STUDIES CONCERNING TELIOSPORE GERMINATION AND THE SUBSEQUENT INFECTION OF CERTAIN PYCNIAL-AECIAL HOSTS OF <u>PUCCINIA</u> <u>RECONDITA</u> ROB. EX DESM. F. SP. <u>TRITICI</u> ERIKSS.

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PUCCINIA RECONDITA ROB. EX DESM. F. SP. *TRITICI* ERIKSS.

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INTRODUCTION

Leaf rust of wheat, incited by Puccinia recondita Rob. ex Desm. f. sp. tritici Erikss., is an important endemic disease in Oklahoma, usually exacting an annual loss of some 5 percent of the state's wheat crop. Most of the varieties of wheat commonly grown in Oklahoma at the present time are susceptible to attack by this organism, and it often happens that when a resistant variety is introduced it appears to become susceptible due to changes in the prevalence of certain races within the population. Such a phenomenon occurred following the introduction of the wheat variety Pawnee and more recently the variety Selkirk. Even more perplexing from the standpoint of the development of resistant varieties is the potential this organism has shown for producing new virulent biotypes or races that are capable of attacking previously resistant varieties of the host. For instance, a new virulence was detected in race 105 of wheat leaf rust in 1951 that enabled some cultures of this race to attack the variety Westar, C. I. 12110, which had previously been resistant to all of the races common in the Great Plains area. This particular virulence, referred to as the "A" virulence in Oklahoma cultures, subsequently appeared in the course of a few years in the other races commonly occurring in the Plains states (i.e. 15, 5, and 9). The source of this new virulence and its spread to the other prevalent races may have been the result of: (1) independent mutations in the several races, (2) a single original mutation which spread by hybridization or vegetative nuclear interchange (heterocaryosis or parasexualism) to other races, or (3) not the result of mutation at all, but rather the unmasking of recessive genes due to hybridization of two races (or the selfing of a single heterozygous race) on the alternate host Thalictrum spp.

The initial experiments were designed to discover the means by which such a series of events might occur in the leaf rust organism. The first objective called for selfing pure cultures of two races (one without and one containing the "A" type of virulence) on the alternate host. This procedure was expected to give some idea of the variability that might be inherent in a single culture. Following this, the influence of selfing upon subsequent heterocaryotic nuclear exchange was to be explored.

All efforts to germinate the teliospores of these two cultures were unsuccessful, however, and successful production of the sexual stage of the organism on Thalictrum was not realized. This difficulty demanded a satisfactory solution before the original objectives could be attained. Therefore, this problem was deemed worthy of concentrated effort, and the bulk of these experiments have been directed toward finding the best means of inducing teliospores to germinate and to produce infection on species of Thalictrum.

Subsequently, the successful germination of some collections of endemic races made it possible to test an extensive collection of Thalictrum species for susceptibility to certain leaf rust populations.

REVIEW OF LITERATURE

Possibilities for experimentation with the pycnial and aecial stages of wheat leaf rust were established in 1921, with the publication of the results of a comprehensive and successful search for the alternate host of this important organism. Jackson and Mains (13), suspecting the alternate host to be a member of the Ranunculaceae, screened many different genera of this family by massive sporidial inoculations. Only species of the genus Thalictrum proved susceptible, and they found that the degree of susceptibility varied widely from one species to another, with Eurasian species often susceptible and American species quite resistant. This original work has since been repeated and supplemented (1, 3, 4, 7, 8, 9, 10, 11, 16, 17, 18). Species of two other genera (both of the Ranunculaceae) have produced aecia of the rust. As in the case of Thalictrum, the Eurasian species Isopyrum fumarioides is much more susceptible than the American species Anemonella thalictroides. This resistance may account for the apparent rarity of the pycnial and aecial stages of wheat leaf rust in America.

Only one collection of wheat leaf rust aeciospores has been reported from a natural infection of the alternate host in America. Levine and Hildreth (15) identified a collection of aecial material from Thalictrum dioicum as race 2 of Puccinia recondita in 1957. However, as a result of a yet unpublished survey by Dr. H. C. Young in 1961, there is considerable evidence that more field infection may occur on American species of *Thalictrum* than has previously been suspected.

Although *Thalictrum* spp. were the first proven to function as alternate hosts of the wheat leaf rust organism under laboratory conditions, the genus *Isopyrum* was first shown to function as alternate host in nature. In 1937, Bryzgalova (4) demonstrated that pycnial-aecial infections on *Isopyrum fumarioides*, which grows in the Lake Baikal region of Siberia, were wheat leaf rust.

Early reports of the discovery of the aecial stage of leaf rust on *Thalictrum* (1, 7) were largely discredited. By 1952, however, D'Oliveira (8) had found *Thalictrum speciosissimum* Loefl. to be commonly infected with rust when growing near old wheat fields in Portugal. His artificial inoculation tests showed at least some degree of susceptibility in 36 of 46 species of *Thalictrum* tested. He also found Eurasian spcies to be the most congenial, with the American species either immune or developing only light restricted infections. Quite surprisingly, *Isopyrum fumarioides*, on which Bryzgalova had had such a very high percentage of successful inoculations with teliospores of Siberian races, was found to be immune to Portuguese races. *Isopyrum* (Anemonella) *thalictroides* was only slightly susceptible to the Portuguese cultures.

Some species of *Thalictrum* seem to be either resistant or susceptible to all collections of leaf rust thus far used in testing. However, Table I shows that there are several species that may vary in response to geographically isolated rust collections.

TABLE I

REACTION OF VARIOUS SPECIES OF THALICTRUM, ISOPYRUM, AND ANEMONELLA TO GEOGRAPHICALLY ISOLATED ENDEMIC CULTURES OF WHEAT LEAF RUST

Species	Natural Habitat	Test Reaction	Rust Source	Authority
A. Species reported susceptible in all tests:				
Thalictrum flavum	S. Europe	VS[1]	Amer.	(16)
do.	do.	VS	Port.	(7)
do.	do.	S	India	(17)
do.	do.	VS	Russia	(11)
Thalictrum speciosissimum	S. Europe	VS	Port.	(8)
Thalictrum calabricum	?	VS	Port.	(8)
Thalictrum foliosum	India	S	India	(17)
Thalictrum laserpitiifolium	?	VS	Port.	(7)
Thalictrum glaucum	S. Europe	S	France	(9)
do.	do.	S	Canada	(3)
do.	do.	VS	Port.	(8)
do.	do.	S	Amer.	(10)
Thalictrum petaloideum	?	VS	Port.	(7)
Thalictrum lucidum	?	VS	Russia	(11)
Thalictrum tuberosum	?	VS	Russia	(11)

[1]Test reactions are graded as follows:

VS--Heavy infection and aecial production (Very Susceptible).

S--Good infection and aecial production (Susceptible).

MS--Good pycnial infection with fair aecial production (Moderately Susceptible).

MR--Fair pycnial infection with occasional aecial production (Moderately Resistant).

R--Fair to poor pycnial infection with no aecial production (Resistant).

VR--Immune; no infection at all (Very Resistant).

