STUDIES ON *USTILAGO HORDEI*

by

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ABSTRACT

Nutritional mutants of Ustilago hordei were used to demonstrate that parasexual recombination occurs within the host plant (Hordeum vulgare) prior to the production of teliospores. The nutritional mutants were also used to show that resistance of the newly-germinated seedling of H. vulgare to U. hordei and of subsequently formed tillers to infection was not correlated, and was probably not controlled by the same gene or genes.

The application of gibberellic acid to H. vulgare was found to cause an increase in the overall tiller height of healthy plants by increasing the elongation of internodal regions 0-1, 1-2, 2-3, and 3-4. There was no increased elongation of internodal regions 4-5 and 5-6. The healthy tillers of diseased plants showed no internodal elongation when gibberellic acid was applied. Diseased tillers, which are usually shorter than healthy tillers, were also unaffected by the presence of gibberellic acid.

The injection of both mating types of U. hordei into the young developing spike of a normally resistant strain of H. vulgare resulted in the production of diseased spikes. This would indicate that blockage to normal infection occurs at the time of seedling penetration, at the level of tiller primordia development, or at the time of spike primordia development.

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INTRODUCTION

Covered smut is a disease of cultivated barley (*Hordeum vulgare* L.) caused by *Ustilago hordei* (Pers) Lagerh. With covered smut of barley, as with most other plant diseases, the infection process and subsequent events which take place during the development and expression of the disease are poorly understood (Fisher and Holton 1957).

The culmination of the covered smut disease of barley is the production of diploid teliospores. In the normal course of events these are disseminated to healthy seeds and the disease process is reinitiated. Immediately following germination each teliospore produces an ordered tetrad from which four haploid sporidial cultures can be developed. The haploid cultures can be grown *in vitro* and compatible pairs brought together to produce a dikaryon, which initiates the infective phase (Figure 1). The dikaryon cannot normally be grown, as such, *in vitro*. Infective hyphae or "suchfaden" are formed by the dikaryon to facilitate its entry into the seedling during germination of the seed.

A number of UV-induced biochemical mutants were produced from *U.hordei* by Hood (1966) and are being maintained in the Botany Department at the University of British Columbia. The use of biochemical mutants affords an opportunity for investigating the nutritional needs of

U.hordei during its infective (dikaryotic) stage. The use of "marker" genes, (one or more mutant genes carried by either or both nuclei of the infective dikaryon), also affords an opportunity for determining whether parasexual recombination occurs within the host plant prior to the production of teliospores. By using marker genes it should be possible to determine whether multiple infections can occur in this specific disease system. Parts of the present study were directed to the problems of nutritional requirement and multiple infection.

Farrar (1958) has reported that the use of gibberellic acid (500 ppm) on Sorghum vulgare inoculated with Sphacelotheca sorghi resulted in a decrease in the percentage of smutted plants. The effects of gibberellic acid on the U.hordei / H.vulgare system were investigated in another part of this study.

Figure 1

Ustilago hordei Life Cycle

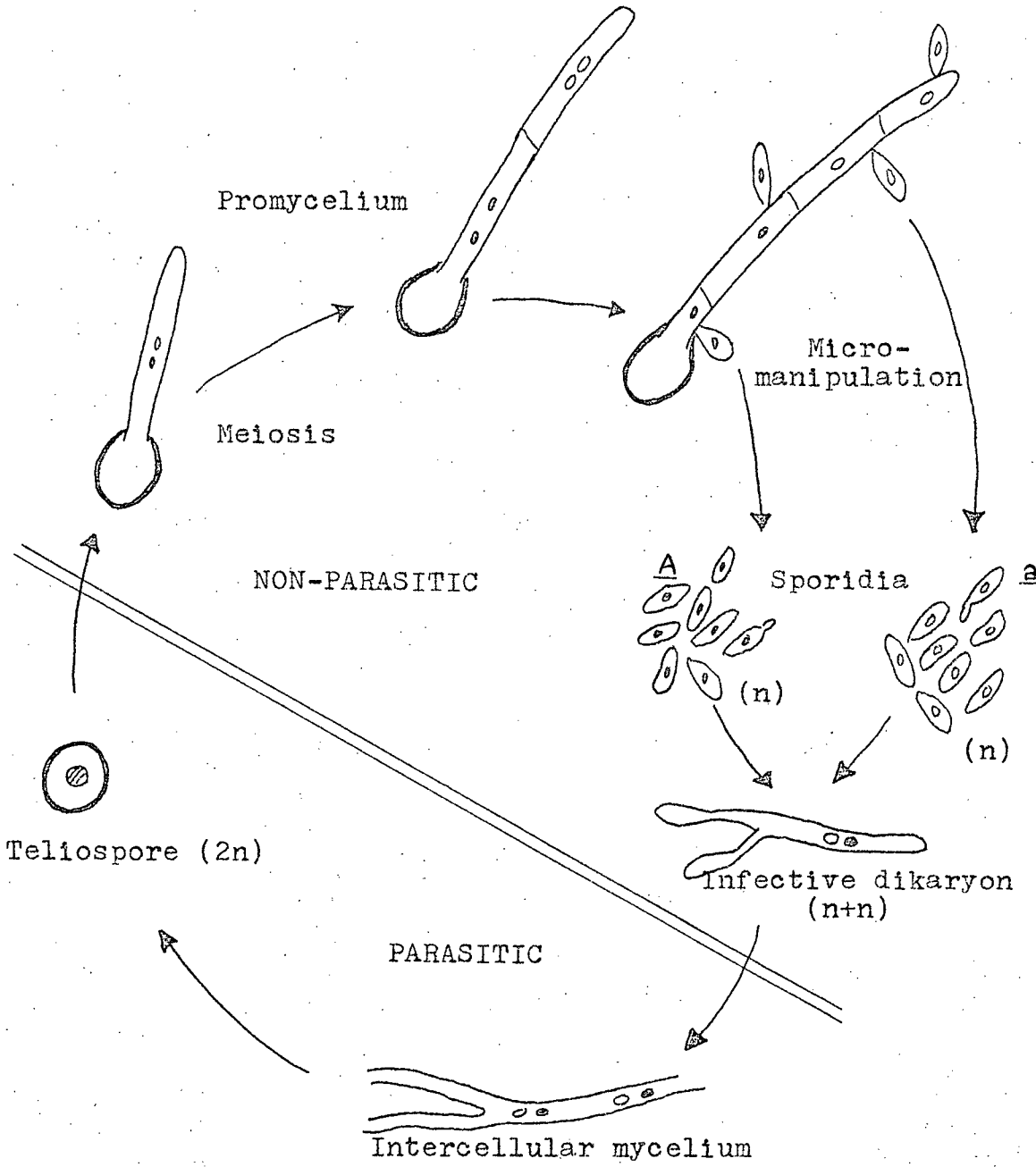
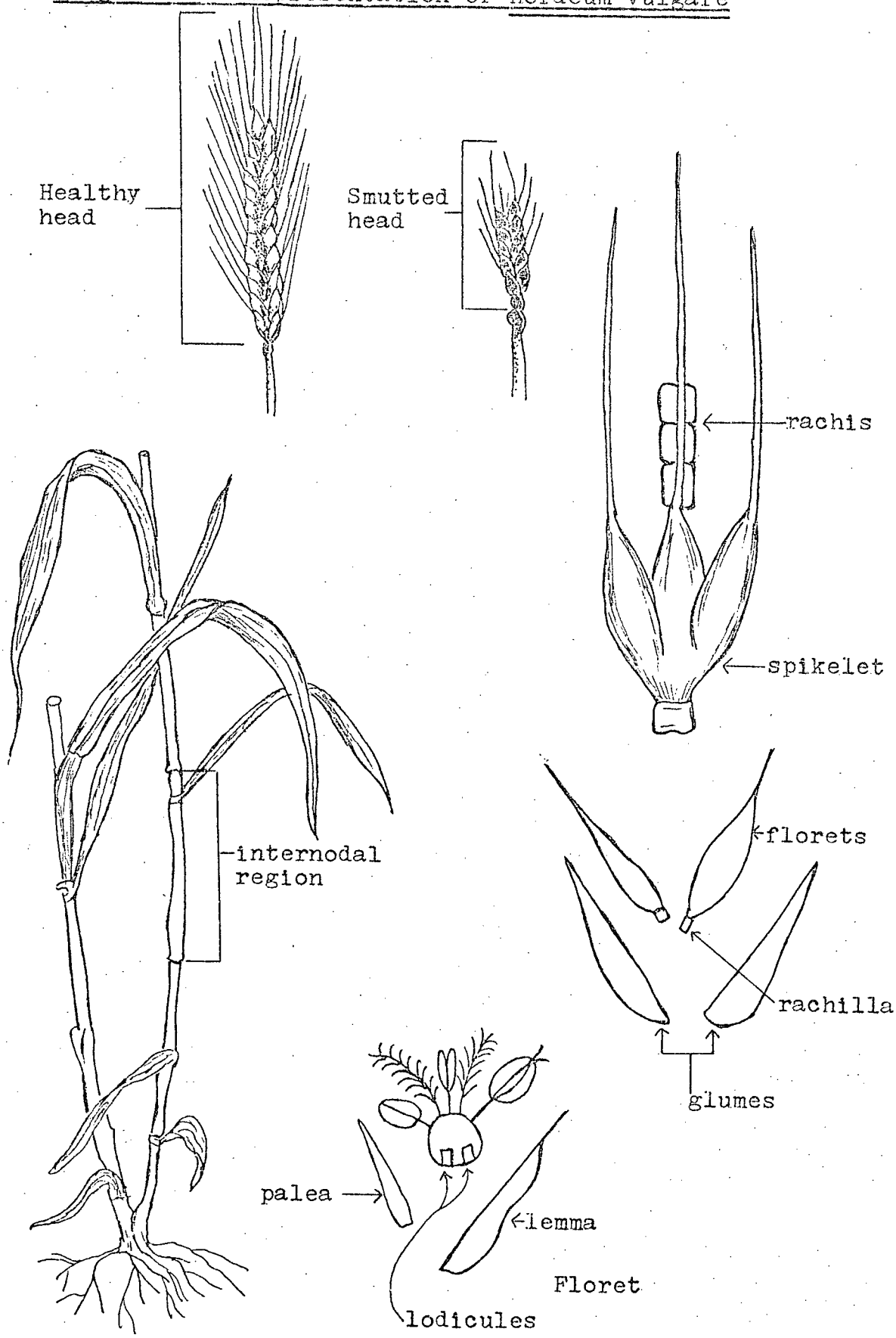


