THE UNIVERSITY OF MANITOBA

A STUDY OF THE INHERITANCE OF RESISTANCE TO ERGOT

(CLAVICEPS PURPUREA) IN TWO WHEATS:

TRITICUM DURUM DESF. CV. CARLETON AND T. TIMOPHEEVI ZHUK.

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by

KENNETH WALKER RILEY

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ABSTRACT

An F_1 monosomic analysis was carried out on the A and B genome

monosomics of Chinese Spring crossed with <u>Triticum timopheevi</u>,

(Manitoba accession number 4B289) and <u>T</u>. <u>durum</u> desf. cv. Carleton,

in an attempt to study the inheritance of the resistance of these

two wheats to ergot, <u>Claviceps purpurea</u>.

Chromosomes 1B and 3B of Carleton were found to condition re-

sistance to ergot. A small part of the resistance of <u>T</u>. <u>timopheevi</u>

was found to be conditioned by chromosome 3B.

A major part of the <u>T</u>. <u>timopheevi</u> resistance was expressed in

crosses with other tetraploids. It was thought that the suppression

of the <u>T</u>. <u>timopheevi</u> resistance may be conditioned by the D genome

of Chinese Spring.

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1.0 INTRODUCTION

Ergot, a disease caused by <u>Claviceps purpurea</u> (Fr) Tul, has been
found on most native grasses and on all cereal crops in Canada
(Seaman 1971). Seeds of the infected hosts are replaced by hard black
bodies called sclerotia. Although ergot is best known as a disease of
rye (<u>Secale cereale</u>), it also causes considerable economic loss in
durum wheat, <u>Triticum durum</u> and spring wheat, <u>Triticum aestivum</u>.

The sclerotia of Claviceps contain toxic alkaloids which have
caused epidemics throughout history. Rapid consumption of grain in-
fected with ergot causes convulsion of smooth muscles which may result
in paralysis and death. Slow continued consumption of these alkaloids
causes constriction of blood vessels, resulting in gangrene. The
epidemics were known in the middle ages as St. Anthony's fire and have
caused thousands of deaths (Barger 1931). In animals, similar symptoms
may occur which have been confused with hoof and mouth disease
(Kingsbury 1964). Continuous feeding of grain with 0.07% ergot con-
tamination has increased abortion rates and reduced growth rates in
pigs and rats (Ingliss and Phillips 1971, Campbell and Burfening 1972).

Ergot in wheat has been a recurrent problem in Western Canada.
Conners (1953) found ergot in 7.0% of wheat fields surveyed in Manitoba,
10.4% in Saskatchewan and 13.3% in Alberta. Current data on carlots of
wheat moving to terminal ports which were downgraded or rejected on
account of ergot were kindly supplied by Mr. S. Safneck of the Inspection

Division of the Winnipeg Grain Exchange. During the 22 month period

from March 1970 to December 1971, a total of 124 carlots of wheat were

rejected (Figure 1). However in the 11 month period from February 1,

1972 to January 1, 1973, 443 carlots of wheat were downgraded (Figure 2).

(A carlot is downgraded if a 500 gram sample contains more than 7 pieces

of ergot sclerotia. Rejection occurs if the sample is more than 0.25%

ergot.) Durum wheat was more heavily infected than red spring wheat,

with 285 and 158 carlots downgraded respectively. Heaviest losses oc-

curred in Saskatchewan and in southwestern Manitoba.

Japan has recently announced that it will no longer accept imports

of wheat containing more than .04% ergot. Countries in Europe have

announced a similar policy (Anonymous 1972). Therefore wheat exports

to these countries in the future will have to be virtually free of ergot.

Ergot is also a problem in the development of triticale and in male

sterile wheat lines which are being developed in the hybrid wheat pro-

gram at the University of Manitoba.

Platford and Bernier (1970) and Ratanopas (1973) have shown that

some wheat cultivars and wheat relatives possess resistance to ergot.

Two of the most resistant strains identified are the durum cultivar

Carleton and a strain of _Triticum timopheevi_ designated as 4B289.