Table I (Continued)

Species	Natural Habitat	Test Reaction	Rust Source	Authority
B. Species varying widely in reaction:				
Thalictrum delavayi	Asia	R	India	(17)
do.	do.	VS	Port.	(8)
do.	do.	VS	Amer.	(16)
Thalictrum dipterocarpum	Europe	VS	Port.	(8)
do.	do.	R	Amer.	(16)
do.	do.	VR	Port.	(7)
do.	do.	S	Canada	(3)
Thalictrum minus	Eurasia	VS	Port.	(7)
do.	do.	R	Amer.	(16)
do.	do.	R	Russia	(10)
do.	do.	VS	Russia	(11)
[2]T. ruthenicum T. elatum T. adiantifolium T. nutans	do.	VS	Russia	(11)
Thalictrum ruthenicum (minus?)	?	VS	Port.	(7)
Thalictrum foetidum	?	MS	Russia	(11)
do.	?	VS	Port.	(8)
Isopyrum fumarioides	Siberia	VS	Siberia	(4)
do.	do.	VR	Port.	(8)
C. Species moderately susceptible to resistant:				
Thalictrum dasycarpum	America	MR	Amer.	(16)
do.	do.	MS	Canada	(3)
do.	do.	MR(outdoor)	Canada	(3)
Thalictrum dioicum	America	MS-VR	Amer.	(16)
do.	do.	R	Canada	(3)
Thalictrum polygamum	America	R	Amer.	(16)
do.	do.	R	Canada	(3)
Thalictrum polycarpum	America	MR	Amer.	(16)
Thalictrum angustifolium	Eurasia	MR	Amer.	(16)
do.	do.	MR	Russia	(11)
Thalictrum aquilegifolium	Eurasia	VR	Port.	(7)

[2]These species listed as synonymous to T. minus by Eremeyeva (11).

Table I (Continued)

Species	Natural Habitat	Test Reaction	Rust Source	Authority
Thalictrum aquilegifolium	Eurasia	MR	Amer.	(16)
do.	do.	MR	Russia	(11)
do.	do.	R	Russia	(10)
Thalictrum simplex	?	R	Russia	(10)
do.	?	R	Amer.	(16)
do.	?	VR	Port.	(7)
Thalictrum hemense	?	VR	Port.	(7)
Thalictrum palmatum	?	VR	Port.	(7)
Thalictrum trigynum	?	MS	Russia	(11)
Thalictrum corynellum	?	MS	Russia	(11)
Thalictrum ambiguum	?	MS	Russia	(11)
Thalictrum saxatile	?	MS	Amer.	(16)
Thalictrum javanicum	India	MS	India	(16)
Thalictrum neurocarpum	?	R	India	(16)
Thalictrum cultratum	?	R	Amer.	(16)
Thalictrum paniculatum	?	R	Amer.	(16)
Thalictrum fendleri	?	R	Amer.	(16)
Thalictrum occidentale	America	R	Amer.	(16)
Thalictrum elegans	?	R	Amer.	(16)
Thalictrum venulosum	America	VR	Canada	(3)
Isopyrum biternatum	America	VR	Amer.	(16)
Isopyrum thalictroides[3]	?	MR	Port.	(8)
Anemonella thalictroides[3]	America	R-MS	Amer.	(16)

[3]Possibly the same species. All names used are those listed by the authors cited.

Craigie (6) states that after barberry leaves are 12 days old they are highly resistant to infection by sporidia of Puccinia graminis tritici. Such a limitation as aging leaf tissue is not referred to in literature relating to infection of Thalictrum leaves by wheat leaf rust sporidia. Brown and Johnson (3), however, did propose that all species of Thalictrum are more resistant under outside conditions. In their greenhouse tests Thalictrum dasycarpum produced aeciospores capable of infecting Little Club wheat. This same species failed to show any infection at all in outdoor inoculation tests.

Several instances are recorded of successful infection of certain Thalictrum species with field grown teliospores (1, 3, 7, 13, 15). However, variations have been noted between individual collections of rust with respect to their capacity to produce effective sporidial inoculum. Jackson and Mains original work brought this out quite clearly. The collections of telia they used were made in early summer and placed outdoors to overwinter. Of their first year collections, 10 of 20 gave good germination the following March. The same proceedure was followed a second year, and 51 of 80 collections germinated. Apparently no difficulties were encountered in germinating those collections that were in germinable condition, the others being discarded. Then, as now, little was known about why some groups of spores fail to respond to repeated attempts at germination. There still has been no experimental demonstration of a given set of conditions that can be depended upon to produce germination of any given collection of teliospores.

A natural wintering technique has been used by a number of investigators to break dormancy (16, 20, 21). Some have doubted the necessity of wintering--at least in the case of spores of some collections or perhaps a portion of the spores of most collections. There is evidence to support both contentions. Johnson (14) has provided ample data to show that the teliospores of Puccinia graminis tritici germinate over an extended period of time, with only a relatively small portion of the total population germinating at any one time. Other stem rust investigations have indicated that teliospores formed late in the season germinate earlier and more abundantly than spores of the same race formed early in the growing season (5).

Whatever the reason, some spores do germinate without a rest period. D'Oliveira first reported the germination of wheat leaf rust teliospores without a rest period in 1940 (7). This was confirmed by Brown and Johnson (3) in 1949. They reported germination of field grown teliospores of leaf rust in less than three months, without overwintering. The same investigators reported inducing germination of teliospores of three separate races only one week after collection. These spores were collected from greenhouse grown cultures. Samborski[1] even concludes that freezing is best avoided in leaf rust teliospores, as it may induce dormancy in the portion of spores that would otherwise germinate readily. Furthermore, he concludes, artificially controlled freezing does not duplicate the overwintering conditions that normally induce some races to

[1]Personal communication.

make the physiological changes necessay to end their dormancy. Vakili (20), on the other hand, reported complete failure in all attempts to germinate greenhouse grown cultures of several races during the first summer after collection in May. A wintering period outdoors succeeded in breaking the dormancy, however, and germination was detected the following January.

In perhaps the most comprehensive experiments relating to the influence of environmental conditions in inducing teliospore germination, Johnson (14) found freezing to be advantageous in conditioning germination of spores formed at higher temperatures. Alternate freezing and thawing, he states, has been investigated without showing positive results. These experiments were conducted on wheat stem rust, and may or may not apply to wheat leaf rust.

There are indications that several other factors besides wintering (or lack of it) may affect teliospore response. In reference to greenhouse grown material, Samborski[1] found teliospores grown in the spring to germinate more quickly than those grown in the winter.

Vakili (20) found that certain races of wheat leaf rust differed in earliness of teliospore formation. He also observed instances in which the host variety and/or rust reaction type affect telial formation. Race 104B formed telia abundantly on the variety Knox (intermediate reaction type), but only sparsely on Axminister and Norka (both fully susceptible to this race). This same phenomenon of telia formation occurring on resistant varieties earlier than on susceptible varieties also has been observed on detached wheat leaves on benzimidazole solution.[2]

On the other hand, Zimmer and Schafer (22) have concluded from their data that the rate of telial formation of Puccinia coronata on oats is entirely independent of the type of reaction on the host, the range of virulence of the parasite, host variety, or stage of host maturity. Only temperature consistently affected the rate of telial formation, with telia forming 10 days sooner at 80°F. than at 60°F. Zimmer, Schafer, and Gries (21) had found earlier indications that environmental conditions during the formation of teliospores of Puccinia coronata did affect subsequent germinability. Natural overwintering preconditions abundant germination in field grown spores, but failed, in conjunction with many other treatments (freezing and thawing, wetting and drying, etc.), to bring about germination in greenhouse grown spores.

Higher temperature also favors early telial formation of Puccinia graminis tritici (14), but there is evidence to show that a high rate of telial formation may even decrease the percentage of germinable teliospores. Johnson (14) found that teliospores formed at 55-60°F. germinated better and sooner than those formed at 70-75°F. In one race spores formed at 70-75° did not germinate at all. Thus the optimum temperature for spore formation may not be optimum for conditioning good germination.