Figure 2

Diagrammatic Representation Of Hordeum vulgare



MATERIALS AND METHODS

A. General Techniques

1. Barley Varieties

Four varieties of barley were used in this study. These, listed in order of increasing resistance to the parental strain of U.hordei used in this study, are as follows: Vantage, Himalaya, Conquest, and Excelsior (Shand, personal communication).

2. Ustilago hordei Cultures

The two wild-type (prototrophic) strains are designated E_3a and I_4A (Hood 1966). As the mutant strains were all derived from one or the other of these two parental strains, E_3a and I_4A are maintained as "standard" strains for testing for auxotrophy and for mating type. Mating occurs between "A" and "a" strains to produce the dikaryon which, if it is infective, can initiate the disease.

The auxotrophs used in the experiments all had a single nutritional requirement not present in the parental strains. They all grew on complete medium and on minimal medium with the addition of the required supplement. The mutants normally showed no growth on minimal medium or on minimal medium that had been supplemented by a nutritional factor other than the one required. Only those nutritional

mutants for which both mating types were available were chosen for this study.

3. Media

The media used were of two types; complete and minimal.

i Complete medium

20 ml	salt solution
1000 ml	distilled H_2O
50 ml	tryptophane
5 gm	casein hydrolysate (vitamin and salt free)
5 gm	yeast extract
10 gm	dextrose
20 gm	agar
10 ml	vitamin solution - added after autoclaving media to prevent vitamin breakdown

mixture steam autoclaved at 121°C for 15 minutes

ii Minimal medium

20 ml	salt solution
1000 ml	distilled H_2O
20 gm	agar
10 gm	dextrose

mixture steam autoclaved at 121°C for 15 minutes
nutritional supplements were added after autoclaving

iii Salt solution

123 gm	Na_3 citrate $\cdot$ $2H_2O$
250 gm	KH_2PO_4- monobasic
100 gm	NH_4NO_3- anhydride
10 gm	$MgSO_4 \cdot 7H_2O$
5 gm	$CaCl_2 \cdot 2H_2O$
5 ml	trace element solution
750 ml	distilled H_2O
2 ml	chloroform

iv Trace element solution

5 gm	citric acid $1H_2O$
5 gm	$ZnSO_4 \cdot 7H_2O$
1 gm	$Fe(NH_4)_2 \cdot (SO_4)_2 \cdot 6H_2O$
0.25 gm	$CuSO_4 \cdot 5H_2O$
0.05 gm	$MnSO_4 \cdot 1H_2O$
0.05 gm	H_3BO_3- anhydride
0.05 gm	$NaMoO_4 \cdot 2H_2O$
1 ml	chloroform
95 ml	distilled H_2O

v Vitamin solution

100 mg	thiamin
50 mg	riboflavin
50 mg	pyridoxine
200 mg	Ca pantothenate
50 mg	p-amino-benzoic acid
200 mg	nicotinic acid
200 mg	choline chloride
400 mg	inositol
50 mg	folic acid
1000 ml	distilled H_2O

vi Nutritional supplements

Stock solutions;

amino acids 500 mg added to 75 ml H_2O and 25 ml ethanol

bases 50 mg added to 75 ml H_2O and 25 ml ethanol

vitamins 5 mg added to 100 ml H_2O

The above solutions were passed through a sterile millipore filter (0.22u) and stored in a sterile flask at 4°C. For use as nutritional additives, 2 ml of stock solution was added per 100 ml of hot, freshly autoclaved minimal medium.

4. Maintenace of Cultures

Cultures were stored on silica gel following the method of Perkins (1962) as modified by Shand (personal communication). Fresh cultures of the strain desired were obtained by placing several crystals of silica gel on a slant of complete medium and incubating at 22°C. After approximately 14 days the culture had grown sufficiently to permit transfer to supplemented minimal medium (hereafter called min(+)). After one week of growth these could be stored on slants, as stock cultures, at 4°C for up to six weeks.

To obtain the culture for inoculation, a portion of the stock culture was transferred to 50 ml of min(+) medium in a 250 ml Erlenmeyer flask and allowed to grow at 22°C on a shaking platform. When the cell count reached approximately 10^5 cells per ml, the cells were diluted, rapidly shaken, and the liquid spread on min(+) agar plates to obtain isolated colonies arising from a single cell. A colony was then selected, tested for its deficiency, placed on a slant, allowed to grow for one week and transferred to liquid medium from which the inoculum was obtained.

5. Nutritional Testing

To determine whether or not the mutant had remained constant, or to determine the requirements of an unknown mutant, nutritional testing was carried out.

The mutant to be tested was grown on complete medium for 5 days and then transferred by the replica plating method to minimal medium and to the various test plates. A single-mutant culture should show no growth on minimal, and growth on only one of the min(+) PLATES. Multiple mutants must be tested on a series of plates in which the suspected combinations of the nutritional requirements are represented.

6. Seed Preparation

Seed to be inoculated was first given an anaerobic/formaldehyde treatment to eliminate contamination by unwanted microorganisms. An Erlenmyer flask was half filled with barley seed and the flask filled with water. After six hours the water was drained off. A rubber balloon was placed over the neck of the flask and the flask incubated at 22°C for 48 hours. The conditions in the flask are such that aerobic organisms such as loose smut (Ustilago nuda) are able to germinate. The conditions however soon became anaerobic with the subsequent death of aerobic organisms. Following this treatment the seeds were dried, immersed in a 1/400 formaldehyde/water

solution for one hour, washed in running tap water for ½ an hour, dried on paper towels, and stored in glass jars for later use.