The objectives of this study were to confirm the chromosomal loca-

tion of the resistance of Carleton and to determine the mode of inherit-

ance and the location of the resistance of _Triticum timopheevi_. The

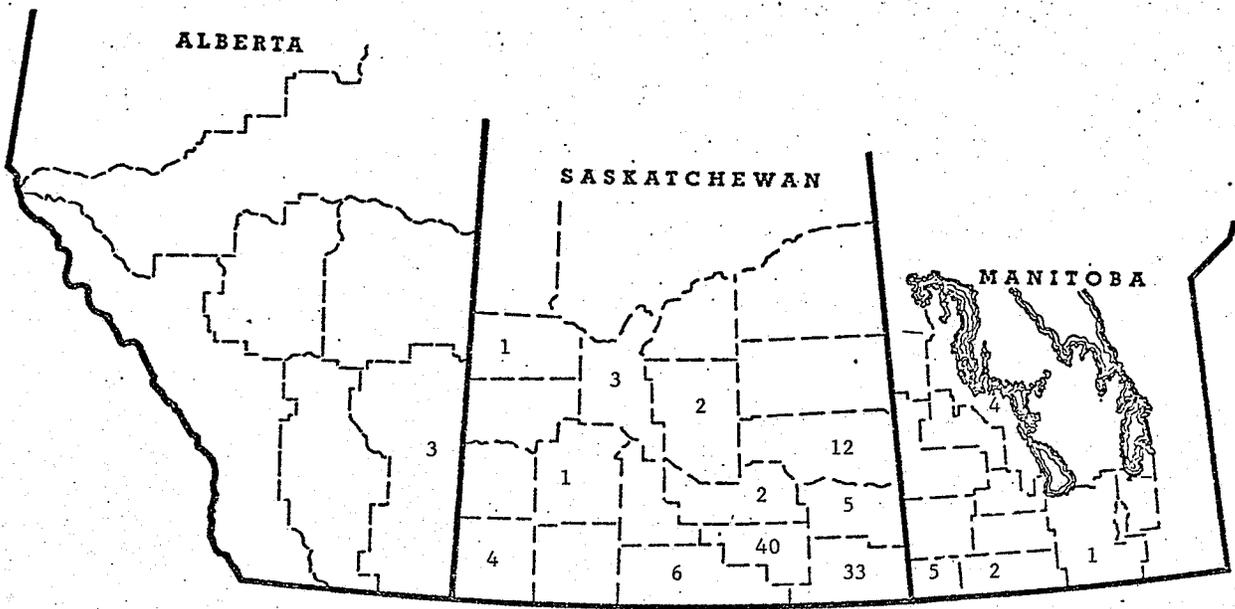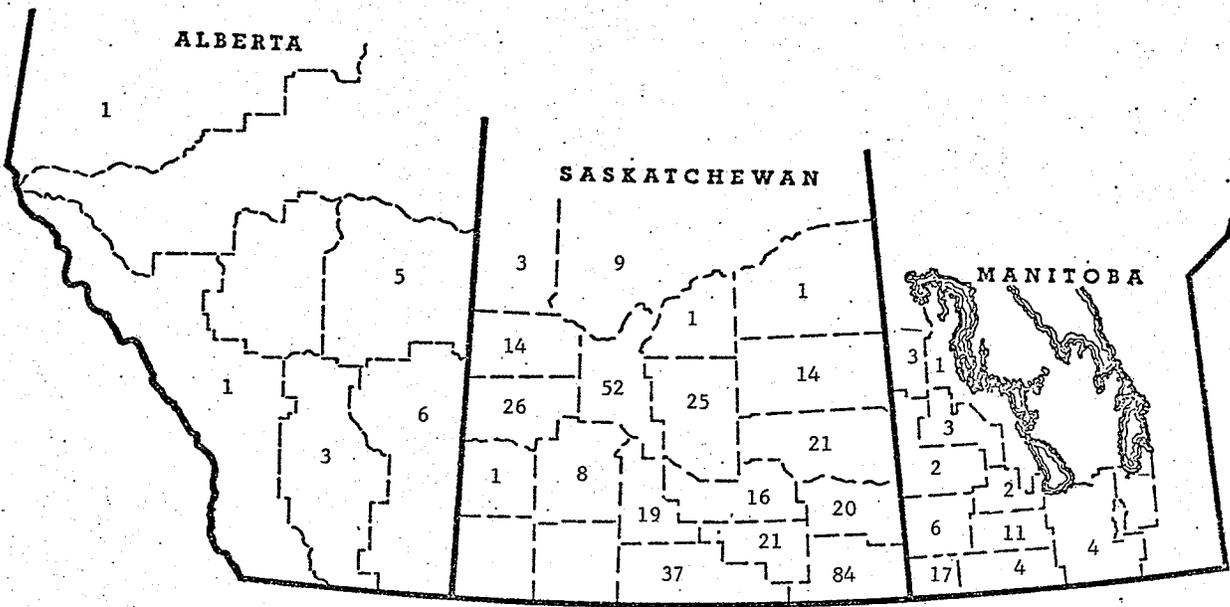Figure 1. Carlots per Crop District of Durum and Red Spring Wheat Rejected on Account of Ergot, March 1970 to December 1971.

