INHERITANCE OF RESISTANCE TO COVERED SMUT IN SEVERAL HYBRIDS OF BARLEY

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Charles Cottingham

1958

This is to certify that the

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INHERITANCE OF RESISTANCE TO COVERED SMUT

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CHARLES COTTINGHAM

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by

CHARLES COTTINGHAM

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

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Department of Botany and Plant Pathology

1958

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The probable origin of barley has been suggested as Ethiopia and Asia. Barley is attacked by many fungi which are instrumental in decreasing yields. Covered smut caused by Ustilago hordei (Pers.) Lagerh. is one of these fungi.

This investigation was concerned with the inheritance of resistance to race 6 of U. hordei, and the inheritance of morphological characters, in four non-commercial varieties of barley: Pannier, Odessa, Chevron and Jet. Pannier and Odessa have 6-row heads, covered caryopsis and blue aleurones. Chevron has a 6-row head, covered caryopsis and a white aleurone. Jet has a 2-row head, naked caryopsis, and a black aleurone.

Inoculations of seeds were made by the vacuum, and the hand-dehulled methods. These methods placed the covered smut spores within close proximity of the elongating coleoptiles which was necessary to initiate infection.

Inheritance of resistance to U. hordei race 6 in the crosses between Pannier x Odessa, Pannier x Chevron and their reciprocals, and Pannier x Jet was explained on the basis of two factor pairs. One factor pair was found to govern the characters, covered versus naked caryopses, and black versus blue aleurone in the cross, Pannier x Jet.

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INTRODUCTION

Barley was cultivated as early as the Stone Age by the lake dwellers of Switzerland. The probable origin of barley has been suggested as Ethiopia and Asia. The cultivated species of barley may have originated from Hordeum agricorithon, a wild 6-row barley with a brittle rachis found in eastern Tibet or Hordeum spontaneum, Koch a 2-row type.

Barley is grown throughout the world, and thrives best in the temperate regions where cool climates prevail. Barley ranks fourth in economic importance among the cereal crops, and is exceeded in value only by wheat, oats and corn. The principal barley-producing states are Minnesota, North Dakota, California, South Dakota, Nebraska, Wisconsin and Michigan. More than 70 million bushels of barley are used annually for malting purposes. Barley is also used as food for humans, and feed for animals.

Barley is attacked by many microorganisms. Among the important fungi attacking barley are the smuts, loose, semi-loose, and covered smut. Covered smut of barley, caused by Ustilago hordei (Pers.) Lagerh. attacks the barley plant and replaces the head and kernels with smut spores. The purpose of this thesis is to study the inheritance of resistance to race 6 U. hordei in four varieties of barley, as well as the inheritance of specific morphological characters in one of the crosses (Pannier X Jet).

REVIEW OF LITERATURE

A. History of Covered Smut Organism Ustilago hordei (Pers.) Lagerh.

A history of U. hordei was prepared by Kellerman and Swingle (21). They reported that Pliny and Theophrastus probably knew of oat smut; but in their writing, all smuts were confused with the stinking smut of wheat. Tragus illustrated the organism in 1552, and placed it in the genus, Ustilago.

Lobeluir, in 1591, was one of the first investigators to separate the smuts of barley from those of oats. He referred to the forms on barley as Ustilago polystichi, and U. hordei distychi. There was no means of knowing whether he included one or both of the barley smuts under these names. Bauchin, in 1596, also separated barley from oat smut, calling the former Ustilago hordeace. Persoon, in 1801, was the first to give a valid description of the barley covered smut fungus. His description of the organism was "Uredo (Ustilago) segetum a Uredo hordei, Pseudoperidia subelliptica reguloso sulvere latente." This reference to the powder as latente would indicate that he had the barley covered smut fungus.

After Persoon, many investigators copied his varietal names without change. Tulasne, in 1847, called the smut of barley Ustilago carba a Vulgaris o Hordeacea. Jensen, in 1888, was the first to clearly separate covered smut from the other smuts on barley under the names Ustilago segetum var. hordei tecta Jensen, and *Ustilago segetum* var. *tecta*. He later called it *Ustilago tecta hordei* Jensen. This classification differentiated between *Ustilago hordei* and *Ustilago nuda*, but not *Ustilago nigra*.

Kellerman and Swingle reported the synonyms of *Ustilago hordei* as in Table 1 (21). However, in 1889, the binomial was changed to its present name, *Ustilago hordei* (Pers.) Lagerh. (12).

