontis in the
genus Plantago is the species lanceolata (9). Two other species have
been tested, P. media and P. major, and were found to be inactive (3).
Two other species, P. surshii and P. virginica were found growing in
areas adjacent to plantings of C. magennisii at Stillwater, Oklahoma,
that were bearing aecial infections. Numerous attempts were made to in-
fect C. magennisii in the greenhouse with aeciospores collected from
these species, but all attempts failed. Healthy plants of these two
species of Plantago were then brought into the greenhouse and placed
under mats of telial material collected from C. magennisii. The telial
mats were alternately wet and dried and the plants sprayed with water in
attempts to induce sporidial development and infection. No evidence of
pycnial or aecial lesions developed. Although these studies were not
exhaustive, it seems evident that these two local species of Plantago
will not serve as an alternate host for P. cynodontis.

Race 1
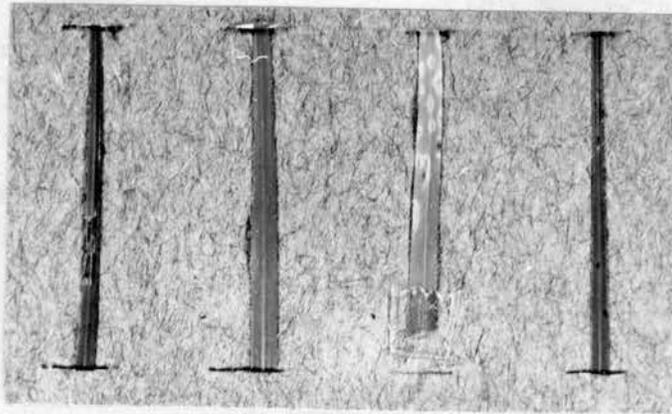
Sunturf   Acc.   Acc.   Acc.
              491   700   497

Race 2

Sunturf   Acc.   Acc.   Acc.
   491   700   497

Figure 4

THE REACTION OF RACES 1 AND 2 OF PUCCINIA CYNODONTIS
ON FOUR VARIETIES AND SELECTIONS OF BERMUDAGRASS

Race 3
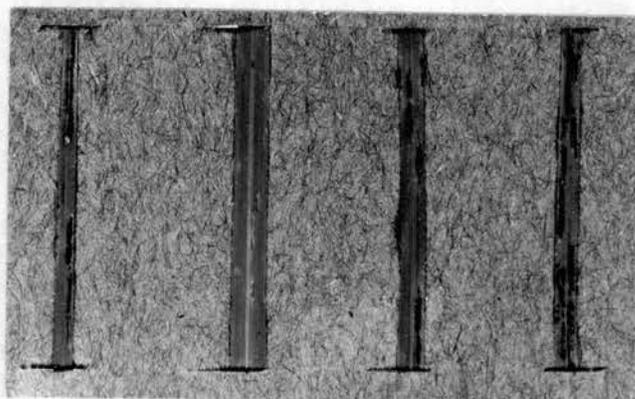
Sunturf   Acc.   Acc.   Acc.
             491   700   497

Race 4
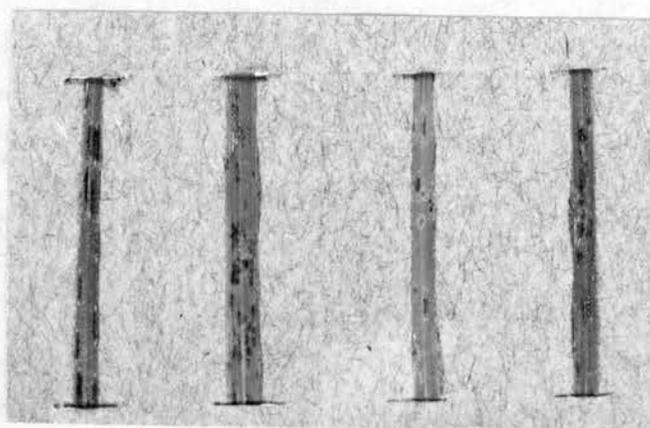
Sunturf   Acc.   Acc.   Acc.
             491   700   497

Figure 5

THE REACTION OF RACES 1 AND 2 OF PUCCINIA CYNODONTIS
ON FOUR VARIETIES AND SELECTIONS OF BERMUDAGRASS

DISCUSSION

The fungus used in these studies rather closely fitted the description given by Arthur (2) for the uredial and telial stages. It was found, however, that some problems might arise in the identification of the teliospores unless the telial lesion is allowed to mature. It is believed that often the immature teliospore does not possess the thickened cap at the crown of the upper cell.