Shifman (18) cites 15-20° C. (59-68°F) as the optimum temperature for telial formation by wheat leaf rust. Whether or not this is the optimum for the

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[2] B. L. Keeling and H. C. Young, Jr. (unpublished data).

formation of germinable spores is a question lacking experimental proof. Mehta (17) attributes the rarity of germinable teliospores in India to formation in, or subsequent exposure to, high temperatures, but cites no experimental data as proof. It would be hard to conclude from existing data whether temperature or other environmental conditions during the formation of the teliospores may actually injure the teliospores or modify their period of dormancy. Johnson (17) alluded to something of this nature by noting that freezing was of value in conditioning germination of stem rust teliospores which were formed at high temperatures.

Chemicals have been shown to be of value in breaking the dormancy of many spores. Little data seems available in reference to rust teliospores, although Gottlieb (12) states that benzaldehyde has been used to induce germination of wheat stem rust teliospores. This statement could not be confirmed, however, as his reference citation is apparently incorrect.

Shifman (18) advocates sudden change in temperature, from 20-25°C. to 5-6°C., or the reverse, to induce normal germination of wheat leaf rust teliospores. Other investigators advocate repeated cycles of wetting and drying, or short periods of soaking (14, 6, 20), to induce germination, and Bryzgalova (4) has listed 9-14°C. as the optimum temperature for germination of wheat leaf rust teliospores collected in Siberia.

MATERIALS AND METHODS

Teliospores for the first two experiments were grown under controlled temperatures in air conditioned greenhouse rooms. A separate room isolated from other rust cultures was used to keep each race as pure as possible. The rust races used were those designated as "9" and "15A". The reaction of these races on the differential varieties used in 1961 is listed in Table II. Race 9 was grown on the wheat variety Little Club, while 15A was grown on Kenya Farmer, a variety susceptible only to races having the "A" type of virulence. Seed of both varieties was obtained from the Department of Botany and Plant Pathology at Oklahoma State University.

Some 75 plants of several different species of Thalictrum were used for rust inoculations. Plants of seven exotic species (Thalictrum speciosissimum, T. speciosissimum illuminator, T. flavum, T. dipterocarpum, T. minus adiantifolium, and T. aquilegifolium) were grown from seed obtained from Thompson and Morgan Nurseries, Ipswich, England. The American species, Thalictrum dioicum, T. sparsiflorum, and T. alpinum were grown from seed obtained from the University of Minnesota, and additional plants of the former were grown from crowns furnished by Dr. J. G. Dickson of the University of Wisconsin. Several unidentified American species collected from 13 different sites along the Eastern foothills of the Rocky Mountains were furnished for testing by Dr. H. C. Young, Jr., Oklahoma State University.

TABLE II

REACTION TYPE OF RACES 9 AND 15A ON THE INTERNATIONAL AND SUPPLEMENTAL LEAF RUST DIFFERENTIALS

Wheat Variety	Reaction Type[1] and Classification[2]			
	Race 15A		Race 9	
	Type	Class	Type	Class
International Differentials[3]				
Malakof	0	R	4	S
Carina	0	R	1-2	R
Brevit	0-1	R	1-2	R
Webster	0	R	4	S
Loros	0-1	R	4	S
Mediterranean	4	S	0-1	R
Hussar	0-1	I	1-2/	R
Democrate	4	S	0-1	R
Supplemental Differentials[4]				
Lee	4	S	0-0;	R
Westar	4	S	0	R
Sinvalocho	0-0;	R	0-0;	R
Waban	0-1	R	0-0;	R

[1] Numerical Scale of Reaction Severity Ranging from 0 to 4.

[2] Classification of Reaction Type: (S) - Susceptible; (I) - Intermediate; (X) - Indeterminate Reaction; (R) - Resistant.

[3] From the SIXTH REVISION OF THE INTERNATION REGISTER OF PHYSIOLOGIC RACES OF PUCCINIA RECONDITA ROB. EX DESM. (Formerly P. rubigo-vera tritici) USDA ARS 34-27, 1961.

[4] From the NORTH AMERICAN 1961 SET OF SUPPLEMENTAL DIFFERENTIAL WHEAT VARIETIES FOR LEAF RUST IDENTIFICATION. Plant Disease Reporter 45 (60: 444-446, 1961.

Plants of these Thalictrum species were found to mature and die back rapidly at temperatures above 80°F., but maintained vegetative vigor while often remaining in the rosette stage for considerable lengths of time at 65-75°F. Plants that have matured seed stalks, or otherwise lost vegetative vigor, often respond with vigorous new growth from the crown when held at 45°F. for a few weeks and then returned to 70-75°F.

Good growth conditions for the plants and at least a partially controlled environment for the experiments were obtained in thermostatically-controlled, air conditioned greenhouse rooms, about 7' x 20' x 10' in size. These glass-partitioned interior rooms were further insulated against outside temperatures by a clear plastic lining. Although the plastic lining reduced the light intensity somewhat, it had a beneficial insulating effect. Although temperatures climbed above 90°F. during the two or three hours of maximum July sun exposure, they soon reverted to the thermostatically controlled level. Nine months of the year fairly constant temperatures could be maintained throughout the entire day.

For isolating (and sometimes inoculating) small groups of plants, moist chambers, about 24" x 30" x 30" in size, were constructed with clear polyethylene plastic. Air movement was provided by muslin ventilators on the sides.

Individual plants of Thalictrum were usually inoculated and incubated inside cylinders of clear, hard plastic 15"-25" in length and about 5 1/2" in diameter. The leaves or filter pads containing the inoculum were suspended from small insulated wires fastened to the top of the cylinder. The cylinders were covered at the top by either large petri dishes or by multiple layers of paper toweling,

RESULTS

Experiment 1. No particular difficulty was encountered in growing teliospores of pure races to be used in germination and infection tests. Wheat plants were first inoculated with rust when two months old, and the infections were reincubated and spread every 7 to 14 days thereafter. When the plants were three months old the temperature was lowered from 76°F. to 68°F., and held at that level to comply with Shifman's (18) recommendation for optimal telial production. Telia of both races 9 and 15A appeared when the plants were 3 1/2 months old, and mature telia were harvested each week for over a month.

Two weeks before the last telia were harvested urediospores were still abundant, and these were used to inoculate leaf rust differentials to estimate the purity of the cultures. The presence of two off-type pustules in an estimated 10,000 were found in race 15A. Slightly more contamination was found in race 9, as 13 off-type pustules were identified among an estimated 10,000 pustules on the differentials. However, contamination in this degree (1 in 1,000 or less) was considered to be insignificant for purposes of these experiments.

Telia of races 9 and 15A were each divided into several groups, and each group subjected to many variations of environmental conditions to determine the most favorable set of conditions for the breaking of dormancy and the induction of germination. The wheat leaves containing the telia were first glued to glass slides with weather strip adhesive. This type of bonding was soon discarded in favor of rubber bands because of the possibility of some chemical diffusing from the adhesive and affecting spore germination. Eventually the leaves were macerated with a fine scapel, and the teliospores filtered through fine screened funnels to remove leaf trash. The spores were then recovered on glass fiber filter paper by means of a Buchner funnel and suction flask.

The treatments receiving primary attention in this experiment were temperature variations and repeated cycles of wetting and drying.

Many combinations of environmental conditions were tried during the course of one year in an effort to induce germination in at least one of the groups of teliospores. Tables III and IV show the variations in treatment that were given to the separate groups of spores including: storage temperatures (maximum, minimum, and mean), the duration of storage, the number of attempted inoculation of _Thalictrum_ plants, and the temperature at which these inoculations were attempted. The species of _Thalictrum_ used in this experiment and the number of times inoculated are listed in Table V. To give a better picture of the continuity of the experiment, figures are given for two six-month periods of treatment, rather than for the entire year.