B. Environmental Relationships

Infection by covered smut cannot begin until two preliminary processes are completed (20). First, the coleoptile must be exposed. This process required from 24-72 hours. Secondly, the smut spore must germinate and produce sporidia and infection tubes. This process was completed within 48 hours. Infection of the coleoptile was no longer possible after the coleoptile had ruptured and the first green leaf had pushed one centimeter beyond it (20).

Planting the inoculated kernels deeper in the soil has been shown to produce more smut infected plants than shallow plantints (20, 40). This was probably due to the longer exposure to the pathogen by the susceptible tissues (20, 40). When seeds were planted deeply in wet soil the balance of host and parasite was disturbed. The fungus became destructively parasitic, killing some of the already weak seedlings at an early age. This prevented a portion of the infected plants from showing smutted heads in the crop (11, 20). A four year experiment produced 2.7 to 115 times

TABLE 1

SYNONYMS OF *U. HORDEI*

Year	Synonym
1552	*Ustilago fragus* - De Stisp
1591	*Ustilago polystichi* - Lobelius
1591	*Ustilago hordei distichi* - Lobelius
1596	*Ustilago hordeacea* - C. Bauhin
1767	*Chaos Ustilago* - Linne
1791	*Reticularia segetum* - Bulliard
1801	*Uredo (Ustilago) segetum a Uredo Hordei* - Persoon
1809	*Caeoma segetum* - Link
1813	*Ustilago segetum* - (Bulliard), Dittmar
1815	*Uredo carbo a Hordei* - De Candolle
1833	*Erysibe uera a Hordei* - Wallroth
1837	*Uredo carba Hordei* - Philippar
1847	*Ustilago carbo a Vulgaris o Hordeaceae* - Tulasne
1856	*Ustilago segetum b Hordei* - Rabenhorst
1888	*Ustilago segetum* var. *tecta* - Jensen
1888	*Ustilago segetum var. hordei tecta* - Jensen
1888	*Ustilago hordei* - Rabenhorst
1889	*Ustilago hordei* var. *tecta* - Jensen
1890	*Ustilago tecta hordei* - Jensen
1890	*Ustilago Jensenii*

as much covered smut in Tennessee Winter barley sown at three inches in depth as in the same variety sown at a half inch (20).

Hyphae from spores on the outer surfaces of the caryopses of covered barley varieties were delayed in penetrating the host (36). Although the spores are normally present in that location, penetration was prevented by adverse conditions occurring at emergence time in very shallow planted seed (36). A higher percentage of infection was obtained in experiments with hand-dehulled barley when the temperature and moisture of the soil were under control during the period of infection. The amount of infection was influenced by the low soil temperature at the time of planting (10).

C. Inoculation Methods

Methods of increasing the percentage of infection by *U. hordei* in field trials were needed to study the inheritance of resistance of barley varieties (42). Attempts to obtain infection by applying spores to the kernels at planting time met with little or no success (42). Some varieties showed slight infection while others did not (18, 33).

Barley kernels were then dehulled by hand, and spores were applied directly to the kernels. This method produced approximately 25 percent more diseased heads than the checks which were dusted with the smut spores without dehulling (1, 18, 42). High infection rates were obtained by the hand-dehulled method in the susceptible variety Chevron (22).

Barley kernels dehulled with concentrated sulphuric acid and inoculated with the covered smut fungus produced approximately the same percentage of infected plants as with hand-dehulled, inoculated kernels. Complete dehulling was not found necessary for good infection. A thin hull over the entire kernel produced optimum results (4, 5, 6). However, the use of concentrated sulphuric acid led to complications which made exact analysis of the reaction of the hybrids impossible (19). Increased severity of infection by U. hordei aided by the removal of the hull resulted in extreme distortions of seedlings and consequent failure to emerge (19). Barley seed dehulled with a smut dockage machine produced no greater infection than unhulled seed (45).

Techniques for inoculation without dehulling have been reported (8, 28, 34, 37, 38). The spore suspension (vacuum) method of inoculation produced 75 percent infection in field plantings of spring and winter barleys (28, 34, 38). Eighty to 100 percent infection was obtained by employing a Waring blender equipped with dull non-cutting blades (8). The seeds were placed in the blender which contained a spore suspension and churned for 15 to 30 seconds. The time of churning depended upon hull toughness (8).