Satisfactory urediospore germination was obtained over a wide range of temperatures (7°C - 30°C) so long as the light intensity during the germination process did not exceed 200 ft-c. It was found that light intensities over 500 ft-c cause a marked decrease in germination regardless of the temperature. Light in these studies was a much more significant factor than temperature in the germination of urediospores of P. cynodontis.

Although urediospore germination would proceed over a broad temperature range, a rather long wetting period was required to produce a satisfactory degree of infection regardless of the temperature. Trace amounts of infection were obtained with wetting periods as short as three to four hours, but wetting periods as long as 10 hours were required for 50 percent infection or more. Light was critical, particularly, early in the wetting period, and any light intensity over 200 ft-c restricted the amount of infection and extended the required wetting period.

Light intensity was also more critical than temperature in determining the time from infection to pustule eruption. At this stage, however, a light intensity of 3000 ft-c or more was required, at any given temperature for the shortest incubation period. A light intensity below 3000 ft-c delayed pustule eruption regardless of the temperature. There appeared to be certain temperature limits, however. Only trace amounts of infection occurred at $5^{\circ}C$ and 1000 ft-c or at $34^{\circ}C$ and 3500 ft-c.

The urediospores of P. cynodontis had a surprisingly high survival ability when stored at $0^{\circ}C$. The only pre-storage preparation the spores received was three days of air drying, yet after fourteen months in storage the germination was as high as 70 percent. The urediospore wall in P. cynodontis is rather thick compared to other graminacious rusts and this may well account for its survival ability in storage. Bermudagrass has a long dormant period in many areas of its culture in the United States, and the ability to survive these dormant periods would be necessary for the survival of the pathogen in nature.

The experiment on urediospore survival indicated a viability of only 25 percent after 12 months of storage, and 60 percent viability in the same storage container after 14 months of storage. Such a difference seems unlikely and was probably due to a self-inhibitor of germination. No particular care was exercised in measuring either the amount of spores or water used in these germination tests. Self-inhibitors of urediospore germination are known to occur and according to Allen (1) and Wilson (10) the percent of germination of urediospores in their studies was inversely proportional to the amount of spores present.

No specific test for the presence of self-inhibitors was made in this study, but it does seem possible that the reduced germination after 12 months of storage was caused by the presence of a self-inhibitor.

The selections of C. dactylon used in the identification and nomenclature of races of Puccinia cynodontis in this investigation may never have any significance in breeding new varieties of this grass. Their importance rests in the fact that with them variants within P. cynodontis have been shown to exist. The importance of pathogenic or physiologic specialization in a breeding program for disease resistance is well recognized. The fact that specialization exists in P. cynodontis will have important significance in turfgrass improvement programs utilizing this grass. These studies also could indicate that C. dactylon rather than C. magennisii or C. transvaalensis would provide the best source of resistance. It should be emphasized, however, that only a minimal number of selections in each species were tested.

The inability to demonstrate that either P. purshii or P. virginica would serve as an alternate host in this study tends to eliminate teliospores found in turfgrass plantings in this area. The species P. lanceolata remains the only known alternate host in this genus, and while it does occur, at least in the eastern areas of Oklahoma, nothing is known concerning the infection of this species with P. cynodontis in nature in this area.

SUMMARY

1. The uredial and telial states of _Puccinia cynodontis_ are described.

2. Urediospores were found to germinate well over a wide range of temperature (5°C to 30°C), but darkness or at best a light intensity of less than 200 ft-c was required for this process.

3. Similarly, the process of infection during the wetting period would proceed over a wide range in temperature (at least 17°C to 30°C), but again, minimal light intensities were required. The required wetting period (10 hours) for satisfactory infection severities was rather long compared to other graminacious rusts.

4. The minimum incubation period from inoculation to pustule formation was determined to be approximately five days at 30°C with 3000 ft-c of light. Light was required during incubation and any intensity less than 1000 ft-c led to a greatly extended incubation period. A temperature of 34°C with 3500 ft-c of light greatly altered the host-parasite interaction.

5. Urediospores were found to store well at 0°C, maintaining 60 to 70 percent viability over a period of 14 months.

6. Physiologic specialization was shown to exist and four races were isolated and named, and a key was made for their identification.

7. No pycnial or aecial development could be obtained on _Plantago purshii_ or _Plantago virginica_. Aeciospores found on these species in

the field could not be induced to go to _Cynodon magennisii_.

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