A few treatments not mentioned in the tables also were used. These included abrupt changes in temperature, chemical treatments, and some minor variations in inoculation procedure. The moisture sources used for inoculation

TABLE III

PRE-GERMINATION TREATMENT OF INDIVIDUAL GROUPS OF TELIOSPORES OF TWO WHEAT LEAF RUST RACES, MARCH THROUGH AUGUST, 1961

Culture	Duration	Temperature min.	Temperature max.	Temperature mean	Attempted Inoculations at 40°F	50°F	60°F	70°F	80°F	Total
15A-1	56 da.	32	125	55	2	18	0	0	0	20
do.[a]	10 da.	62	68	65	0	0	0	0	0	0
15A-2	21 da.	18	60	34	0	4	15	1	0	20
do.[a]	20 da.	10	88	70	0	0	0	0	0	0
15A-3	69 da.	20	75	37	0	2	18	2	0	22
15A-4	69 da.	30	75	37	0	2	18	2	0	22
15A-5	96 da.	32	38	35	0	0	0	0	0	0
do.[a]	8 da.	30	85	55	0	0	0	0	0	0
do.[a]	85 da.	80	90	85	0	0	0	0	0	0
do.[a]	11 da.	30	32	35	0	0	0	0	0	0
15A-6 (storage trts. sim. to 15A-5 above)					0	1	2	0	0	3
15A-7&8	215 da.	30	40	36	0	0	0	0	0	0
do.[a]	5 da.	75	130	100	0	0	0	0	0	0
do.[a]	4 da.	40	50	75	0	0	0	0	0	0
do.[a]		62	120	92	0	0	0	0	0	0
9-1	14 da.	30	78	58	0	0	0	0	0	0
do.[a]	21 da.	18	64	34	1	2	11	1	0	15
9-3a	45 da.	30	65	50	0	1	2	0	0	3
do.[a]	53 da.	58	90	84	0	0	0	0	0	0
do.[a]	36 da.	10	95	34	1	1	11	1	0	14

[a]Some groups of teliospores were stored more than once under varying conditions. Some storage periods were not followed by test inoculations until September as will be indicated by the figures in the table. (Table IV).

TABLE IV

PRE-GERMINATION TREATMENT OF INDIVIDUAL GROUPS OF TELIOSPORES OF TWO WHEAT LEAF RUST RACES, SEPTEMBER, 1961, THROUGH FEBRUARY, 1962

Spore Group	Attempted inoculations at: 40°F.	50°F.	60°F.	70°F.	80°F.	Total Attempted Inoculations
15A-1a[a]	0	5	10	3	0	18[d]
15A-1b[a]	0	6	10	1	1	18
15A-4a[b]	0	5	10	1	0	16
15A-4c[b]	0	5	8	2	1	16
15A-6	0	0	0	0	0	0
15A-7	0	6	9	1	1	17
15A-8	0	4	9	2	1	16
9-1	0	0	3	8	0	11
9-3a[c]	0	0	5	5	0	10
9-3b[c]	0	0	5	5	0	10

[a] Subdivisions of Group 15A-1 (Table III)

[b] Subdivisions of Group 15A-4 (Table III)

[c] Subdivisions of Group 9-1 (Table III)

[d] Most of the inoculations listed in this table were multiple plant inoculations, rather than single plant inoculations as in Table III.

TABLE V

THALICTRUM SPECIES USED FOR TEST INOCULATIONS

WITH TELIOSPORES OF LEAF RUST RACES 9

AND 15A IN EXPERIMENT 1

Thalictrum species	Numbers of Race 9 Inoculations		Number of Race 15A Inoculations	
	Mar. to Aug. '61	Sep. '61 Feb. '62	Mar. to Aug. '61	Sep. '61 Feb. '62
T. flavum[a]	10	8	20	20
T. speciosissimum[a]	8	17	3	2
T. ssm. illuminator[a]	0	0	5	18
T. dipterocarpum[a]	8	9	4	8
T. dioicum	25	0	9	28
T. minus adiantifolium	11	9	17	0
T. alpinum	9	8	3	1
T. sparsiflorum	2	0	5	10
T. aquilegifolium	0	0	0	4
unidentified spp. (T. dasycarpum?)	0	0	2	16
Totals	73	51	68	107

[a] Indicates species previously known to be highly susceptible to leaf rust.

included distilled water (used in most cases), tap water, and distilled water acidified with .001 molar nitric acid. In a few instances distilled water containing a minute droplet of Tween 20 (a surface tension depressant) was used. In most instances a deVilbiss atomizer or pressure spray gun was used to moisten the leaves of the plant to be inoculated. Occasionally no moisture was applied and germination and subsequent infection was dependent upon natural condensation.

A few chemical treatments were tried in May, 1961. Spore groups 15A-1, 15A-2, 15A-4c, 15A-5, 15A-6, 15A-7, 15A-8, 9-1, and 9-3a were reserved as controls. Only distilled water was used on these cultures. Groups 15A-4b and 9-3b were treated with benzaldehyde, the former group with the pure chemical, and the latter with 10^{-5} dilution. Group 15A-4d was treated with .001 molar nitric acid, and group 15A-4a with 100 mg./liter of gibberellic acid.

Since these chemical tests were so few and so limited in scope, no real evaluation was possible.

Periodic inoculation and inspection for signs of infection on several available species of Thalictrum was used as the main criterion of spore germination. Several of these species have long been known to be congenial alternate hosts to the wheat leaf rust organism and should have shown some sign of infection if there had been any appreciable percentage of teliospore germination. More than 200 test inoculations, however, failed to produce a single recognizable infection.

In addition to the inoculation tests, attempts were made to germinate the teliospores of the various groups on microslides where they could be examined for signs of germination at a 430X magnification. These trials also failed to give any evidence that either race used in this experiment germinated to any appreciable degree in response to any of the treatments used during the entire year of experimentation.

Experiment 2. This was an attempt to duplicate the environmental conditions that might logically occur in nature as the optimum conditions for germination of leaf rust teliospores and the subsequent infection of *Thalictrum*. Leaf rust teliospores are generally assumed to germinate and infect in the spring, so an arbitrarily chosen set of environmental conditions should include: (1) moderate to cool mean temperature, with a fairly large diurnal temperature range, (2) a low temperature near the optimum for teliospore germination, (3) slight air movement - to obtain distribution of the light sporidia, but not enough wind to blow them away; and (4) high humidity, which would be necessary to maintain the moisture on the teliospores and leaves of the *Thalictrum* plants for many hours after the initial wetting.

Only race 9 was used for this experiment since, by this time, telial material available for inoculum was becoming somewhat limited. Additional telia of this race were brought out of refrigerated storage (one year), transferred to filter discs, and then bulked with the cultures of race 9 already used for one year in Experiment 1 to increase the total inoculum.

The inoculations were carried on inside one of the plastic moist chambers previously described. These were situated inside one of the thermostatically-controlled greenhouse rooms. The moist chamber was vented to provide slight air movement and was equipped with a hygrothermograph to provide a continual record of temperature and humidity. The plants and spores were wet down for inoculation with a fog nozzle that delivered a fine mist spray.

The spores were placed in various sites inside the moist chamber--some suspended near the top, with others near ground level. Some were placed on flats of soil, some on peat moss, while one filter disc of teliospores was soaked in the supernatant fluid of ground wheat leaves. Contaminant fungi were allowed to grow at will among the spore cultures. These variations were tried to test possible interaction of outside materials (commonly associated with the teliospore in nature) in conditioning their germination.

The atmospheric conditions were allowed to vary somewhat also as natural conditions are not likely to be rigid. Between inoculations, the temperature was held in the 70-80° F. range, with the relative humidity fluctuating between 40 and 60%. During inoculations (which began at 5 P.M. at about 3-day intervals) the temperature was dropped to the 42-50° F. range, with the relative humidity fluctuating between 70 and 100%. The duration of these inoculations varied from 12 to 48 hours, but usually was about 16 hours.