Figure 2. Carlots per Crop District of Durum and Red Spring Wheat Downgraded on Account of Ergot, Feb. 1 1972 to Jan. 1 1973.

fact that $\underline{T}$. $\underline{timopheevi}$ has shown considerable resistance to numerous wheat pathogens prompted a comprehensive literature review of this species.

2.0   LITERATURE REVIEW

2.1   The Nature of the Pathogen

The life cycle of Claviceps purpurea has been reviewed by Dickson (1956), Heald (1933), Barger (1931) and Bove (1970). The ergot sclerotia either fall to the ground, or are planted with the grain in the spring. After a period of moist cool conditions, one to several stromata are produced. A stroma is composed of a stipe one-half to one inch long which bears a perithecium. The mature perithecium contains many long, narrow asci. Each ascus contains 8 needle-shaped ascospores which are forcibly ejected and carried by water or air currents to a floret of a susceptible host. The spore germinates in the moisture in the floral cavity within 24 hours, and penetrates the ovary wall near the base of the ovule (Kirchhoff 1929). Campbell (1958) found that the fungus grows intercellularly for two or three days, around the outer integuments of the ovary. In 4-5 days the fungus begins to grow intra-cellularly. It penetrates the barrier of integuments surrounding the ovule, and begins to produce conidia and exude honeydew on the surface of the ovary. Gravity and insects spread the honeydew and conidiospores to other florets. A compacted mass of large hyphae form an absorbing structure in the vascular bundles of the rachilla (Dickson 1956). As the hyphal mass advances through the ovary, it differentiates into thickened, septate hyphal filaments, which become more compact and push outwards. The production of honeydew and conidia stop. The superficial

hyphae darken and form a cortex, completing the formation of a sclerotium (Tulasne 1853, as reported in Heald, Dickson and Bove).

2.2 Host-Parasite Relationships

Claviceps purpurea is capable of parasitizing many species and genera of the Gramineae including common forage and cereal crops. However there are conflicting reports concerning host specificity. Stager (1923) believed that there were several species of Claviceps, and classified C. purpurea into three races. One race, designated P_1, was capable of infecting the cultivated cereals. Sprague (1950) suggested that seven races of C. purpurea had been found but felt that a complicated maze of races and subraces within C. purpurea probably existed.

Campbell (1957) found no indication that races of Claviceps purpurea existed that were host specific, as he was able to infect rye, wheat, and barley with all except one of 421 isolates from wild grasses. However variability in cultural colonies indicated that races in the cultural sense do exist. Campbell found that the technique used in inoculation is important, and suggested that Stager may have reported some species as being immune if he failed to observe infection.

Ratanopas (1973) found host-parasite interaction between different Claviceps isolates and different wheat varieties, which indicated that vertical resistance existed and therefore different genes for resistance were likely to exist in different wheat hosts.

2.3 Nature of Resistance to Ergot

Graminaceous hosts with tight florets, or with florets that remain open for a short time can provide resistance to infection by excluding spore entry. Abe and Kono (1957) found rye to be more susceptible than other cereals because the florets remain open longer. Campbell (1957) suggested that the morphology and development rhythm of the plant, i.e. if flowering occurs when the head is still in the boot, are of utmost importance in providing resistance to infection.