7. Mating Type Test

To test the mating type a liquid culture of each of the two parental types was prepared, (10^7-10^8 cells/ml). Each liquid culture was then spread evenly over the surface of a separate minimal or a minimal (+) plate, and the cells were allowed to grow for 2 days at 22°C, creating a lawn of growth over the surface of the plate. Several separate drops of the culture to be tested (liquid-conc 10^7-10^8 cells/ml) were placed on the plates and incubated at 20°C for 72 hours. The presence of infection hyphae (aerial hyphae) indicated a compatible reaction (i.e. that the two cultures were of opposite mating type).

8. Planting

Field:

The seeds in the fields were planted in 10 foot rows, 120 seeds per row, then thinned to 100 plants per row. Seeding was done either by hand or with a V-belt planter.

Greenhouse:

Pots with a diameter of 6½ inches were planted with twelve seeds, then thinned to six plants per pot. The pots were placed under lights and the day length adjusted to correspond to that of the normal growing season.

9. Bleach Treatment of Spores Before Germination

Bleach treatment is a method for surface sterilization of spores to prevent bacterial contamination of the media. The spores to be treated were suspended for 1 hour in 2 ml of sterile water. After this period, 0.5 ml of a 5 percent solution of chlorine bleach was added, the spores were shaken for 30 seconds, and then the solution was poured onto a filter paper in a Buchner funnel where they were washed for 2-3 minutes with sterile distilled water. The filter paper with the spores was then added to a flask of complete medium and allowed to shake for 1 hour, after which time the filter paper was removed and the flask allowed to continue shaking.

10. Isolation and identification of teliospores

Teliospores were removed from each panicle after the head had been allowed to dry for several days placed into complete liquid medium, and grown for 48 hours in a New Brunswick Psycrotherm R-27 shaker incubator at 23°C. The medium contained tetracycline at a concentration of .01 mg per ml to inhibit bacterial growth. At the end of 48 hours the resulting culture (i.e. a mixture of teliospores and haploid sporidia derived from them), was diluted to 500 sporidia per ml and spread at a concentration of 50 sporidia per plate on complete medium. After 72 hours the colonies arising from individual

sporidia were transferred to complete media and were allowed to grow for 48 hours. They were then replicated onto the various media for testing (colonies which produced suchfäden were presumed to have arisen either from a teliospore or from a compatible pair of sporidia).

B. Specific Techniques

1. Multiple Infection

To demonstrate the effect of inoculating barley seed with several separate mutants, ten mutant cultures showing the highest pathogenicity on Vantage in the 1967 University of British Columbia field tests (Shand personal communication) were crossed, in compatible combinations, with both wild and mutant partners. The resulting dikaryons were heterozygous (with wild partner) or homozygous (with mutant partner) for the mutant deficiency. In a second series of inoculations, all the mutant cultures of a single mating type were mixed together and this mixture was used in compatible combination with the wild-type culture to produce an inoculum capable of producing a number of different dikaryons. The teliospores produced from these crosses were collected and an attempt was made to identify the nutritional deficiencies of the resulting sporidia.

The relationship between the percentage of plants smutted and the percentage of tillers smutted was also investigated.

2. Effects of Gibberellic Acid on *Ustilago hordei* Host-parasite System

To test the effect of the fungus upon the overall

length of the tillers of the barley plant, one hundred seeds of Vantage barley were infected with I_4A and E_3a. The seeds were planted in rows, 15 seeds per row, and thinned to 10 plants per row (approximately one foot between each plant).

After the plants had matured they were pulled up and the length of the tillers measured from the crown to the base of the spike.

The effect of gibberellic acid (500 ppm) was determined by using four experimental categories of plants. Each category contained 100 plants.

1. control: uninoculated seeds; no gibberellic acid
2. gibberellic acid control: uninoculated seeds; plants sprayed with gibberellic acid throughout growth.
3. I_4A and E_3a: seeds inoculated with the fungus: plants not treated with gibberellic acid.
4. inoculated gibberellic: plants from seed inoculated with the fungus; sprayed with gibberellic acid throughout growth.

The percentage of smutted tillers and smutted plants was determined. Using *t* and *F* tests the effect of gibberellic acid on tiller length and internodal elongation was measured.

3. Injection of Resistant Strains

Seeds of Vantage, Himalaya, Excelsior, and Conquest

barley were divided each into three groups and planted in the greenhouse. The first group of seeds was planted after inoculation with I_4A and E_3a, and acted as a control group to show whether or not infection occurred through the seed of each variety. The second group of seeds was planted and after germination the plants were injected every second day with a mixture of I_4A and E_3a; this group served to show whether inoculation by injection could result in development of the disease. The third group contained uninoculated seeds that were allowed to grow and the resulting plants were not injected with the fungus; this group served as the uninoculated control group.

RESULTS AND DISCUSSION

A. Multiple Infection

In order to determine whether an infected tiller can carry more than one genotype of the pathogen, plants were inoculated with a mixture of haploid (gametic) cultures and either of the wild mating types. All haploid cultures were of a single mating type but each carried a different genetic marker. As the inoculum was capable of forming a number of heterozygous dikaryons, each with a different marker, the recovery from a single infected tiller of two or more genetic markers would indicate that two or more dikaryotic infections had taken place. The relationship between infectivity taken on the basis of total plants and infectivity taken on the basis of total tillers was also determined. Virulence of a smut culture is usually estimated on the basis of percentage of smutted tillers per row or plot. Thus, an estimate of fifty percent smutted tillers could mean either that fifty percent of the plants were healthy and fifty percent completely diseased, or that all plants were diseased with an average of fifty percent smutted tillers per diseased plant. The procedures in this series of experiments were designed with this problem in view.

A high percentage of the total number of inoculated seeds usually gave rise to plants that had no smutted

tillers. The first three cross types of Table I show the kind of result that is usually obtained: approximately half the plants showed no evidence of the disease; about 1/5 of the plants showed disease in all tillers; and the remaining plants showed both healthy and diseased tillers.

Plants that have been infected with a heterozygous dikaryon (e.g. I_4A X pantothenic acid V359a) showed a varied percentage of tillers infected, depending on the cross (Table I). The percentage of plants with no infected tillers (Table II) may be as high as 70 percent, as with pantothenic V359a X I_4A, Adenine V175a X I_4A, argenine X52a X I_4A, niacin X95a X I_4A, and niacin X95A X E_3a, or as low as 20 percent with histidine V466a X I_4A, and leucine U50a X I_4A. The variations may have been due to differences in infectivity or to differences in later stages of the parasitic phase, but further studies would be necessary to establish which was the case.

Crosses made to E_3a in all cases but one (histidine V466) gave a much higher percentage of totally infected plants than did crosses to I_4A. The crosses made to I_4A varied from zero to 20 percent of the plants totally infected (except histidine V466) whereas the same crosses made to the E_3a varied from 25 percent to 50 percent of the plants being totally infected (except pyridoxine V26, serine V5, and niacin X95). This may indicate that the nucleus of the A mating type carries a greater genetic

Table I

Cross Type	Percentage Of Plants With The Following Percentage Of Infected Tillers						
	0	1-19	20-39	40-59	60-79	80-99	100
e_a X A*[1]	43	6	13	6	9	8	16
I_A X a*[2]	44	5	12	11	8	2	18
A* X a*	57	6	13	7	3	2	12
E_a X Pan V359A	25	13	-	12	-	-	50
E_a X Pdx V26A	57	28	-	-	15	-	-
E_a X Ad V175A	57	-	-	-	-	-	43
E_a X Pro V234A	56	-	-	11	-	-	33
E_a X Ser V5A	60	7	-	-	13	13	7
E_a X Arg X52A	28	-	-	15	-	15	42
E_a X His V466A	40	20	10	10	-	-	20
E_a X Leu U50A	42	30	-	-	-	-	28
E_a X Nia X95A	67	-	17	8	-	-	8
E_a X Met V241A	42	-	-	15	15	-	28
I_A X Pan V359a	70	-	10	10	-	-	10
I_A X Pdx V26a	50	16	-	-	-	16	18
I_A X Ad V175A	70	-	10	-	-	10	10
I_A X Pro V324a	57	15	-	-	28	-	-
I_A X Arg X52a	67	11	11	-	11	-	-
I_A X Ser V5a	57	15	14	-	14	-	-
I_A X His V466a	20	10	-	10	20	10	30