No infection of <u>Thalictrum</u> was noted during the entire experiment despite the fact that several plants of species known to be susceptible to leaf rust were included.

Experiment 3. Field grown teliospores were used as inoculum in this experiment. Two sources of infected straw were used. One of these was 1961 straw of an unknown variety (or varieties) that overwintered in an outdoor straw pile. The other source was Bison wheat which had served as the spreader variety in a rust nursery at Stillwater, Oklahoma, in 1962. This variety is susceptible to all races of wheat leaf rust to which it has been tested.

The infected wheat straw was placed on 1/2" galvanized wire mesh suspended above the Thalictrum plants to be inoculated inside plastic moist chambers. The chambers were situated outdoors, but in a partially shaded site. The temperature inside the moist chamber was recorded on a thermograph.

The straw and Thalictrum plants to be inoculated were wet thoroughly with a fine mist spray. The experiment was started April 30 and continued through June 21, 1962.

This test was designed to simulate the natural etiology of the rust disease on the alternate host with the single exception of controls placed on the time and duration of the wetting periods of plants and straw.

The first wetting period attempted was successful, for infection was noted well within the time reported to be required to produce infection (7-9 days). However, after the appearance of the first group of pycnial pustules, infection stopped abruptly, and although alternating cycles of wetting and drying were continued for six more weeks, no more pustules were observed.

As in Experiment 2, the plants and straw were wet at 5 P.M. Both usually would be damp the following morning, but would dry early in the day, and moisture would be applied again in early evening. The dates of inoculation, the maximum, minimum, and mean temperature for each day, and an hourly record of temperature on the three days when the successful inoculations occurred are recorded in Tables VI and VII. An abrupt rise in daily mean temperature is apparent immediately following the first three days of inoculations.

Thalictrum species showing infection in this experiment were: T. speciosissimum illuminator (3 plants), T. speciosissimum (1 plant), and T. flavum (1 plant). In the same experiment single plants of the following species were not infected: T. dioicum, T. speciosissimum illuminator, T. dipterocarpum, T. alpinum, T. sparsiflorum, and an unidentified American species. Also, two additional plants of T. flavum did not become infected.

Only 29 pycnial pustules were observed, and since several of supposedly susceptible species escaped infection, this was not considered an adequate test to determine the reaction of the three American species used (T. dioicum, T. alpinum, and the unidentified species) to wheat leaf rust.

The results described above were obtained with the overwintered (1961) source of inoculum. A similar test using the 1962 Bison as a source of inoculum was set up during the last two weeks of this particular study. In this case, no infection was noted on the four plants of supposedly susceptible Thalictrum species which were used. However, successful infection was obtained with this same inoculum in a later study and the wetting and drying cycles during this period may have had some influence on subsequent teliospore germination.

TABLE VI

DAILY TEMPERATURE RECORD DURING THE INOCULATION

PERIOD, MAY 1 THROUGH JUNE 21, 1962

Date		Temperature				Date		Temperature			
Month	Day	High	Low	Mean[a]	Range in °F.	Month	Day	High	Low	Mean	Range in °F.
May	1[a]	92	58	75	34	May	27	104	75	90	29
	2[a]	90	54	72	36		28[b]	90	79	85	11
	3[a]	94	64	79	30		29	100	70	85	30
	4	98	63	81	35		30	105	70	88	35
	5	101	70	86	31		31[b]	100	76	88	24
	6	102	71	87	31	June	1	94	74	84	20
	7	102	69	86	33		2	85	69	77	16
	8	101	70	86	31		3	82	70	76	12
	9[b]	105	72	89	33		4	97	66	82	31
	10	112	77	95	35		5	102	76	89	26
	11[b]	107	78	93	29		6	103	74	89	29
	12	110	78	94	32		7	93	74	84	19
	13	111	75	93	36		8	88	73	81	15
	14	106	73	90	33		9[b]	88	70	79	18
	15	99	73	86	26		10	93	74	84	19
	16	98	76	87	22		11	98	75	90	29
	17	104	74	89	30		12[b]	104	75	90	29
	18[b]	100	74	87	26		13	94	70	82	24
	19	106	74	90	32		14	95	68	82	27
	20	101	77	89	24		15	94	73	84	21
	21[b]	104	76	90	28		16	98	74	81	24
	22	105	74	90	31		17	102	78	90	24
	23	109	77	93	32		18	99	69	84	30
	24	106	76	91	30		19[b]	101	75	88	26
	25	111	79	95	32		20	102	75	89	27
	26	107	75	91	32		21[b]	100	76	88	24

[a] Indicates dates of successful inoculation.

[b] Indicates dates of unsuccessful inoculations

[c] All mean temperatures computed from high and low readings rather than the average of 24 hourly readings.

TABLE VII

HOURLY TEMPERATURE RECORD FOR THE

INOCULATION PERIOD MAY 1 THROUGH MAY 3, 1962

Time	May 1	May 2	May 3	Mean Temperature[b]
1 A.M.	60	58	65	May 1 - 71.4
2 A.M.	59	58	65	May 2 - 72.5
3 A.M.	59	57	65	May 3 - 77.6
4 A.M.	59	56	65	
5 A.M.	58	54	65	
6 A.M.	59	55	66	
7 A.M.	60	60	68	
8 A.M.	70	80	80	
9 A.M.	87	88	86	
10 A.M.	89	80	85	
11 A.M.	83	85	87	
12 A.M.	87	90	94	
1 P.M.	92	89	92	
2 P.M.	84	89	93	
3 P.M.	82	87	90	
4 P.M.	80	91	92	
5 P.M.[a]	84	84	95	
6 P.M.	86	80	82	
7 P.M.	75	74	78	
8 P.M.	67	70	74	
9 P.M.	63	67	72	
10 P.M.	59	66	70	
11 P.M.	59	65	69	
12 P.M.	62	58	64	

[a] Indicates hour of inoculation each day.

[b] Mean temperatures represent the average of the 24 hourly readings and are slightly different from those listed for the same dates in Table VI.

Experiment 4. This group of tests was performed in one of the plastic-lined, temperature-controlled, greenhouse rooms previously described.

Several different groups of Thalictrum plants were inoculated with rust simultaneously, each group in a separate moist chamber.

The source of inoculum was the same as that used in Experiment 3. In addition, two new collections of 1962 straw were used. One was from the variety Mediterranean which is resistant to race 9, but susceptible to the other races common in this area, and the other was from an unnamed selection (62-5) which is resistant to certain races and susceptible to others.

Temperature recordings and inoculations were made in the same manner as the previous experiment and a continuous temperature record was kept (Table VIII). The experiment started July 9 and was continued through September 3, 1962.

The group of tests composing Experiment 4 was designed primarily to verify the apparent correlation between the rise in daily mean temperature and the abrupt cessation of infection experienced in the previous experiment. Therefore the temperatures were carefully controlled during this series of tests.