The effect that fertilization of the ovary has on conditioning resistance has been noted by several workers. In France, Rapilly (1968) noted that non-fertilized ovaries of wheat are highly susceptible. Futrell and Webster (1965) found that ergot was more likely to affect sterile heads of sorghum. They believed that the ovary becomes resistant when fertilized, and that unfertilized ovaries produce an excessive amount of flowering hormones which cause their susceptibility. Campbell (1958) found that no infection in barley occurred if inoculation was delayed until the anthers appeared, and that resistance was rapidly obtained after fertilization. Purinik and Mathre (1971) found that unfertilized male sterile barley can be infected up to 15 days after normal anthesis, while fertilized male sterile barley becomes completely resistant 10 days after fertilization.

Ratanopas (1973) found that fertilization increased resistance, which was expressed as an increase in partially infected and aborted

florets and a decrease in sclerotia and honeydew.

Resistance can also be expressed before fertilization occurs, when the ovary is most susceptible. Ratanopas (1973) carried out his inoculations two days before anthesis and found some wheat varieties more susceptible than others. Puranik and Mathre (1971) inoculated male sterile wheat with a capillary dropper into the floral cavity, and found the wheat variety Chris to be the most resistant. Chris was also most resistant when grown in the field under natural conditions of infection.

Platford and Bernier (1970) found two wheat varieties, Carleton and Kenya Farmer, which possessed a high level of resistance when inoculated two days before anthesis. Resistance was expressed by a decrease both in number of sclerotia formed and honeydew production.

2.4 Studies of the Inheritance and Transfer of Ergot Resistance

Robinson (1959) working with sugar cane in Queensland crossed a resistant cultivated variety with a susceptible, frost-resistant wild species, Sacchaum spontaneum. The F_1 plants were susceptible to a race of Claviceps purpurea called false floral smut. The wild species was found to be homozygous for susceptibility which was dominant.

Bennett and Bashaw (1960) crossed a well-adapted variety of the pasture grass Paspalum dilatatum which was susceptible to ergot (Claviceps paspali, Stevens and Hall) to an immune species, P. malacophyllum. The F_1 plants were highly resistant, indicating dominant inheritance of resistance. Highly resistant and immune plants were

obtained from an F_2 population which was segregating for ergot resistance.

Kalsdyan-Avanestan (1967) in Russia, tested the resistance of several interspecific Triticum crosses to Claviceps purpurea. He found that resistance was recessive in some F_1's but dominant in others. Dominant inheritance to resistance was found in Triticum polonicum x T. timopheevi, T. timopheevi x T. aestivum var Awnless 1, T. durum var africanum x T. timopheevi. It was concluded that ergot resistance in wheat is an unstable quantitative character controlled by more than one gene.

Ratanopas (1973) examined the resistance of the durum wheat variety Carleton and the spring wheat variety Kenya Farmer to 58 isolates of Claviceps purpurea. He found evidence that the resistance of Kenya Farmer and Carleton are genetically different and that both horizontal and vertical resistance are present.

Platford (1973) found that the resistances of the durum wheat Carleton and the hexaploid wheat Kenya Farmer were both recessive. He tested the Kenya Farmer-Chinese Spring substitution lines and the F_1's of the A and B genome monosomics of Chinese Spring by Carleton and found that some of the resistance was conditioned by chromosome 6B of Kenya Farmer and 1B of Carleton.

2.5 The Origins of Triticum Timopheevi

Triticum timopheevi Zhuk. is a cultivated tetraploid wheat found in parts of Soviet Georgia. Crosses with other tetraploid wheats produce a sterile F_1 even when chromosome pairing is almost perfect. This

has sparked interest in the origin of T. timopheevi. Lilienfeld and

Kihara (1934) suggested that T. timopheevi had a different progenitor

to the other tetraploids (AABB), and designated the T. timopheevi

genomes as AAGG. However Kostoff (1941) believed that the T. timopheevi

differs only in degree of divergence and renamed the T. timopheevi com-

plement as AAββ . Sachs (1953) suggested that cryptic structural

hybridity, i.e. many very small non-homologous chromosomal segments

could account for the sterility of F_1 hybrids even when pairing was good.