The most effective covered smut inoculum consisted of spores and extensive mycelial ramifications on the pericarps of the caryopses beneath the seed hull (37). The occurrence of this subhull inoculum in nature explained why surface disinfectants of

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seed barley have been relatively ineffective in covered smut control (37).

D. Resistant Varieties

O. A. C. Number 21, Atlas, Velvet, Sacramento, Glabron, Leiorrhynchum, Wisconsin Barbless Number 38, Shaw, Sol and Success were six-row, covered barley varieties which showed some resistance to covered smut (1). Spartan, Golden Pheasant, and Huron two-row covered varieties, and the hull-less varieties Himalaya, New Era, Russian, Mongolian, and Burbank were moderately resistant to covered smut (1).

The susceptible variety Odessa (C. I. 934) showed fewer smutted spikes when inoculated kernels were field planted than greenhouse planted inoculated kernels. However, under more favorable environmental conditions the smut percentage in greatly increased. The variety Chevron (C. I. 1111) produced intermediate percentages of covered smut (22). Jet (C. I. 967) was an intermediate resistant variety with resistance governed by both morphological and physiological characters (22). The highly resistant variety Pannier (C. I. 1330) produced no smutted plants in its progeny (22). These differences may be based upon gene combinations which govern degrees of resistance or susceptibility to the pathogen (22).

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E. Inheritance of Morphological Characters

Covered versus Naked Caryopses

A study of the cross between Canadian Thrope x Guy Mayle produced a 3:1 ratio in the F_2 generation with covered caryopses dominant. Canadian Thrope was a 2-row, covered variety and Guy Mayle was a 6-row, naked variety. It was concluded that a single factor difference existed between covered and naked caryopses (25). Similar results have been found by other investigators (3, 7, 9, 16, 29, 31, 41).

Black versus Blue Aleurone

Black versus blue aleurone was governed by a single factor pair in a number of crosses reported. Black was dominant (3, 7, 9, 15, 16, 27, 29, 31, 44).

F. Inheritance of Resistance to Race 6 *U. hordei*

Numerous morphological differences among barley varieties led to a study of the relationships between these morphological characters and resistance or susceptibility to covered smut (14). Little or no correlation was found between factors for smut susceptibility and those for height of plant in a cross between the barley varieties Glabron x Trebi (14). Little or no correlation between lemma color and other morphological characters and the factor for resistance was found (14).

No infection was found in the F_2 generation of a cross between Cape x Kwan (28). Infection ranged from 0 to 27.5 percentage classe

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classes in the F_3 families. Sixty-one families showed no infection. It was concluded that Kwan contained more than two dominant genes for resistance to covered smut (28). Inheritance of resistance to race 6 covered smut in the cross between Chevron x Brachytic and Colsess x Brachytic was found to be governed by one major factor pair. In the F_1 generation of the Chevron x Brachytic cross, resistance was dominant. A 3:1 ratio was obtained in both crosses by segregation of the F_2 based upon F_3 progeny tests. Smut reaction was found to be independent of five plant characters (32).

Variety
Odessa
Jet
Chevro
Pannie

[a] Peri

[b] Flag

TABLE 2

REACTION OF PARENTAL VARIETIES TO *USTILAGO HORDEI* (PERS.) LAGERH. RACE 6 (22)

Variety	C. I. No.	Smut Reaction	Temp.	Smut Inf. Plants-Percent
Odessa	934	Susceptible	24 C	94
Jet	967	Resistant-Intermediate[a]	24 C	20
Chevron	1111	Intermediate[b]	24 C	53
Pannier	1330	Resistant	24 C	0

[a]Pericarp removed over embryo in this variety.

[b]Flag leaf only showed smut sori.

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MATERIALS AND METHODS

A. Crosses Involved

The covered smut, resistant variety Pannier (C. I. 1330), was crossed with a susceptible variety, Odessa (C. I. 934), a susceptible variety, Chevron (C. I. 1111), and a resistant variety, Jet (C. I. 967). (Table 2) After pollination the heads were covered with aluminum foil to prevent chance pollination. F_1 plants were grown in pots in the greenhouse to provide F_2 seed.

Because of the differences in the morphological characteristics of Jet and Pannier, seeds from individual F_2 plants were grown in separate rows in the field. Seed from individual F_2 plants of the other crosses was also grown in separate rows in the field, but no studies were made of morphological characters.