1 A* = mixture of all A type mutants

2 a* = mixture of all a type mutants

Table II

Percentage Of Plants With 0 Percent And 100 Percent Of Tillers Infected

Mutant			Wild type	Percentage of plants with no diseased tillers	Percentage of plants with 100% of tillers diseased
pantothenic acid	V359	A	E_3a	31	50
		a	I_4A	70	10
pyridoxine	V26	A	E_3a	60	0
		a	I_4A	50	20
adenine	V175	A	E_3a	65	40
		a	I_4A	70	15
proline	V324	A	E_3a	60	35
		a	I_4A	60	0
argenine	X52	A	E_3a	30	40
		a	I_4A	70	0
serine	V5	A	E_3a	60	10
		a	I_4A	60	0
histidine	V466	A	E_3a	40	20
		a	I_4A	20	30
leucine	U50	A	E_3a	40	30
		a	I_4A	20	20
niacin	X95	A	E_3a	70	10
		a	I_4A	70	0
methionine	V241	A	E_3a	40	30
		a	I_4A	50	20

Table III

Ratio of Smutted Plants to Smutted Tillers

Cross	Percentage Plants with Smut	Percentage Plants with all Tillers Smutted	Percentage Smutted Tillers of Smutted Plants	Ratio of Percent Smutted Plants to the Percent Smutted Tillers
E_a X pantothenicV359A	75	50	60	1.25
E_a X pyridoxineV26A	43	0	8	5.38
E_a X adenineV175A	43	43	46	0.94
E_a X prolineV324A	44	33	16	2.92
E_a X arginineX52A	71	43	52	1.36
E_a X serineV5A	40	6	18	2.21
E_a X histidineV466A	60	20	19	3.10
E_a X leucineU50A	57	28	27	2.09
E_a X niacinX95A	33	8	12	2.68
E_a X methionineV241A	57	28	37	1.53
I_A X pantothenicV359a	30	10	28	1.07
I_A X pyridoxineV26a	50	12	17	2.98
I_A X adenineV175a	30	10	21	1.45
I_A X prolineV324a	43	0	9	4.92
I_A X arginineX52a	33	0	6	5.72
I_A X serineV5a	43	0	7	5.88
I_A X histidineV466a	80	30	39	2.07
I_A X leucineU50a	86	14	50	1.72
I_A X niacinX95a	29	0	14	2.08
I_A X methionineV241a	50	14	22	2.32

potential for pathogenicity. The variability in the percentage of diseased plants, using different heterozygous dikaryons, is also shown by the data of Table III.

The percentage of total tillers smutted was nearly always less than the percentage of total plants smutted (Table III). Using the data of Table III, the correlation between infectivity on the basis of plants (i.e. percentage of total plants with one or more infected tillers) and on the basis of tillers (i.e. percentage of infected tillers in plants with at least one tiller known to have been infected) was calculated. The results are given in Table IV. The resultant correlation coefficient (0.35) is low and it can be concluded that the percentage of diseased tillers and percentage of diseased plants are not interdependent. The low correlation would indicate that if resistance of the host to U. hordei was genetically determined, the resistance to infection of the seed and subsequent infection of the tiller were not related, and probably were not controlled by the same gene or genes. The ability of the fungus to infect the secondary primordia seemed not to be correlated with the ability to penetrate the original primordia. This could be a result of the presence of different genes governing the two events.

The fungus may possess a gene (or genes) for infectivity that counteracts the gene(s) for resistance. Variability in both these genetic systems may account

Table IV

Correlation Coefficient For Diseased Tillers Versus Diseased Plants

X_1 = percent plants with smut
X_2 = percent smutted tillers of smutted plants
EX_1 = 980
EX_2 = 567
$Ex_1x_2/(n-1)$ = covariance
$b_{1,2}$ = regression coefficient
$b_{2,1}$ = regression coefficient
$r_{1,2}$ = correlation coefficient

$$Ex_1x_2 = EX_1X_2 - \frac{EX_1X_2}{n} = 30,895.00 - 555,660/20 = 3,112.00$$

$$b_{1,2} = \frac{Ex_1x_2/(n-1)}{Ex_2^2/(n-1)} = \frac{163.78}{711.08} = 0.23$$

$$b_{2,1} = \frac{Ex_1x_2/(n-1)}{Ex_1^2/(n-1)} = \frac{163.78}{308.42} = 0.53$$

$$r_{1,2} = (b_{1,2})(b_{2,1}) = (0.23)(0.53) = 0.12 = 0.35$$

Confidence Limits; P=0.95 (-0.1 to +0.65)

for some of the correlation. Because the correlation was positive one would expect that a culture that is going to infect more seedlings is also going to give a larger number of infected tillers per diseased plant following this infective event.

To investigate the possibility of mitotic recombination in U. hordei, ten biochemical mutants were chosen (Table V). To insure that these mutants were stable the tests shown in Table V were carried out.

In addition to the crosses listed in Table I, crosses were also made to produce the homozygous dikaryon of each mutant. Smutted tillers were produced only after inoculation with heterozygous dikaryons (Table I); these tillers were collected and a random selection of teliospores was germinated and the resulting sporidia were tested for biochemical deficiencies. The expected sporidial types were recovered in all cases. In the cross E_3 X A^* several recombinant sporidia were also recovered (Table VI).

Testing of the crosses I_4A X a^* and A^* X a^* yielded the two wild mating types only. The isolation of both mating types from both crosses demonstrates that recombination had occurred, as only a single wild mating type was used during infection of the seeds. Isolation of the various biochemical mutants from these two crosses was not successful, probably because of the isolation techniques used. The isolation involved randomly selected

Table VI

Resultant Sporidial Types Obtained for the Cross E_3a X A*

Mutants showing absolute requirement for supplements.

1 adenine
2 adenine, serine, pyridoxine
3 adenine, proline, histidine
4 adenine, pyridoxine

Mutants that show slow growth unless certain requirements added--requirements essential for any growth shown before the brackets, requirements that must be added to achieve growth equivalent to that demonstrated by the parental types shown in brackets.

1 adenine, pantothenic acid (niacin, proline, serine, and pyridoxine)

2 adenine, serine (niacin, proline, pantothenic acid, pyridoxine and arginine)

3 adenine, proline (serine, and histidine)

4 adenine, proline (serine, pantothenic acid, arginine, and histidine)

Table V

Growth of Mutant Cultures on Various Media

Mutant	Growth on Complete Medium	Growth on Minimal Medium	Growth on Minimal Plus Nutritional Requirement	Mating with E_3a	Mating with I_4A
pantothenicV359a	+	-	+	-	+
V359A	+	-	+	+	-
pyridoxineV26a	+	-	+	-	+
V26A	+	-	+	+	-
adenineV175a	+	-	+	-	+
V175A	+	-	+	+	-
prolineV324a	+	-	+	-	+
V324A	+	-	+	+	-
arginineX52a	+	-	+	-	+
X52A	+	-	+	+	-
serineV5a	+	-	+	-	+
V5A	+	-	+	+	-
histidineV466a	+	-	+	-	+
V466A	+	-	+	+	-
leucineU50a	+	-	+	-	+
U50A	+	-	+	+	-
niacinX95a	+	-	+	-	+
X95A	+	-	+	+	-
methionineV241a	+	-	+	-	+
V241A	+	-	+	+	-
E_3a	+	+		-	+
I_4A	+	+		+	-

+ indicates growth
- indicates no growth

individual teliospores which had been allowed to germinate and produce sporidia. The sporidia were then isolated and allowed to grow on plates. Resultant colonies were chosen and identified by nutritional testing. The wild type colonies probably outgrew the mutants, and thus prevented the isolation of mutants by this method. This problem could be overcome by the early separation, by micromanipulator, of the products of a single tetrad.

Crossing each of the mutants individually with the opposite wild mating type yielded non-mutants of both mating types as well as the original mutant; however, no recombinant mutant of the opposite mating type was recovered.

The micromanipulator was used to separate the products of the tetrads produced by the E_3a X A^* cross. Teliospores were isolated and placed onto small (2 cm^2) blocks of complete agar medium, allowed to germinate and then, using a micromanipulator, individual sporidia were isolated and placed on individual agar blocks. These sporidia were allowed to grow and the resultant colonies were identified as to their nutritional requirements.