Wagenaar (1961, 1966) proposed an asynaptic genetic system, which could

account for the poor pairing of T. timopheevi hybrids, which arose in

the original tetraploid population and separated the Timopheevi group

by a sterility barrier. Any structural changes would have arisen follow-

ing the formation of this genetic barrier. Feldman (1966a) examined

heteromorphic associations in the F_1 between Chinese Spring ditelo-

centrics and T. timopheevi. He suggested that five major translocations

had occurred in the course of divergence between the timopheevi complex

and the emmers. He proposed that the A genome was a stable "pivotal

genome" and hybridization between the B genome and other diploid species

may have occurred. Bozzini and Giorgi (1969) showed that the karyotypes

of the T. timopheevi group and the Emmer group belong to two distinct

groups. Electrophoresis of seed proteins of the two groups show strong

differences at four or five loci (Johnson 1967). Most of these differ-

ences were attributed to the G(B) genome. Feldman (1966b) found the

5B diploidization mechanism to be expressed in $\underline{T}$. $\underline{timopheevi}$ suggesting that this mechanism may have arisen in a common progenitor.

It is proposed (Zakubziner 1958, Harlan and Zohary 1966) that the $\underline{T}$. $\underline{timopheevi}$ group arose from a non-weedy, sub-dominant group of wild wheats centred in Turkey, Iran and Iraq, while the cultivated emmers arose from a weedy, agressive race in Jordan and Palestine. Johnson (1967) suggested that $\underline{T}$. $\underline{dicoccoides}$ may have been the wild progenitor of both groups.

2.6 Behavior of Triticum Timopheevi in Crosses

The F_1 hybrids of $\underline{T}$. $\underline{timopheevi}$ by all other Triticum species have been found to be highly sterile (Sachs 1953) (Bell & Lupton 1955) (Allard 1949), including $\underline{Triticum}$ $\underline{araracium}$ and $\underline{T}$. $\underline{dicoccoides}$, which pair well in crosses with $\underline{T}$. $\underline{timopheevi}$ (13-14 bivalents at meiosis). F_1 seed set between 35% (Fedesenko 1970) and 75% (Allard (1949) has been obtained when $\underline{T}$. $\underline{timopheevi}$ was used as the male parent in crosses with Chinese Spring. However, when $\underline{T}$. $\underline{timopheevi}$ is used as the female, shrivelled F_1 seed with poor germination are produced (Khodyrev 1969, Allard 1949). F_1 plants are somewhat female fertile and viable seed set of 2.2% has been reported (Allard 1949) when backcrossing the F_1 to the vulgare parent.

The pairing in the F_1 of a durum or vulgare by $\underline{T}$. $\underline{timopheevi}$ cross is variable, with the bivalent associations ranging between 4 and 14 pairs and averaging about 9-10 bivalent and 1 trivalent association per

cell during meiosis (Sachs 1953, Love 1941, Wagenaar 1961, Allard 1949).

The F_1 gametes of a vulgare by $\underline{T}$. $\underline{timopheevi}$ cross contained 15 to 21

chromosomes (Allard 1949). In the BC_1F_1 and further backcross genera-

tions, increased pairing was observed, accompanied by greater fertility

and return to the chromosome number of the recurrent parent (Allard

1949). A seed set of 56% was found after the third backcross (Allard

1949).

In crosses with $\underline{T}$. $\underline{monococcum}$ (AA) and $\underline{T}$. $\underline{timopheevi}$, between 5 and

7 bivalents were found at meiosis, (Sachs 1953, Wagnaar 1961) indicating

that the A genomes of $\underline{T}$. $\underline{timopheevi}$ and the other triticums are fairly

homologous.

Fedesenko (1969) found the poor fertility in vulgare x $\underline{T}$. $\underline{timopheevi}$

hybrids manifested in low pollen germination, abnormal pollen tube growth

and abnormal mitosis in the endosperm after fertilization occurred.

Meiotic instability may persist even after a return to the parental

chromosome number. Semeniuk (1947) examined the F_5 and F_6 lines of

Pridham's Steinweidel by $\underline{T}$. $\underline{timopheevi}$ cross and found 4.8 to 53.8% of

the cells had univalents. Chromosome instability could be recognized

by abnormalities at anaphase I and II, micronuclei at interphase, and

presence of aborted pollen.