A portion of the F_2 seed of Pannier x Odessa and its reciprocal was inoculated with race 6 U. hordei and grown to maturity in the greenhouse in 1956. A random sample of these F_2 plants furnished seed for the F_3 progeny which were field grown to observe morphological characteristics. Two tests were conducted with the remaining portion of the F_2 seed to determine its reaction to covered smut. The F_2 plants of the Pannier x Jet and Pannier x Chevron crosses were not tested for smut reaction.

B. Field Trials of F_3 Plants

In the spring of 1957 seeds from single F_2 plants were planted in the field in separate rows. These rows were designated as F_3 families. The F_3 families were harvested and packaged as individual plants within a family. Each envelope contained the seed from a single plant of a single F_3 family. Readings of plant type were made for the plant characteristics of each F_3 family.

C. Inoculation Methods

Inoculations were made by the vacuum method in the F_2 of the Pannier x Odessa cross. The barley kernels were placed in a spore suspension in vials and inoculated with *U. hordei*. Following inoculation, the seeds were spread out on blotting paper and allowed to dry before planting in 6-inch pots in the greenhouse. Readings were made on the number of infected plants.

The hand-dehulled method was used for the other tests, since this method was found to give the highest percentage of infected plants (22). The pericarps were removed from the embryos in Jet, which removed the morphological resistance factor. Spores of race 6 of *U. hordei* were applied to moistened kernels of barley to obtain the maximum spore load. The emerging coleoptiles were dusted with spores twice before they elongated the length of the caryopses. This elongation required from 48 to 72 hours. Dehulled, inoculated kernels were placed in petri plates which contained moistened filter paper inside both the cover and bottom of the plates.

Water was added periodically to maintain the required humidity for maximum spore germination as well as maximum seed germination. The plates were wrapped in wet paper towels and waxed paper and incubated at 24° C.

Seedlings were removed from the petri plates on the third or fourth day and transplanted into rows in the soil of a greenhouse bench. The seedlings were planted with the coleoptiles pointed upward. Care was taken not to plant the seedlings more than one inch deep. However, some of the plants had distorted leaves and grew in an abnormal manner under the soil surface. Such plants were uncovered and exposed, but many failed to recover. Similar results by other workers have been reported (10, 19, 32). The seedlings were grown at temperatures above 70° F. to aid maximum disease development. Fluorescent lights were provided to illuminate the plants twelve hours per day.

D. Greenhouse Test of the F_4 Plants

In the fall of 1957, the F_4 seeds were hand-dehulled and inoculated with race 6 of U. hordei. The F_4 seeds were planted in a series of five pots, and each pot contained five seeds from a single F_3 plant. Therefore, each series contained a total of 25 seeds from five F_3 plants and represented a single F_4 family.

E. Classification of Diseased Plants

The number of smutted plants was recorded after maturity. Plants with a stunted appearance were examined for smut by splitting the sheaths open and exposing the heads. If any diseased head or tiller appeared, the plant was classified as smutted. Plants on which disease readings could not be taken were omitted from the counts.

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EXPERIMENTAL RESULTS

A. Reactions of Parental Varieties, Odessa, Jet, Chevron and Pannier

The reactions of the parental varieties Odessa, Jet, Chevron and Pannier to race 6 of *U. hordei* in the tests of the F_4 progeny are shown in Table 3. Odessa was highly susceptible to covered smut. Jet, showed a resistant-intermediate reaction, while Chevron was susceptible in its reaction to covered smut. Pannier produced no infected plants and was classified as resistant to covered smut.

B. Inheritance of Resistance to Race 6 of *U. hordei*

Pannier x Odessa Crosses

Inoculation of the F_2 seedlings of the cross Pannier x Odessa and its reciprocal produced 5.2 percent infected plants as compared to 57 percent infected plants in the susceptible Odessa check. This ratio of 456 non-infected to 25 infected plants in the F_2 gives a mathematical ratio of 18.2 to 1 which lies within the probability of a 15:1 ratio. Therefore, the results of the F_2 test indicate that two factors govern the resistance to race 6 of *U. hordei* in the cross Pannier x Odessa.