Using the micromanipulator on the E_3a X A^* cross, eight mutant sporidia were isolated from germinating teliospores; the eight resulting cultures were from eight different teliospores, and represented a very small

sample of the potential variability of this cross. The variation in the amount of growth of these eight cultures when replicated to different media should be noted. As an example of this variation, mutant VIb 9 (Table VII) grew well on media that lack methionine, leucine, or histidine; the mutant had the enzyme systems available to produce its own supply of these requirements. There was no growth on media deficient in adenine or serine; these nutrients must be supplied. The media that were deficient in pyridoxine, pantothenic acid, proline, niacin, or arginine, support very limited growth. There were several possible explanations for this result. Gene interaction may be involved whereby the presence of one gene was suppressing enzyme production of another gene; alternatively, a dosage effect might allow only a small amount of a certain compound to be produced. A recombination could have resulted in the organism receiving only a certain fraction of the entire gene dosage needed for adequate production of the final compound. The life cycle of U. hordei involves the formation of a "double haploid" hypha called a dikaryon (Figure 1). One nucleus of the dikaryon may exert greater influence on the growth of the fungus than the other nucleus. If the controlling nucleus was deficient for a gene, the secondary nucleus may be able to provide a small amount of the deficient product, resulting in minimal growth of

the fungus in the absence of an external supply of the product.

The isolation of multiple mutants indicated that a series of recombinations had taken place between the two nuclei of the dikaryon. It may also indicate that fusion of independent dikaryons had occurred either in the inoculum prior to infection or within the infected plant.

The linkage relationships of the ten markers used in this experiment are not known. If it is assumed that the recombinational event involved linked markers, such a process would require formation of a diploid nucleus followed by mitotic crossing over. To obtain a third marker in the same nucleus, a mitotic crossing over would need to occur between the haploid nucleus with two markers and another haploid nucleus. To obtain a third haploid nucleus, some type of mycelial fusion and nuclear transfer would have to occur. There is however, no evidence that mycelial fusion occurs in basidiomycetes.

The possibility that transformation has occurred is also present. During the inoculation of the seeds, or in the plants, some cells may be destroyed, liberating free deoxyribonucleic acid that could be taken up by the young growing hyphae.

This experiment has also provided the beginnings of a system for studying competition. Table VI shows the mutants that have resulted from the cross $E_3a \times A^*$,

each mutant contains an adenine deficiency. The high frequency of adenine-deficient mutants would indicate a low survival rate for the gene or genes involved in adenine synthesis. This frequency, at which the various mutants appear in the population, could be used as a means of determining genome frequency for the mutant genes.

Table VII

Tests on Three Multiple Mutants

Growth on complete plates minus the following nutrients

Nutrient	Mutant		
	VIb 12	VIb 9	VIa 1
methionine	3	3	3
leucine	3	3	3
niacin	1	1	2
histidine	2	2	2
adenine	0	0	0
proline	1	1	3
serine	1	0	0
pantothenic acid	0	1	2
arginine	2	1	2
pyridoxine	1	1	0

Growth on minimal plates with the following single nutrient added.

Nutrient	Mutant		
	VIb 12	VIb 9	VIa 1
methionine	0	0	0
leucine	0	0	0
niacin	0	0	0
histidine	0	0	0
adenine	0	0	0
proline	0	0	0
serine	0	0	0
pantothenic acid	0	0	0
arginine	0	0	0
pyridoxine	0	0	0
complete	4	4	4
minimal	0	0	0

4 = wild type growth
0 = no growth
1-3 = variation in amount of growth between none and wild type.

B. The Effects of Gibberellic Acid on Inoculated and Uninoculated Barley Plants

The first part of the experiment was a study of tiller elongation following treatment with gibberellic acid. The effect of U. hordei on the total tiller length of plants that were not treated with gibberellic acid was first studied. By measuring the internodal distances on healthy and diseased, treated and untreated plants the effect of gibberellic acid on tiller elongation in barley was also determined.

The second objective of the experiment was to determine if gibberellic acid treatment reduced the percentage of diseased tillers on barley plants inoculated with U. hordei.

1. Plants not treated with gibberellic acid.

Using a table of random number, 108 non-smutted tillers were chosen from one hundred plants with both smutted and non-smutted tillers. When a nonsmutted tiller had been chosen, a smutted tiller from the same plant was selected, again by random numbers. As a student t test was to be used it was essential that the smutted and non-smutted tillers be matched as closely as possible and that no genetic variability affected the calculations of the matched tillers. Lengths of the 108 pairs of tillers are recorded in Table VIII, which also shows the y value (the difference between non-smutted

Table VIII

Tiller Length in Centimeters

Smutted	Nonsmutted	Y	Smutted	Nonsmutted	Y	Smutted	Nonsmutted	Y
70	84	14	40	75	35	55	75	20
58	64	6	50	75	25	50	55	5
52	76	24	40	60	20	40	60	20
52	78	26	55	75	20	50	80	30
30	48	18	40	60	20	50	70	20
52	66	14	45	53	8	65	70	5
55	42	13	30	45	15	40	46	6
40	69	29	48	55	7	45	60	15
50	80	30	40	60	20	50	80	30
48	56	8	56	70	14	50	55	5
60	80	20	60	85	25	60	65	5
65	80	15	30	53	23	34	55	21
45	65	20	30	53	23	60	68	8
50	82	32	60	78	18	30	45	15
40	45	5	50	66	16	35	70	35
38	55	17	35	55	20	55	65	10
60	60	0	45	65	20	70	80	10
50	45	5	55	90	35	20	58	38
60	75	15	40	50	10	30	52	22
40	42	2	60	75	15	40	65	25
45	70	25	50	60	10	44	55	11
45	66	21	40	46	6	50	60	10
43	48	5	60	83	23	60	86	26
52	70	18	55	75	20	34	45	11
33	60	27	30	50	20	30	45	15
57	70	13	45	60	15	30	46	16

Table VIII continued

Smutted	Nonsmutted	Y	Smutted	Nonsmutted	Y	Smutted	Nonsmutted	Y
42	53	11	45	53	8	40	65	25
55	70	15	55	80	25	55	70	15
60	73	13	42	65	23	50	70	20
60	84	24	50	60	10	30	45	15
40	65	25	60	70	10	55	70	15
55	80	25	40	35	5	30	22	8
40	40	0	40	55	15	35	46	11
65	73	8	48	70	22	26	46	11
40	40	0	60	76	16	36	65	29
50	60	10	30	50	20	40	65	25

Table IX

t-test to Determine Equality of the Population Means of Smutted and Nonsmutted Tillers

n = 108
E smutted = 5030.00
E nonsmutted = 6775
E y = 1745

The null hypothesis is that the presence of U. hordei does not effect the tiller length of thr barley plant and therefore the two population means (smutted and nonsmutted) will be equivalent.

u = 0	$(Ey)^2$	= 3,045,025.00	SS	= 9,633.00
n = 108	$(Ey)^2/n$	= 28,194.67	S^2	= 90.02
Ey = 1745	Ey^2	= 37,827.00	S^2/n	= 0.83
$\bar{y}$ = 16.15				

$$S^2/n = 0.83 = 0.911$$

$$t = \bar{y} - 0 / S^2/n = 16.15/0.911 = 17.74$$ with 107 degrees of freedom

At 0.5 percentage point of the t-distribution with 107 degrees of freedom, t 2.625 ; since t-calc. = 17.74,

the hypothesis that the population mean is equal to zero or that the presence of U. hordei does not effect tiller length is rejected.

and smutted tillers).

To test the effect of U. hordei, the null hypothesis was taken to represent that there was no difference in the lengths of smutted and non-smutted tillers. As is shown in Table IX, the calculated value of t (17.74) was considerably greater than the t table value (2.625). From this it was concluded that the null hypothesis did not apply, and that smutted tillers are shorter than those which show no smut. (No completely smutted plant was used in this calculation. However it may be mentioned that plants that were completely smutted were always visibly dwarfed).