The daily temperature during this group of tests did vary to some extent, due primarily to high outdoor temperatures. However, the daily mean temperature reached the high 70's on only a few occasions (by fortunate chance always between inoculations), and ranged as low as 67^{o}F. Daily minimum temperatures ranged from 57-70^{o}F. and the maximum

TABLE VIII

RECORD OF DAILY TEMPERATURES DURING THE INOCULATION PERIOD JULY 9 THROUGH SEPTEMBER 3, 1962

Month	Day	Temperature Record 12MN	6AM	12NN	6PM	Mean	Low	High	Diurnal Range °F
July	9[a]	63	-	95	75	-	-	-	-
	10	63	62	84	78	72	62	86	24
	11	65	64	86	70	71	64	87	23
	12	65	63	83	77	72	63	89	26
	13	64	63	87	73	72	63	88	25
	14[a]	60	60	82	73	69	60	84	24
	15	63	62	65	70	65	61	71	10
	16[a]	71	72	87	70	75	70	87	17
	17	64	64	80	70	70	64	82	18
	18	65	65	87	80	74	65	87	22
	19[a]	65	64	89	78	74	64	91	27
	20	64	63	72	65	66	63	73	10
	21	63	64	92	75	73	63	93	30
	22	64	64	95	79	75	63	96	33
	23	65	64	68	66	66	63	73	10
	24[a]	63	63	76	67	67	63	77	14
	25	62	64	79	67	68	62	79	17
	26	64	66	82	66	70	64	83	19
	27[a]	64	67	72	68	68	64	76	12
	28	61	61	76	75	68	61	77	16
July	29	to	July	31		73[b]	68	81	13
August	1[a]	73	70	77	76	74	70	82	12
	2[a]	74	73	89	64	75	61	91	30
	3	61	59	85	75	70	59	87	28
	4	63	60	94	77	73	60	95	35
	5	62	59	87	78	72	59	90	31
	6[a]	64	59	88	78	72	59	92	33
	7	64	56	88	67	69	55	90	35
	8[a]	60	60	84	80	71	60	87	27
	9[a]	65	59	87	83	73	58	90	32
	10	64	59	88	78	72	58	92	34
	11	62	58	89	77	71	57	90	33
Aug.	12 and Aug. 13					75[b]	64	94	30

Table VIII (Continued)

Month	Day	Temperature Record 12MN	6AM	12NN	6PM	Mean	Low	High	Diurnal Range °F
Aug.	14[a]	65	65	92	72	73	64	92	28
	15	62	62	79	72	68	61	79	18
	16	72	74	90	70	77	70	91	21
	17	68	70	85	68	73	66	86	20
	18	68	72	87	69	74	67	88	21
	19	68	74	90	70	75	67	90	23
	20					75[b]	67	91	24
	21					76[b]	68	92	24
	22	69	69	89	77	78	68	92	24
	23	69	68	89	80	79	68	92	24
	24[a]	62	61	69	75	67	60	85	25
	25	69	67	97	88	80	67	101	34
	26	74	68	99	81	81	67	104	37
	27	75	69	100	85	82	68	104	36
	28	69	68	87	70	73	67	90	23
	29	63	64	87	72	71	63	90	27
	30	64	64	91	76	74	62	92	30
	31	64	64	65	65	65	63	72	9
Sept.	1	70	69	80	72	70	69	82	13
	2	71	71	84	63	72	63	86	23
	3	71	71	77	-	-	70	80	10

[a] Indicates dates of inoculation.

[b] Mean temperature on these dates was estimated from incomplete data, i.e., the high and low temperatures only.

temperatures ranged from 71-104°F. If the mean temperature for the day was calculated using the hourly temperature record, then the mean was always lower than the average of the maximum and minimum temperatures. This was due to the fact that the temperature could be held to within ±2°F. of the established temperature for 12 to 15 hours and the maximum temperature was of rather short duration during midafternoon. Consequently, an average of four readings made at 6 and 12 A.M. and P.M. was thought to be a more accurate representation of the actual mean temperature.

The overwintered 1961 straw, which had ceased to produce infection for over five weeks in the previous experiment, responded promptly with successful infection. The first observed infection was on July 19, 10 days after the first inoculation was made. The test with this source of inoculum was concluded on July 31 after a total of 26 infections had been observed (Table IX).

The tests with the 1962 Bison straw as a source of inoculum were, by far, the most successful. The first infections appeared July 18 and by July 24 five of the Thalictrum plants were extremely heavily infected with pycnia. These plants were moved to separate isolation chambers to insure that the developing aeciospores would not escape. The pycnial infections had been promiscuously fertilized by mixing the exudate at random with a camel's hair brush. Since the race population of the inoculum source was unknown, planned crosses or selfs could not be made.

TABLE IX

RECORD OF THALICTRUM PLANTS INOCULATED WITH TELIOSPORES GROWN ON OVERWINTERED 1961 WHEAT STRAW JULY 9 TO JULY 31, 1962

Plants Tested	Duration of Test	Times Inoculated	Number of Pycnia	Number of Aecia
Thalictrum flavum #2	22 da.	6	10	0[a]
T. flavum #3	22 da.	6	0	0[a]
T. speciosissimum #2	22 da.	6	4	0[a]
T. speciosissimum illum. #2	22 da.	6	12	2[a] [b]
T. aquilegifolium #1	22 da.	6	0	0
T. minus adiantifolium	22 da.	6	0	0
T. dioicum #3	22 da.	6	0	0
T. dioicum #4	22 da.	6	0	0

[a] Promisucous mixing of pycniospores.

[b] One aecium was formed following fertilization from a single pycnia source.

The infections on the plants of supposedly susceptible species of Thalictrum were sufficient (as high as 50 per leaflet) to indicate that Bison straw inoculum would provide a valid test of the susceptibility for a number of American species. At this concentration of available sporidia, it was reasonably certain that there would be no escapes.

The plants of Thalictrum tested with the 1962 Bison source of inoculum and the infections observed on them are listed in Table X. A wide variation in type of infection was observed on plants of moderate susceptibility, which possibly indicated differential response to certain of the sporidial biotypes presented to them. Infection types observed were: (a) necrotic fleck, (b) non-functional pycnial swelling, (c) pycnial infection briefly functional, but discolored, soon senescent, and ringed by necrotic tissue; and (d) brightly colored infections with long duration of production of functional pycnia. These were not sharply defined classes, but rather arbitrary categorization of a continuous intergradation. Plants with at least one functional pynial pustule were considered susceptible and it will be noted that American species varied widely in susceptibility to the races present in this inoculum. Ultimately 15 of 48 plants tested showed at least some degree of susceptibility. In addition, 7 other plants produced flecking suggestive of resistance, but only in cases where the fleck reactions were accompanied by at least one functional pycnial infection were the plants considered susceptible.

The fleck infections could not be definitely confirmed as true rust infections. However, the manner in which they developed and their appearance on the young leaflets 5 to 7 days following inoculation indicated their origin as rust infections. They first appeared as small, slightly swollen chlorotic spots about 1/3 mm. in diameter. Often the chlorotic spots gradually turned necrotic and a small black spot appeared in the center. These flecks usually occurred on the youngest leaves of the plants so affected. In all cases, older leaves were observed to be more resistant to any type of rust infection than younger leaves.

These fleck type reactions were of interest, however, since on some of the American species there would be one to several functional pycnial infections along with a number of the resistant fleck type of infections. This phenomenon indicated two things: (1) that the inoculum source contained more than one race, or at least more than one source of virulence, and (2) the host plant had a differential reaction to the different strains or types of the parasite that were present.

Aecia were rare on the American species appearing on only one plant of _T. dasycarpum_ from Colorado, but even on the susceptible Eurasian species as many as three-fourths of the pycnial infections commonly failed to produced aecia.

Also, it was interesting to note the occurrence of a floral infection on a plant of an unidentified species from Wyoming (II-B-2, Table X). This was a large, highly functional pycnial infection on a plant where the leaves had been quite resistant.