A chi-square test (Table 4) was applied to the data to determine the closeness of fit for a theoretical ratio of 15:1. The chi-square was .921 and the probability was .35 . This indicated

TABLE 3

REACTION OF PARENTAL VARIETIES USED AS CHECKS IN THE F_4 PROGENY TESTS TO RACE 6 OF *USTILAGO HORDEI*

Variety	C. I. No.	Smut Reaction	Temp.	Smut Inf. Plants Percent
Odessa	934	Susceptible	24 C	80
Jet	967	Resistant-Intermediate[a]	24 C	0
Chevron	1111	Susceptible	24 C	33
Pannier	1330	Resistant	24 C	0

[a]Pericarp removed over embryo of this variety.

TABLE 4

CHI-SQUARE TEST OF F_2 PROGENY OF THE CROSS PANNIER x ODESSA

Cross	*U. hordei* Race	Char-acters	Obs. Freq.	Theoretical Ratio	Calc. No. Freq.	X^2	p Value
Pannier x		Res.	456		450.9		
Odessa	6	Susc.	25	15:1	30.1	.921	.35

a good fit for a 15:1 ratio if resistance in the cross Pannier x Odessa is governed by two factor pairs in the F_2 progeny.

Inoculation of the F_4 progeny of 17 F_3 families of the cross Pannier x Odessa demonstrated that 5 of the F_3 families came from homozygous resistant F_2 plants. The 12 remaining F_3 families came from 12 F_2 plants that were either heterozygous resistant or homozygous susceptible. This group of 12 plants cannot be separated into its components with certainty. This gave a mathematical ratio of 5 homozygous resistant to 12 either heterozygous resistant or homozygous susceptible families. These data indicated that two factor pairs were operating for resistance to race 6 of U. hordei in the F_4 of the cross Pannier x Odessa.

The chi-square test for closeness of fit for a 7:9 ratio was 1.378 (Table 5) with a probability of .25. This indicated a good fit for a two factor ratio, and agreed with the F_2 tests.

Pannier x Chevron

Fifteen of the 31 F_3 families inoculated with race 6 of U. hordei proved to be homozygous resistant and came from homozygous resistant F_2 plants (Table 6). The remaining 16 F_3 families were derived from either heterozygous resistant or homozygous susceptible F_2 plants. The latter group of 16 F_3 families cannot be further classified with accuracy. This gave a ratio of 15 homozygous resistant to 16 heterozygous resistant or homozygous susceptible F_3 families which fits a 7:9 ratio with a chi-square of .256 and a probability of .63 that resistance in the cross Pannier x Chevron was governed by two factor pairs.

TABLE 5

CHI-SQUARE TEST OF F_4 FAMILIES OF THE CROSS PANNIER x ODESSA INOCULATED WITH RACE 6 OF U. HORDEI

Cross	U. hordei Race	Char-acters	Obs. Freq.	Theoretical Ratio	Calc. No. Freq.	X^2	p Value
Pannier x		Res.	5		7.4		
Odessa	6	Susc.	12	7:9	9.6	1.378	.25

TABLE 6

CHI-SQUARE TEST OF F_4 FAMILIES OF THE CROSS PANNIER x CHEVRON INOCULATED WITH RACE 6 OF U. HORDEI

Cross	U. hordei Race	Char-acters	Obs. Freq.	Theoretical Ratio	Calc. No. Freq.	X^2	p Value
Pannier x		Res.	15		13.6		
Chevron	6	Susc.	16	7:9	17.4	.256	.63

Pannier x Jet

The F_4 progeny of individual F_3 families of the cross Pannier x Jet were inoculated with race 6 of U. hordei. Nine of these F_3 families proved to be totally resistant which indicated that they came from homozygous resistant F_2 plants (Table 7). The remaining 13 F_3 families proved to be either heterozygous for resistance or homozygous for susceptibility based upon the reaction of the inoculated F_4 plants. The latter two groups were classified together. This gave a ratio of 9 resistant to 13 susceptible families which fits a possible 7:9 ratio with a chi-square of .066 and a probability of .79 that a two factor inheritance was involved (Table 7).

C. Inheritance of Agronomic Characters in F_3 Families

Black versus Blue Aleurone

The results of the chi-square test are shown in Table 8. Five F_3 families were homozygous black and two F_3 families were homozygous blue. The remaining 15 F_3 families were either segregating for black or blue color and could not be further classified with accuracy (Table 9). The chi-square (Table 8) was .052 with a probability of .11 that black versus blue aleurone is governed by a single factor pair, and the black character is dominant.