2. The effect of Gibberellic acid on smutted and non-smutted tillers.

The first objective of the experiment was to determine whether the presence of gibberellic acid reduces the percentage of smutted tillers. The data of Table X would indicate that the gibberellic acid treatment had reduced the percentage of smutted plants. However, the percentage of smutted plants (85%) in the non-treated rows is much higher than normal. The conclusion here is that in this particular case, the presence of gibberellic acid reduced the percentage of smutted plants from 85 to 50 percent. In a similar way, the gibberellic acid treatment reduced the percentage of smutted tillers from 35 to 28 percent. Thus, whether measured in terms of plants or tillers, the gibberellic acid treated plants showed lower levels of the disease. If gibberellic acid treatment resulted in a decrease in the percentage of smutted tillers, it could possibly relate to the difference in relative growth rates of the fungus and the meristem of the host in which it resided.

The second objective of the experiment was to determine the effect of gibberellic acid on total tiller height. For measurement purposes, the ten categories in Table X were used. As different categories contained different total tiller counts, a table of random numbers was used to produce categories with less variability in

Table X

Gibberellic Acid Treatment Categories

1 control plants: seeds not infected

2 gibberellic acid control: seeds not infected

3 seeds infected: plants treated with gibberellic acid
-no smut plants

4 seeds infected: plants treated with gibberellic acid
-plants with some smut-tiller count of unsmutted tillers

seeds infected plants treated with gibberellic acid
-plants totally smutted-no plants in this category

6 seeds infected plants treated with gibberellic acid
-plants with some smut-tiller count of smutted tillers

7 seeds infected no gibberellic acid-no smut plants

8 seeds infected no gibberellic acid-plants with some
smut-tiller count of unsmutted tiller

9 seeds infected no gibberellic acid-plants all smut

10 seeds infected no gibberellic acid-plants with some
smut-tiller count of smutted tillers

Total percentage of smutted tillers on infected gibberellic acid treated plants 97/343 28%

Total percentage of smutted plants in infected gibberellic acid rows 17/34 50%

Total percentage of smutted tillers on infected no gibberellic acid plants 74/210 35%

Total percentage of smutted plants in infected no gibberellic acid rows 17/20 85%

tiller number. Classes 7,8,9 and 10 had very few tillers and the total tiller number for these four classes was used. In all cases only the longest tiller of an individual plant, smutted or unsmutted, was used to provide data for Table XI. Using an F test the relationships between these categories were examined.

The first tests (described in Table XIII), indicate that there was no relationship among the groups. An overall increase in the maximum tiller length over the control as a result of gibberellic acid is shown in Table XIV. If a plant has been inoculated, and some of its tillers develop smut, then gibberellic acid has no effect on the length of the smutted or non-smutted tillers (i.e. the difference in tiller length due to smut is retained Table XV)).

A statistical test was not needed to show that the lengths of smutted and non-smutted tillers were different since $\bar{\bar{y}}$ of the non-smutted tillers was 88.11 (Table XII) and $\bar{\bar{y}}$ of the smutted tillers was 62.00 (Table XII). The control plants, and plants that remained healthy after inoculation were not statistically different (Table XVII). The uninfected tillers of plants showing some infection however were not in the same statistical population as the healthy tillers of the healthy plants (Table XVIII). The experiment showed that gibberellic acid treatment resulted in an increase in the maximum length of uninfected plants. If a

Table XI

Tiller Lengths In Centimetres

Sample	1	2	3	4	6	7	8	9	10
	100	115	95	99	75	105	92	44	75
	106	80	110	95	68	90	95	60	76
	90	120	125	99	44	90	97	75	42
	105	110	112	86	66	90	95	70	72
	85	112	110	85	70		88	43	62
	110	105	115	66	68		90		73
	100	110	112	118	70		90		60
	105	90	90	85	80		90		44
	100	115	110	100	65		92		36
	85	100	105	60	70		75		60
	90	100	120	86	65		90		85
	110	100	105	70	60				
	104	100	105	70	45				
	90	114	110	85	60				
	90	106	100	80	60				
	100	115	100	102	80				
	93	120	105	80	66				
	105	115	80	86	55				
	100	110	106	80	65				
	100	120	107	85	75				

Table XII

Tiller Categories

Sample number	1	2	3	4	6	7	8	9	10
Tiller Length	100	115	95	99	75	105	92	44	75
	106	80	110	95	68	90	95	60	76
	90	120	125	99	44	90	97	75	42
	105	110	112	86	66	90	95	70	72
	85	112	110	85	70		88	43	62
	110	105	115	66	68		90		73
	100	110	112	118	70		90		60
	105	90	90	85	80		90		44
	100	115	110	100	65		92		36
	85	100	105	60	70		75		60
	90	100	120	86	65		90		85
	110	100	105	70	60				
	104	100	105	70	45				
	90	114	110	85	60				
	90	106	100	80	60				
	100	115	100	102	80				
	93	120	105	80	66				
	105	115	80	86	55				
	100	110	106	80	65				
	100	120	107	85	75				
Sample total T	1968	2157	2122	1717	1307	375	994	292	685

Table XII Continued

Sample number	1	2	3	4	6	7	8	9	10
Size of sample n	20	20	20	20	20	4	11	5	11
Sample mean $\bar{y}$	98.40	107.85	106.10	85.85	65.35	93.75	90.36	58.40	62.27
T^2/n	193,651.20	232,632.45	225,144.20	147,404.45	85,412.45	35,156.25	89,821.45	17,052.80	42,656.81

Grand total = 9,333

En = 95

General mean $\bar{\bar{y}}$ = 97.05

$E(T^2/n)$ = 924,487.31

Table XIII

Relationship Among Tiller Heights

G = 9,333

En = 95

$\bar{\bar{y}}$ = 97.05

$E(T^2/n)$ = 924,487.31

G^2 = 87,104,889.00

(I) = G^2/En = 916.893.56

(II) = $E(T^2/n)$ = 924,487.31

(III) = Ey^2 = 933,055.00

among samples (II-I) = 7593.75

within samples (III-II) = 8567.69

total (III-I) = 16161.44

$K-1$ = 5

$En-K$ = 89

$ns^2_{\bar{y}}$ = 1,518.75

s^2p = 96.26

$F = ns^2y/s^2p$ = 1,518.75/96.26 = 15.77

F at 5% - $5/_{89}df$ = 2.3683

$15.77 > 2.3683$

Therefore the population means were not the same.

Table XIV

Relationship Of Gibberellic Acid Treated No-Smut Plants

To Non-Gibberellic Acid Treated No-Smut Plants

G = 6247.00

En = 60

$\bar{\bar{y}}$ = 104.11

$E(T^2/n)$ = 651,427.85

G^2 = 390,025,099.00

(I) = G^2/En = 650,416.81

(II) = $E(T^2/n)$ = 651,427.85

(III) = Ey^2 = 656,575.00

among samples (II-I) = 1011.04

within samples (III-II) = 5147.15

$K-1$ = 2

$En-K$ = 57

$ns^2\bar{y}$ = 505.52

s^2p = 90.30

F = ns^2y/s^2p = 505.52/90.30 = 5.60

F at 5% - $2/_{57}df$ = 3.20

5.60 > 3.20

Therefore the population means were not the same.

Table XV

Relationship Of Healthy Tillers Of Diseased Plants With And Without Gibberellic Acid Treatment

G	= 2711.00		
En	= 31		
$\bar{\bar{y}}$	= 88.11		
$E(T^2/n)$	= 237,255.90		
G^2	= 7,349,521.00		
(I)	$= G^2/En$	= 237,081.32	
(II)	$= E(T^2/n)$	= 237,255.90	
(III)	$= Ey^2$	= 241,155.00	
among samples		(II-I)	= 174.58
within samples		(III-II)	= 3,899.10
total		(III-I)	= 4,073.68
K=1	= 1		
En=K	= 30		
$ns^2_{\bar{y}}$	= 174.58		
s^2p	= 129.97		
F	$= ns^2_{\bar{y}}/s^2p$	= 174.58/129.97	= 1.34
F at 5% - $1/_{30}$ df		= 4.1709	

$1.34 < 4.1709$

Therefore the population had the same means.