TABLE X

REACTION OF THALICTRUM PLANTS MASS INOCULATED WITH TELIOSPORES ON FIELD INFECTED STRAW OF BISON WHEAT

I. Identified American Species

Plant Source	Length of test (days)	Times inoc.	fleck	Infection Recorded pycnia non-func.[a]	func.[b]	aecia	Total
	A. *T. alpinum*						
1. Grant, Colo.	41	9	-	-	19	-	19
2. Grant, Colo.	11	1	-	-	-	-	-
3. Grant, Colo.	11	1	-	-	-	-	-
	B. *T. dasycarpum* (male)						
1. Lyons, Colo.	13	6	153	7	165	5	325
2. Laramie, Wyo.	41	9	15?	-	1	-	16?
3. Lyons, Colo.	20	2	-	-	1	-	1
4. Ft. Collins, Colo.	41	9	-	-	-	-	-
5. Lyons, Colo.	11	1	-	-	-	-	-
	C. *T. dasycarpum* (female)						
1. Ft. Collins, Colo.	20	2	-	-	1	-	1
2. Custer, S. Dakota	27	8	-	-	-	-	-
3. Ft. Collins, Colo.	41	9	19?	1?	-	-	20?
	D. *T. dasycarpum* (sex undetermined)						
1. Ft. Collins, Colo.	21	7	-	-	-	-	-
2. Ft. Collins, Colo.	20	7	-	-	-	-	-
	E. *T. dioicum* (sex not specified)						
1. Jackson, Wyo.	20	7	-	-	-	-	-
2. Wisconsin	26	5	-	?	25	-	25?

II. Miscellaneous American Species

Plant Source	Length of test (days)	Times inoc.	fleck	non-func.	func.	aecia	Total
	A. Species "X" (female)						
1. La Veta, Colo.	27	8	25?	-	-	-	25?
2. Cimarron, N. Mex.	20	7	-	-	-	-	-

Table X (Continued)

Plant Source	Length of test (days)	Times inoc.	fleck	Infection Recorded pycnia non-func.	func.	aecia	Total
3. La Veta, Colo.	41	9	20?	-	-	-	20?
4. Westcliffe, Colo.	41	9	-	-	-	-	-
5. Wetmore, Colo.	41	9	-	-	-	-	-
6. Cimarron, N. Mex.	20	6	-	-	-	-	-
	B. Species "Y" (male)						
1. Steamboat Sprs., Colo.	41	9	?	-	-	-	?
2. Bighorn Mts., Wyo.	27	8	50?	-	1	-	51?
	C. Species "Z" (male)						
1. Westcliffe, Colo.	27	8	?	-	-	-	?
2. Cimarron, N. Mex.	27	8	?	-	3	-	3?
	D. <u>T. dasycarpum hypoglaucum</u>						
1. Stillwater, Okla.	43	12	?	-	-	-	?
2. Stillwater, Okla.	41	9	125	-	2	-	127?
	E. Misc. Unrelated Spp.						
1. Steamboat Sprs., Colo.	27	8	150?	-	-	-	150?
2. Westcliffe, Colo.	41	9	-	-	-	-	-
3. Wetmore, Colo.	41	9	-	-	-	-	-
4. Encampment, Colo.	41	9	4	1	1	-	6
5. Cimarron, N. Mex.	11	1	-	-	-	-	-
6. Cimarron, N. Mex.	41	9	50?	2	11	-	63?

III. Eurasian Species

Species	Source	Length of test (days)	fleck	Infection[c] Recorded pycnia	aecia	Total
1. speciosissimum (4)[d]	Europe	15	-	50+	20+	50+
2. speciosissimum illuminator (2)	Europe	15	-	50+	20+	50+
3. sparsiflorum (1)	Europe	15	8	40	-	48
4. flavum (1)	Europe	15	-	50+	20+	50+

Table X (Continued)

Species	Source	Length of test (days)	fleck	Infection[c] Recorded pycnia	aecia	Total
5. dipterocarpum (1)	Europe	15	4	30	-	34
6. alpinum (2)	Europe	43	-	-	-	-
7. aquilegifolium (1)	Eurasia	20	-	-	-	-

[a] Non-functional, but morphologically distinguishable.

[b] Functional, i.e., having at least one pycnium to exude pycniospores.

[c] Infections on highly susceptible European species were not counted, since as high as 50 per leaflet were observed.

[d] Numbers in parentheses indicate number of plants tested with uniform reaction.

Abundant infection was produced on wheat from the aeciospores taken from the exotic Thalictrum plants. In the evaluation of the role of the alternate host on the variability of the wheat leaf rust fungus in the United States, it was interesting to note that spores from the aecia produced on the T. dasycarpum plant from Colorado (I-B-1) were effective in producing uredial infections on Little Club wheat. Analysis of the races or biotypes derived from these cultures should prove quite interesting, and will be the subject of further intensive study.

Tests with the 1962 Mediterranean straw as an inoculum source also produced infection on the Eurasian species following the first and second inoculations. The first pycnia appeared about July 20, and since these first infections were rather sparse the test was terminated on July 31. Infections produced on the various species of Thalictrum used in this test are listed in Table XI. The only American species included, T. dioicum, was not infected. Aecia were formed on the Eurasian species and spores from these aecia produced uredial infections on wheat. The final test, using the straw of the unnamed spring wheat selection 62-5 as an inoculum source, produced no infection whatever on the 3 Eurasian species used.

TABLE XI

REACTION OF *THALICTRUM* PLANTS INOCULATED WITH TELIOSPORES FORMED ON FIELD INFECTED STRAW OF MEDITERRANEAN WHEAT IN 1962.

Thalictrum species	Duration of test	Times Inoculated	Number of Pycnia	Number of Aecia
Thalictrum dioicum #1	22 da.	6	0	0
T. speciosissimum #1	22 da.	6	135	65[a]
T. sparsiflorum #1	22 da.	6	8	0[a]
T. dipterocarpum #1	22 da.	6	6	1[b]
T. minus adiantifolium #1	22 da.	6	9	0[c]
T. speciosissimum illuminator	15 da.	4	2	1[b]
T. flavum #1	15 da.	4	2	0[b]
T. dioicum #2	7 da.	8	0	0

[a] Promiscuous mixing of pycniospores.

[b] Natural crossing only.

[c] Fertilization from a single source.

DISCUSSION

Teliospore germination in this study was measured in terms of actual infection of the Thalictrum host, which would involve not only teliospore germination *per se*, but the germination of the sporidia produced and the penetration of the host leaf by the germinating sporidia. Each of these various steps may require a specific chain of conditions.

Exhaustive attempts to induce such infection using two specific pure greenhouse-grown cultures of wheat leaf rust teliospores failed completely, while three out of four field collections of telia produced infection with relative ease when placed under the proper conditions. These "proper conditions" apparently encompass a fairly broad range of environmental conditions, although in these tests when the daily mean temperature rose above 75° F. successful infection of Thalictrum ceased rather abruptly.

Other investigators also report varying degrees of ease or difficulty in germinating various teliospore collections. The several investigators have their own "rules of thumb" to insure a greater percentage of successful germination, but there seems to be no way of predicting whether or not a particular culture will germinate readily.

Samborski states[3] that the germinability of wheat leaf rust teliospores is "influenced by inherent factors of the culture and by environmental conditions

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[3] Personal communication.

present during teliospore formation." If this is true, and the weight of evidence seems to support it, then the task of finding the determining factors involved in teliospore dormancy indeed will be difficult, for the "inherent" (i.e. germinal, ultimately expressed as physiological) differences alone represent a large, as yet undetermined, variation. Add to this an interaction of inherent factors with the environmental variations possible, such as varied nutrition (host variety), variations in light quality, intensity, and duration, and temperature and humidity changes, and the problem becomes massive in scope.

Teliospore germination difficulties have been the greatest single hindrance to the study of the genetics in this rust organism and a solution to the problems involved would be welcomed by all investigators in the field.