Covered versus Naked Caryopses

In the cross Pannier x Jet, 6 F_3 families were homozygous covered and four F_3 families were homozygous naked. The remaining

TABLE 7

CHI-SQUARE TEST OF F_4 FAMILIES OF THE CROSS PANNIER x JET INOCULATED WITH RACE 6 OF U. HORDEI

Cross	U. hordei Race	Char-acters	Obs. Freq.	Theoretical Ratio	Calc. No. Freq.	X^2	p Value
Pannier		Res.	9		9.6		
x							
Jet	6	Susc.	13	7:9	12.4	.066	.79

TABLE 8

THE F_3 SEGREGATION OF BLACK AND BLUE COLOR OF KERNELS IN BARLEY HYBRIDS OF THE CROSS PANNIER x JET

Cross	Char-acters	Obs. Freq.	Theoretical Ratio	Calc. No. Freq.	X^2	p Value
Pannier	Black	5		5.25		
x						
Jet	Blue	2	3:1	1.75	.082	.11

TABLE 9

AGRONOMIC CHARACTERISTICS AND SEGREGATION OF F_3 FAMILIES IN THE CROSS PANNIER x JET

	Kernel Color		Kernel Characteristics	
	Blue	Black	Covered	Naked
1	3	20		23
2	7	17	19	5
3	1	9	10	
4	33			33
5	5	15	11	9
6	3	7	7	3
7		20	20	
8	3	6	2	7
9		15	13	2
10	2	10	10	2
11	7	15	20	2
12	16	0	13	3
13	8	21	23	6
14	4	11		15
15	7	19	17	9
16	6	19	20	5
17	4	9	13	
18		9	9	
19	2	10		12
20	1	9	10	
21		5	3	2
22		23	23	

F_3 families were either segregating for the covered or the naked characteristic (Table 9). A chi-square test was applied to the homozygous characters to determine the closeness of fit for a 3:1 ratio where a single factor pair is involved (Table 10).

The chi-square was 1.20 with a probability of .28 that covered versus naked caryopses in the cross Pannier x Jet was governed by a single factor pair, and the covered character was dominant.

TABLE 10

F_3 SEGREGATION OF COVERED VERSUS NAKED CARYOPSES IN HYBRIDS OF THE CROSS PANNIER x JET

Cross	Characters	Obs. Freq.	Theoretical Ratio	Calc. No. Freq.	X^2	p Value
Pannier	Covered	6		7.5		
x						
Jet	Naked	4	3:1	2.5	1.20	.28

DISCUSSION

The study of inheritance of covered smut resistance in barley was complicated by several factors. The coleoptile must be penetrated by the fungus in its early growth to produce infection. Therefore, a method of placing the spores of the fungus in close proximity of the emerging coleoptile must be employed. Either the vacuum or the hand-dehulled method was employed for this purpose. However, the hand-dehulled method presented further complications because the fungus became increasingly destructive when applied directly to the young coleoptiles. This resulted in severe distortions of the leaves, failure of many plants to emerge properly, and death of many plants before and immediately after emergence. Other investigators have found similar results (10, 19, 32).

A second complication was the lack of an inoculation method which would consistently give 100 percent infection with susceptible individuals. Consequently, only 57 percent infection was obtained by the vacuum method in the susceptible Odessa checks with F_2 population studies, while 80 percent infection was obtained in the susceptible Odessa checks in the F_4 population studies using the hand-dehulled method.

Inoculation of F_2 seedlings of the cross Pannier x Odessa produced only 5.2 percent infection from a population of 481 plants.

The low percentage of covered smut infection in this cross was partially explained by the inefficiency of the vacuum method of inoculation, and the death of inoculated susceptible plants. Foot rot and seedling blight caused by *Helminthosporium sativum* and *Fusarium* sp. reduced the number of surviving individuals in certain rows.

The barley variety Pannier had an immune reaction to race 6 of *U. hordei*. No attempted breakdown of its resistance to race 6 has been successful. In the three other varieties, Odessa, Chevron, and Jet increased infection was obtained by increasing the inoculum load and raising the temperature from 16° C. to 28° C. However, infection was obtained in all crosses made between Odessa, Chevron or Jet and Pannier. A 15:1 ratio for resistance to race 6 of *U. hordei* was obtained in all of these crosses with Pannier. Consequently, it was concluded that an allelic series (P_1 P_1) existed in Odessa, Chevron, Jet and Pannier which provided weak protection of race 6 of *U. hordei*. In addition Pannier had a gene (P_2 P_2)which provided immunity to race 6 of *U. hordei*. The weakest character was found in Odessa (P_1'''' P_1''''), the next weakest in Chevron (P_1''' P_1'''), and the strongest in Jet (P_1'' P_1''). (See Table 11.) When severe inoculation techniques were employed the resistance of all three varieties to race 6 of *U. hordei* was broken (22).