Table XVI

Relationship Of Smutted Tillers Of Diseased Plants With And Without Gibberellic Acid Treatment

$G = 2284$

$En = 36$

$\bar{y} = 62.00$

$E(T^2/n) = 145,122.06$

$G^2 = 5,216,656.00$

$(I) = G^2/En = 144,907.11$

$(II) = E(T^2/n) = 145,122.06$

$(III) = Ey^2 = 150,260.00$

among samples $(II-I) = 214.95$

within samples $(III-II) = 5,137.74$

total $(III-I) = 5,352.89$

$K-1 = 2$

$En-K = 33$

$ns^2\bar{y} = 107.47$

$s^2p = 155.68$

$F = ns^2\bar{y}/s^2p = 107.47/155.68 = 0.69$

F at 5% - $2/_{33}df = 3.3000$

$0.69 < 3.3000$

Therefore the populations had the same means.

Table XVII

Relationship Of Non-Inoculated (Healthy) Plants With Inoculated (Healthy) Plants

G = 2343.00

En = 24

$\bar{\bar{y}}$ = 96.07

$E(T^2/n)$ = 228,807.45

G^2 = 5,489,649.00

(I) = G^2/En = 228,735.37

(II) = $E(T^2/n)$ = 228,807.45

(III) = Ey^2 = 230,151.00

among samples (II-I) = 72.08

within samples (III-II) = 1,343.55

$K-1$ = 1

$En-K$ = 22

$ns^2_{\bar{y}}$ = 72.08

s^2p = 61.07

$F = ns^2y/s^2p$ = 72.08/61.07 = 1.18

F at 5% - $1/_{22}df$ = 4.3009

1.18 < 4.3009

Therefore the population had the same means.

Table XVIII

Comparison Of Healthy Tillers Of Diseased And Healthy Plants

$G = 2962$

$En = 31$

$\bar{\bar{y}} = 95.6$

$E(T^2/n) = 284,472.65$

$G^2 = 7,772,444$

$(I) = G^2/En = 250,724$

$(II) = E(T^2/n) = 284,472.65$

$(III) = Ey^2 = 284,982.00$

among samples $(II-I) = 33,748.65$

within samples $(III-II) = 509.35$

$K-1 = 1$

$En-K = 30$

$ns^2_{\bar{y}} = 33,748.65$

$s^2p = 16.95$

$F = ns^2/y/s^2p = 33,748.65/16.95 = 4.170$

F at 5% $1/_{30}df = 4.170$

Therefore the population means were not the same.

plant was diseased the maximum tiller length of the gibberellic acid treated and untreated tillers was not statistically different. Healthy tillers of diseased plants were not as tall as the healthy tillers of healthy plants. Gibberellic acid seemed to produce an effect that was countered by the development of the disease. Smutted tillers were usually shorter than non-smutted tillers, whether or not they have been treated with gibberellic acid.

To determine the effect of gibberellic acid on internodal elongation in the barley plant, the six categories shown in Table XIX were used. From the data, bar graphs were prepared that showed the spread of the internodal distances in the six categories (Figure 3 to Figure 7).

The first bar graph, Figure 3, compares gibberellic acid treated with untreated (control) plants. The gibberellic acid treatment resulted in increased elongation in internodal regions 0-1, 1-2, 2-3, and 3-4, (crown and first node, first and second node, second and third node, and third and fourth node), whereas regions 4-5 and 5-6 showed no increased elongation. The average internodal distances for the final two nodes of both the gibberellic acid treated and untreated controls were very similar (Figure 3).

The next two graphs illustrate the interaction

Table XIX

Internodal Length

Row Type	Internodal Distance					
	Node Number					
	1	2	3	4	5	6
Control	2	5	7	12	23	33.5
	5.5	8	10	13	25	38
	2.5	6	10	13	20.5	31.5
	5.5	11.5	13.5	17.5	28.5	38
	1.5	5.5	10	12.5	22.5	36.5
	3.5	6.5	9	12.5	18	34
	4.5	10	11	12	17.5	22
	6	9	10.5	12	19.5	29.5
	5.2	10.5	12.5	15.5	24.5	38.5
	3	8	13	17.5	30	44
	4	10.5	14.5	18.5	31.5	35
	2	7.5	16.5	17.5	29	41.5
	6.5	9.5	11.5	12	22	23.3
	3.5	7	9.5	12.5	20	32.5
	5	10	14.5	16.5	25	38.5
average	/4.0	/8.3	/11.5	/14.3	/23.8	/34.4

Table XIX

	1	2	3	4	5	6
Smutted Infected No Gibberellic Acid	3	10	13	17.5	20	14
	4	9	15.5	16	13.5	16.5
	5.5	12	14.5	20	12	17
	4	11.5	15	17.5	16	13
	3	10	14	15	12	4
	3	6.5	7	7.5	7.5	6
	2	9	14.5	17.5	13.5	5
	2.5	9	11	14	18	10.5
	2	7	9.5	17.5	16	10
	3	10.5	14	17	17	10
average	/3.2	/9.5	/12.8	/16	/14.6	/10.6

Table XIX

	1	2	3	4	5	6
No Smut Infected No Gibberellic Acid	3	10.4	11.5	12.5	10	30
	3	6	11.5	16	26	34
	4	11	13.5	15	23	29
	5.5	13	16.5	18	20	21
	4	10	14	15	21	28
	4	11	13	15	21	23
	3.5	9.5	14	16.5	24	26
	4.5	11.5	12.5	14	15	18
	3	8	12	14	23	34.5
	3.5	10	13	15	21	27
average	/3.8	/10	/13.2	/15.1	/20.4	/27

Table XIX

	1	2	3	4	5	6
Smutted Infected Gibberellic Acid	3	9	13	15.5	17	10
	3	8	15.5	18	22	8
	2.5	5.5	15	19.5	26.5	15
	1	4.5	14	18	21	23
	3	6.5	14.5	18.5	21	12
	2	4	9	15	17.5	13
	4.5	10.5	15	18	11	8
	3	6.5	13	13	8.5	3
	3	4.5	13	17	22.5	21
	3	2	11	15.5	16	8
	1	7	13	14	15.5	7
	4.5	5.5	8	17	21	8
	3.5	5	15	17.5	19.5	9
	4.5	11.5	15.5	17.5	20	12.5
	2.5	4	10.5	17	17.5	12
average	/2.9	/6.3	/13	/16.7	/18.4	/11.3

Table XIX

	1	2	3	4	5	6
No Smut Infected Gibberellic Acid	7	13	18	20.5	20.5	17.5
	5	11	15	18.5	27.5	41.5
	6.5	10	14.5	16	24	30
	7	12	16.5	17	24	38.5
	2.5	9	16	18.5	23	30
	4.5	11.5	14.5	17	22.5	19
	4.5	8	13	17	16.5	30
	7.5	14.5	16	18.5	22	25.5
	4.5	10.5	15	17	21	40
	8	12	15	17.5	21.5	38
	9	12	16	17.5	17.5	20.5
	9.5	13.5	19	21.5	27	20.5
	7	11.5	16	18	23	32.5
	8	10	14	18	26	39
	6.5	12	15.5	18	25	31.5
average	/6.5	/11.4	/15.6	/18	/22.7	/30.3

Table XIX

	1	2	3	4	5	6
Gibberellic Acid	3.5	7.5	11	16	14.5	29.5
Control	5.5	10.5	14	16	23	33
	5.5	10.5	14	18	22.5	30
	8	13.5	18.5	18.5	28	37.5
	3	11	15	17.5	26	45.5
	2.5	10	15.5	20	26	36
	9.5	14	19	20	26	35
	4	11	16.5	20.5	28	39
	7	13	19.5	20.5	25	26
	9	15	17	20	22	28.5
	6.5	12.5	16.5	18	26.5	37.5
	2	10	15.5	19.5	26	32
	4.5	9.5	14.5	16.5	19	36.5
	7	12.5	17	17.5	19.5	20.5
	6	10	14	17.5	18	29
average	/5.6	/11.4	/15.8	/18.4	/23.3	/33.