2.7 Triticum timopheevi as a Source of Disease Resistance

Triticum timopheevi has been found to possess immunity or resist-

ance to a wide range of wheat pathogens. These include $\underline{P}$. $\underline{graminis}$,

<u>P</u>. <u>recondita</u>, <u>P</u>. <u>striiformis</u>, <u>Tilletia levis</u>, <u>T</u>. <u>tritici</u>, <u>Erysiphe</u> <u>graminis tritici</u>, <u>Ustilago tritici</u> and Fusarium and Septoria species (Jacubziner 1958, McIntosh and Gyfaras 1971, Allard and Shands 1954).

Another indication of the wide resistance of <u>T</u>. <u>timopheevi</u> can be found in its reaction to diseases in the International Spring Wheat Rust Nursery which tests 600 to 900 accessions each year in 20 to 30 countries. An accession of <u>Triticum timopheevi</u>, D-357-1 from Russia has consistently been one of the most resistant entries. In the past 10 years its resistance has only been overcome by races of <u>Puccinia</u> <u>graminis</u> and <u>P</u>. <u>recondita</u> in Brazil, Bolivia, Peru, Chile, and in South Africa and it has been attacked by septoria in South Africa. Its resistance to <u>Erysiphe graminis</u> and Helminthosporium has apparently remained intact.

Jacubziner (1958) reported that this "complex resistance" of <u>T</u>. <u>timopheevi</u> is effective against most physiologic races of these diseases, and also is a stable resistance, maintaining resistance in the field to brown rust (<u>P</u>. <u>recondita</u>) and yellow rust (<u>P</u>. <u>striiformis</u>) for over 50 years.

The reactions to the races of diseases to which <u>T</u>. <u>timopheevi</u> had been found to be tested is summarized in Table 1.

2.8 <u>Disease Resistant Timopheevi Derivatives</u>

Shands (1941) produced a line of winter wheat which he reported as resistant to leaf rust, stem rust and mildew. Selections were made from

Table 1. Resistance of Triticum timopheevi to Plant Diseases.

Resistance to Stem Rust, Puccinia graminis

Race	9	10	11	15	15B	15B-IL	17	19	21	29	32	34	36	38	39	40	48	50	56	100	113	116	120	125	139	152	189	198	Author	Source
Reaction	I	I	I	R			I	MR	R	I	I	I		I	R	R	I		R	I	MR		I		I		I	I	Newton et al. (1940)	
					MS																R								Bell & Lupton (1955)	
				R				S	R			R						R				R		R					Jacubziner (1969)	
			MS	R	S		S					MR																R	Watson & Stewart (1956)	CI 11802
						R																					S		Garcia-Rada et al. (1942)	
																													McIntosh & Gyarfas (1971)	CI 11802

Resistance to Leaf Rust, P. recondita

Race	1	9	11	20	21	26	27	28	29	52	76	77	95	116	122	135	138	174	185	Author	Source
Reaction	I	I	I				I	I	I	I	I									Newton et al. (1940)	
				I	I								I		I		I	I	I	Zacubziner (1961)	
						R						R				R	R			Watson & Stewart (1956)	CI 11802

Resistance to Stripe Rust, P. striiformis

Race	2	2B	5	7	8
Reaction	R	R	R	S	S

Resistance to Powdery Mildew, Erysiphe graminis

Race	3	4	5	9	10	12	13	15	18	19	Author
Reaction		I									Bell & Lupton (1955)
	I			I	I	I	I	I	I	I	Jacubziner (1959)
			R								Allard & Shands (1954)

Resistance to Loose Smut, Ustilago nuda

Race	2	4	5	8	9	10	11	12	13	14	15
Reaction	I	I	I	I	I	I	I	I	I	I	I

Resistance to Bunt, Tilletia tritici

Race	13	15	16	Author
Reaction	I	I	I	Jacubziner (1969)

Reaction types:
I   Immune
R   Resistant
MR   Moderately resistant
MS   Moderately susceptible
S   Susceptible

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the progeny of a single BC_1F_2 seed.

Allard and Shands (1954) produced two cytogenetically stable spring wheat lines CI 12632 and CI 12633 from a cross between a Chinese Spring by Illinois line and T. timopheevi. The two lines were selected following two backcrosses and eight generations of selfing. The lines were highly resistant to powdery mildew (Erysiphe graminis tritici) and most races of stem rust except 15B.