The proposed genotypes of Pannier, Jet, Chevron and Odessa for reaction to race 6 of *U. hordei* are presented in Table 11. In the F_2 test of the cross Odessa x Pannier, the data was obtained

TABLE 11

PROPOSED GENOTYPES FOR PANNIER, JET, CHEVRON, AND ODESSA

Variety	Genotypes			
Pannier	P_1	P_1	P_2	P_2
Jet	P_1''	P_1''	p_2	p_2
Chevron	P_1'''	P_1'''	p_2	p_2
Odessa	P_1''''	P_1''''	p_2	p_2

by the direct reaction of the F_2 embryo. However, in the F_3 family ratings, F_4 plants were read for smut reaction. The genotypes of the F_3 families of the cross Pannier x Jet which would give resistant and/or susceptible plants in the F_4 are shown in Table 12. Any combination of homozygous (P_1 P_1), or (P_2 P_2) produced all resistant plants. All other combinations produced some susceptible plants for a 7-resistant to 9-susceptible ratio in the F_4 generation of all crosses.

Since Pannier was totally resistant, the homozygous condition of P_1 P_1 P_2 P_2 prevailed. In all cases of crosses between Jet, Chevron or Odessa x Pannier, infection was obtained in the offspring. Jet was resistant in its reaction to U. hordei as a check in this experiment. However, its resistance has been broken under severe inoculation techniques (22). Consequently, a weaker allele was proposed without the benefit of the stronger Pannier gene (P_1'' P_1'' p_2 p_2). The allele for Chevron was P_1''' P_1''' p_2 p_2. The weakest allele (P_1'''' P_1'''' p_2 p_2) was found in Odessa which was highly susceptible to race 6 of U. hordei.

The study of morphological characteristics in the cross Pannier x Jet was conducted because Jet was different in more than one morphological characteristic. One factor pair was found to govern covered versus naked caryopses, and black versus blue aleurone. The factors for covered and black aleurone were dominant over those for naked and blue aleurone.

TABLE 12

THE REACTION OF F_4 PROGENY FROM F_3 GENOTYPES
OF THE CROSS PANNIER x JET
TO RACE 6 OF U. HORDEI

Genotypes*		Reaction to Race 6 of U. hordei
P_1 P_1 P_2 P_2	1	All resistant plants
P_1 P_1 P_2 p_2	2	All " "
P_1 P_1 p_2 p_2	1	All " "
P_1 P_1'' P_2 P_2	2	All " "
P_1 P_1'' P_2 p_2	4	Some susceptible plants
P_1 P_1'' p_2 p_2	2	Some " "
P_1'' P_1'' P_2 P_2	1	All resistant plants
P_1'' P_1'' P_2 p_2	2	Some susceptible plants
P_1'' P_1'' p_2 p_2	1	All susceptible plants

* P_1 P_1 , good resistance

P_2 P_2 , good resistance

P_1'' P_1'', fair resistance

p_2 p_2 , poor resistance

The chi-square for black versus blue aleurone was .052. This was within the probability range for closeness of fit for a 3.1 ratio, if these two characters are governed by a single factor pair, and black is dominant.

SUMMARY AND CONCLUSIONS

1. *Ustilago hordei* (Pers.) Lagerh. was the causal organism of covered smut disease presented in this study. Pannier, Chevron, Odessa and Jet were the parental varieties of barley used. Pannier was resistant, and Chevron had a susceptible reaction. Odessa was highly susceptible, and Jet was resistant in its reaction to covered smut. The added morphological resistance factor of Jet was eliminated in this experiment by removing the pericarps from the caryopses.

2. The F_2 and F_4 families in all crosses produced ratios which indicated that resistance was governed by two factor pairs.

3. The chi-square tests indicated that one factor pair controlled the morphological characters for covered versus naked caryopsis and black versus blue aleurone.

4. A reduction in stand counts was apparently caused by destruction of some susceptible plants by *U. hordei*, as well as other soil borne organisms.

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