In spite of the difficulties, the efforts of Zimmer and Schafer (21) seem to make some headway on this same problem with crown rust, and their experimental design would apply equally well to other rusts.

The experiments just concluded indicate that optimum temperature conditions for the germination of leaf rust teliospores followed by successful sporidial infection of the genus Thalictrum are met when the diurnal temperatures range from a 60-65°F. low to an 85-95°F high. Two lines of evidence can be presented to support this contention: (1) When the diurnal cycle was held in this approximate range by means of thermostatic controls, extremely heavy infections were obtained on susceptible host plants of Thalictrum. (2) Nineteen separate attempts to inoculate susceptible test species were made with a collection of overwintered straw during the period of May 1 to July 31. During every one of the nine inoculation periods which resulted in successful infection the overnight low temperature recorded the following morning was 64°F. or below. The unsuccessful attempts at infection were all made during temperature cycles in which the overnight low was 70°F. or above. This evidence strongly indicates that an extended period of time at or below 70°F. is necessary for a successful infection cycle.

Moderate fluctuation in temperature serves several useful purposes. A continual drop in temperature overnight helps to maintain humidity at a maximum. A daily afternoon rise in temperature clears away excess humidity after each inoculation period is ended. Such wetting and drying cycles probably condition more teliospores to germinate in the next wetting period.

Prolonged periods of high humidity often render Thalictrum plants susceptible to leaf blights. Since unhealthy leaves rarely become infected, regular drying cycles help maintain the host plants in acceptable condition for testing.

The present study has not changed the basic concept that American species of Thalictrum are largely resistant to wheat leaf rust populations. It has presented considerable evidence to show that there are certain races that will attack many of them, and that a few appear to be susceptible to the major segment of leaf rust populations in Oklahoma. Not all of the differences between separate species of Thalictrum can be brought out in figures. Table X does reveal that resistant type infections seldom, if ever, appear on the highly susceptible European species: Thalictrum flavum, T. speciosissimum, and T. speciosissimum illuminator. On the other hand, it does not give any indication of size, longevity, vigor, color, or morphology of the pycnial infections, and these seem to be important criteria to be used in judging relative susceptibility. For example, an isolated infection on one of the three species mentioned above will be greatly swollen, orange-red in color, and may grow to be 1 centimeter or more in breadth. It may eventually produce 100 or more functional pycnia and may continue to function vigorously for several weeks.

American species rarely supported infections over 3 mm. in diameter; they often were less richly colored and became senescent sooner. On Thalictrum dioicum necrosis rapidly develops around the growing infection to restrict its size and shorten its life. Necrosis also developed around infections on T. dasycarpum, but it was not so rapid and damaging to the vigor of the infection as in the case of T. dioicum.

Since it was not so designated by the supplier, the origin of the T. sparisiflorum used in these tests is not known. Certain forms of this species, according to Boivin (2), are native both to Eastern Siberia and Western North America. Its identification will be of interest since it was the only possible native American species that supported infections that approached those on the highly susceptible species in longevity and color (but not in size). For this reason it was regarded as the most susceptible of the 'native' species, and equal in susceptibility to T. dipterocarpum to races occurring in this test.

Thalictrum alpinum, a native species of dwarf plant type (plant I-A-1 of Table X) appeared to be moderately susceptible, perhaps equal to T. dioicum.

The idea that species of the alternate host may not respond uniformly to various races, being susceptible to some and resistant to others, is not new. Mains (16) had suspected something of this nature thirty years ago.

Mention was made in the last experiment of the "black dot fleck" type of reaction that was noted on many American plants of Thalictrum when subjected to leaf rust sporidial inoculation. This type of reaction was interpreted to be parallel to the necrotic fleck (0;) type of reaction that the dicaryon stage of the organism produces on many varieties of wheat.

The plant designated as I-B-1 in Table X produced an extremely interesting pattern of infection. More than 100 pycnial infections were present, and these ranged from large and massive to extremely small and non-functional. These small, non-functional, pycnial infections seemed to intergrade with numerous black-spot fleck reactions. This pattern of infection is shown in Figure 1. This continuous intergradation of infection type--which paralleled the X type reaction of the dicaryophase on wheat--seemed to support the interpretation of the black-spot flecks as incipient infections of a race of the haplophase to which the Thalictrum plants were resistant.

Several times a pycnial infection that was as vigorous as those found on the most susceptible Eurasian varieties occurred upon an American species. A prime example of this was the floral infection on plant II-B-2 (see figure 2) that produced many pycnia which were functional for several days. In this

Figure 1. Fully matured pycnial infections on a native Thalictrum plant. The leaflet at the upper right shows some of the types of reaction (from necrotic flecks to the pycnial clusters) produced on a plant of T. dasycarpum by mass sporidial inoculation.

Figure 2. Vigorous pycnial infection found on a pistil of an unidentified species of Thalictrum (I-F-2, Table X). The leaf tissues of this plant were highly resistant to infection.

case, a chance sporidium either found a highly susceptible tissue on an otherwise resistant plant or perhaps carried a virulence that the other sporidia present did not contain.

These tests have shown American species of Thalictrum to be somewhat more susceptible to leaf rust than previous investigators have reported. Is there, then, reason to suspect that American leaf rust populations are now different from the European populations, from which they have descended, with respect to alternate host range? An analysis of Table I shows the effects of geographic isolation upon alternate host relationships. Thalictrum dipterocarpum, Isopyrum fumarioides, and others show widely different reactions to isolated populations of rust, and may demonstrate cases of local specialization. Local specialization, if it truly occurs, signifies a dynamic population.

What is to be expected when vigorous, single infections of Puccinia recondita f. tritici appear in nature on the species of Thalictrum normally infected by other leaf rust forms (See Mains) whose uredial hosts are native grasses such as species of Elymus, Hordeum, Agropyron, and Bromus? Will natural hybrids occur? Shifman (18) reports success in hybridizing two of these forms with form tritici, but apparently American workers have neglected research along these lines. However, the fact that wheat breeders are incorporating sources of rust resistance derived from grasses makes this an all the more interesting and vital topic for future research.

SUMMARY

1. Exhaustive efforts failed to produce any detectable germination in teliospores of two races of wheat leaf rust grown in pure culture in the greenhouse. Separate lots of the spores subjected to many variations of treatments did not respond by germinating.

2. Teliospore germination was obtained in both overwintered and freshly cut straw grown in the field. The latter bore spores that germinated profusely enough to test a number of American species of *Thalictrum* for relative susceptibility to leaf rust.

3. American species of *Thalictrum* were found to vary widely in response to inoculation with leaf rust sporidia. Nearly half of the plants produced one or more functional pycnia. The phenomenon of a large, highly vigorous infection occurring on floral tissue of an otherwise highly resistant plant was observed.

4. These tests indicate that *Thalictrum dasycarpum* has a higher degree of susceptibility than *T. dioicum*.

5. *Thalictrum sparsiflorum* was found to be the native species most susceptible to races of leaf rust used in this test. *Thalictrum alpinum*, a native species of dwarf plant type, showed moderate susceptibility.

6. A wide variation in type of infection was observed on plants of moderate susceptibility, which possibly indicated differential response to certain of the

sporidial biotypes presented to them. Infection types observed were: (a) necrotic fleck, (b) non-functional pycnial swelling, (c) pycnial infections briefly functional, but discolored, soon senescent, and ringed by necrotic tissue, and (d) brightly colored infections with long duration of production of functional pycnia. These were not sharply defined classes, but rather an arbitrary categorization of a continuous intergradation.

7. Several hours at or below 70° F. seems to be necessary for successful inoculation of Thalictrum by Oklahoma leaf rust teliospores.

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