Moderate resistance to loose smut (Ustilago), and resistance to some races of leaf rust were also transferred, but the bunt (Tilletia) resistance was not transferred. Both the stem rust and post seedling mildew resistance (to races of mildew) were thought to be conditioned by a single dominant gene on chromosome 2B as determined by monosomic analysis (Nyquist 1957 and 1962). Seedling mildew resistance was conditioned by another independent gene (Nyquist 1963).

Pridham in Australia crossed the spring wheat variety Steinweidel with T. timopheevi which resulted in the variety Timvera. Timvera was found to possess similar stem rust resistance to that of CI 12633 and CI 12632. However not all the T. timopheevi rust resistance was transferred (Watson & Luig 1958).

Watson and Stewart (1956) found that the leaf rust resistance to four races (26, 95, 135, 138) was transferred in the Steinweidel cross, but not in the Illinois by Chinese Spring cross.

An Australian T. timopheevi derivative called CI 13005 from a cross

between a hexaploid variety Cheyenne by Shands 473 (T. timopheevi) was found to give moderate resistance to race 15B in seedling and adult stages (Atkins 1967 as reported by McIntosh and Gyarfas 1971).

Line W (W 3563) is a Steinweidel T. timopheevi reselection which is reported to be resistant to all races of stem rust in Australia (McIntosh and Gyarfas 1971).

The incorporation of mature plant stem rust resistance derived from T. timopheevi into winter wheat has been reported from the Soviet Union (Skurygina 1970).

McIntosh and Gyarfas (1971) compared the stem rust reactions of three derivatives and several accessions of T. timopheevi. By using selected North American and Australian races, they were able to differentiate a number of genes or factors for stem rust resistance. Gene SrTt 1 is present in CI 12632, Timvera and CI 13005. Gene SrTt 2 is present in line W (W3563). A third factor is present in CI 13005 which confers moderate resistance against the new subraces of the 15B complex. The nineteen T. timopheevi accessions were placed into four different reaction classes. T. araraticum was placed in a fifth class. It is believed that a great amount of resistance for stem rust which exists in T. timopheevi still remains to be exploited.

3.0 MATERIALS AND METHODS

3.1 Monosomic Analysis - Plant Materials

The materials utilized in this study included:

(I) The hexaploid Triticum aestivum L. variety Chinese Spring,
and the fourteen monosomic lines of the A and B genomes of this variety.
Chinese Spring has been found to be susceptible to ergot when tested at
the University of Manitoba (Platford, 1973).

(II) T. durum desf. variety Carleton is a tetraploid produced
from a Vernal Emmer by Mindum cross, and has been found to possess re-
sistance to ergot (Platford & Bernier 1970).

(III) T. durum desf. variety Stewart 63 has the same parentage
as Carleton, but is more susceptible to ergot (Platford 1973).

(IV) T. timopheevi Zhuk. variety Nigrum Manitoba accession number
4B289 is a tetraploid wheat obtained from Zhukovsky in Russia. This
accession has maintained a high level of resistance in greenhouse and
field screening (Bernier 1973).

3.2 Handling the Parents and F_1 Generation:

Root tips from ten germinated seeds of each of the fourteen Chinese
Spring monosomic lines of the A and B genomes were collected and fixed.
The chromosome number for each plant was determined at mitotic metaphase
using the Feulgen technique for staining the chromosomes. The monosomic
seedlings were grown in the growth cabinet along with T. timopheevi and

3.3 Inoculation:

The inoculum for this experiment originated from an isolate of

Claviceps purpurea on Manitou wheat and is designated M-4. A conidio-

spore suspension of this isolate was maintained by the Plant Pathology

Section. The inoculum was made up every second day by diluting a por-

tion of the conidiospore suspension with distilled water to a spore

density of 10^4 conidiospore per cc.

Heads were inoculated two days before anthesis according to the

method outlined by Platford (1973) when the stigma on a primary floret

near the centre of the head was beginning to appear feathery. The outer

glumes of ten florets were removed and these florets were inoculated by

inserting a hypodermic syringe through the lemma, without touching the

pistil, and filling the floral cavity with approximately .02 ml. of

conidial suspension. After inoculation, each head was covered with a

glassine bag and fastened securely to maintain high humidity around the

head. At least six heads of each monosomic line and each check variety

were inoculated, except Chinese Spring 1A x Carleton, Chinese Spring 2B x

Carleton and Chinese Spring 1A x T. timopheevi for which five heads were

inoculated, and Chinese Spring 2A x Carleton which had 4 heads inoculated.

3.4 Rating the Infection:

The rating system was based on methods developed previously by

Platford and Bernier (1970), and by Ratanopas (1973).

The honeydew produced by each inoculated head was rated visually

two weeks after inoculation as follows:

1. no visible honeydew.

2. honeydew confined to glumes.

3. honeydew exuding outside glumes in

    small drops.

4. large drops of honeydew running

    down the head.

Four weeks after inoculation, the ten inoculated florets were examined. The numbers of florets were recorded which fell into each of the following reaction classes:

A sclerotium - was judged to have been produced when the ovary had been completely engulfed by mycelium.

A partial infection - occurred when the infection stopped before engulfing the entire ovary.

An abortion - occurred when no infection or seed was found in a floret.

Seed set - was the occurrence of a normal seed in the inoculated floret.

The sclerotia were subdivided into the numbers which fell into each of the following size classes:

1. the number of sclerotia smaller than a normal seed.

2. the number equal in size to a normal seed.

3. the number larger than a normal seed.

An index system was developed by Ratanopas (1973) in order to classify and quantify the overall reaction of an inoculated head. This index is based on three components, honeydew production, size of sclerotia, and number of sclerotia. The index rating system which was used in this study is presented in Table 2. The most susceptible component had the strongest influence in determining the index rating. In order to assure an unbiased rating of the reaction, the chromosome numbers were not assigned to the plants until after the rating had been carried out.

A sclerotia size index from 0 to 100 was calculated from the number of sclerotia in each of the 3 size classes by using the following formula:

$$\frac{\sum_{i=1}^{3} N_i \times R_i}{T \times 3}$$

where   i = the size class

   N_i = number of sclerotia in the ith size class

   R_i = size class multiplier 1, 2, 3

   T = total number of sclerotia on a head

3.5 Analysis of Results:

The reaction of the 34 chromosome F_1 heads were compared with the 35 chromosome plants which had arisen from the same Chinese Spring monosomic line. Therefore the plants used in each comparison were identical

Table 2. Rating System for Disease Caused by _Claviceps_ _purpurea_.

(for ten inoculated florets on a head)

Reaction Index	Quantitative Index	Infection Type [+]	Frequency of Sclerotia	Honeydew Reaction
Immune	0	No infection	0	1
Very Resistant	1	AR	0	1
Resistant	2	$AR + S_1 + S_2$	4 or less	1-2
Moderately Resistant	3	Mainly S_1 and S_2 a few S_3	6 or less	1-2
Moderately Susceptible	4	S_2 and S_3	8 or less	3
Susceptible	5	Mainly S_3	more than 8	4

[+] Infection types.

AR - Abortive Reaction = Abortions + partial infections

S_1 - Sclerotium smaller than seed

S_2 - Sclerotium equal in size to a seed

S_3 - Sclerotium larger than seed.

except for the absence of one chromosome.

The Mann-Whitney test was carried out on each set of comparisons. The index of each head in a comparison was ranked, and the ranked values of the 34 and 35 chromosome heads were compared. This test does not assume that the data is normally distributed. Similar tests were also carried out on the three components of the index.

3.6 **Cytological Analysis:**

The pairing relationships of at least 10 pollen mother cells from 2 or more F_1 plants were scored at F_1 meiotic metaphase for each of the following crosses: Chinese Spring x _T. timopheevi_; Chinese Spring x Carleton; Carleton x _T. timopheevi_; and Chinese Spring^2 x _T. timopheevi_.

3.7 **Supplementary Crosses:**

F_1 plants of the following crosses were tested for resistance to the M-4 isolate of ergot: Carleton x _T. timopheevi_; Stewart 63 x _T. timopheevi_; _T. timopheevi_ x rye (_Secale cereale_ L. accession OD 174); two plants of Chinese Spring^2 x _T. timopheevi_. Two triticale lines, 6A20 (a Carleton x rye cross) and 6A190 (Stewart x Prolific rye) were also tested. Between 6 and 20 heads were tested for each cross, except the Chinese Spring x _T. timopheevi_^{2} cross for which 2 heads were tested. The viability of the inoculum was checked by inoculating a susceptible check variety on each date when inoculations were carried out.