Explanation of Facing Figure

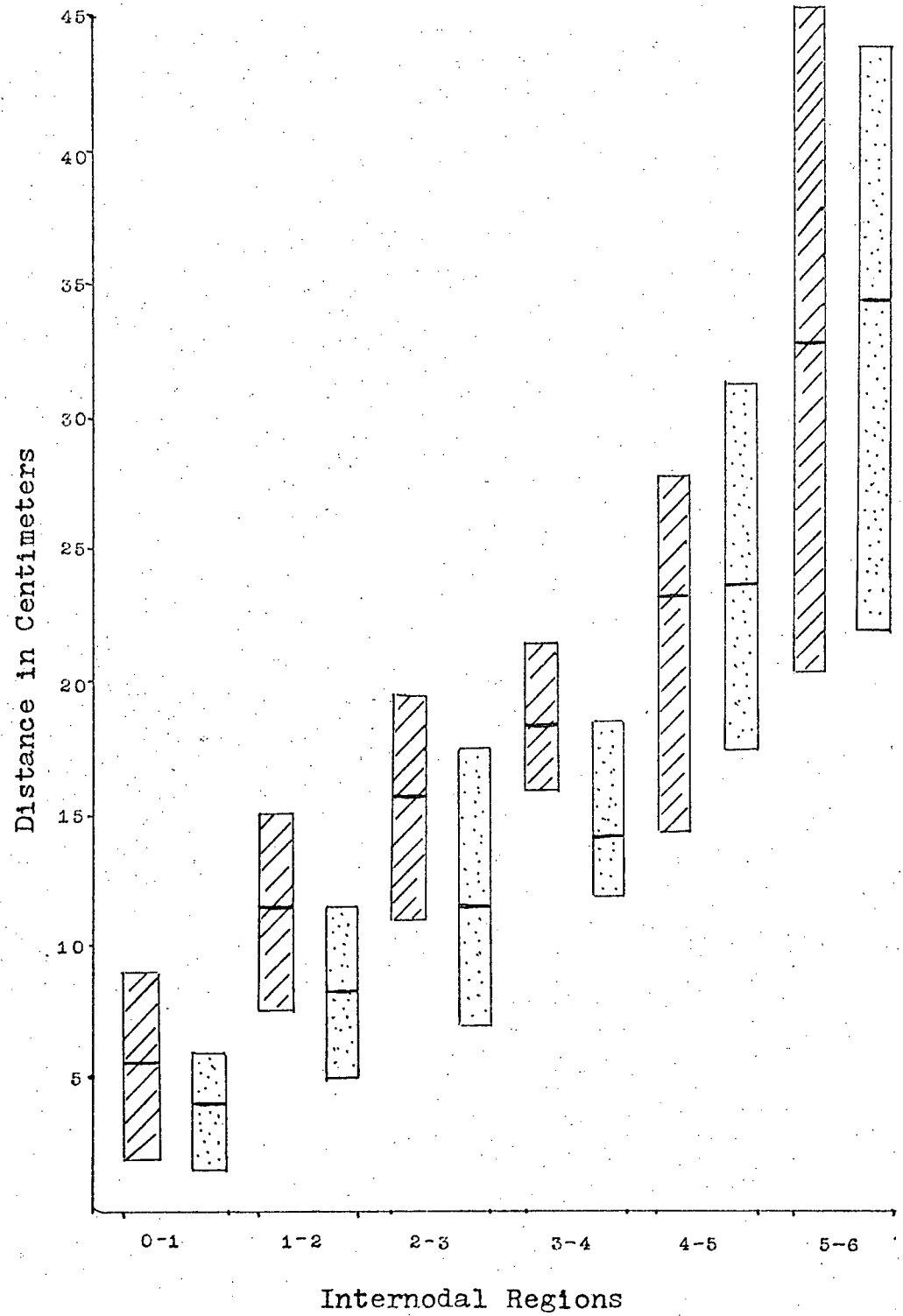
Gibberellic Acid Treated, Control Plants = 

Nongibberellic Acid Treated, Control Plants = 

Average Elongation of Each Internodal Region = —

Figure 3

Comparison of Internodal Variation of Gibberellic Acid Treated, Control plants with Nongibberellic Acid Treated, Control Plants.



Explanation of Facing Figure

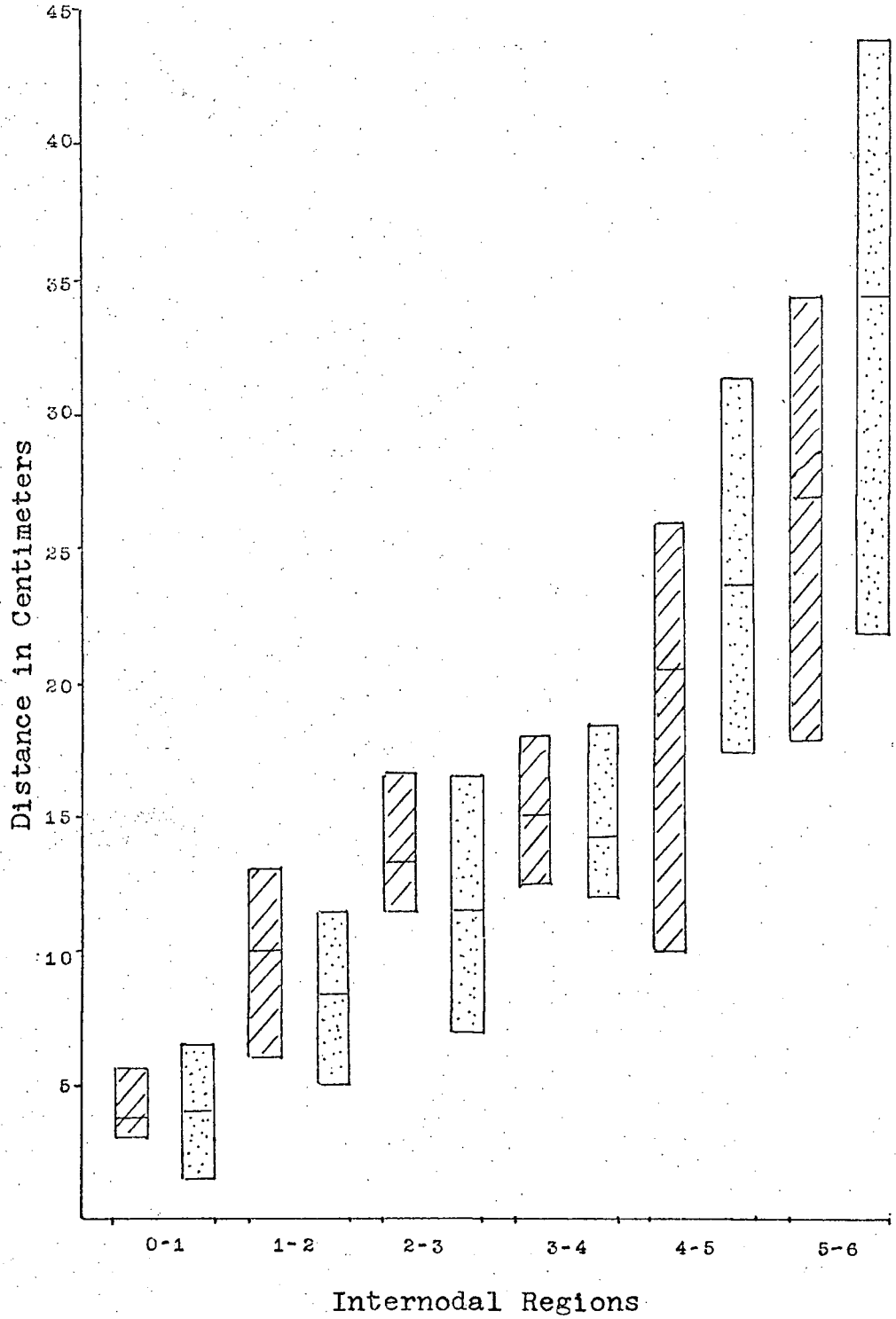
Nongibberellic Acid Treated, Diseased Plants = 

Nongibberellic Acid Treated, Control Plants = 

Average Elongation of Each Internodal Region = —

Figure 4

Comparison of Internodal Variation of Healthy Tillers of Nongibberellic Acid Treated, Diseased Plants with Nongibberellic Acid Treated, Control Plants.



between the disease and gibberellic acid treatment. Figure 4 compares the non-treated control with the healthy tillers of diseased plants. The internodal distances were similar except for the upper two, where the control plants exhibited greater elongation. The failure of elongation of internodes 4-5 and 5-6 of the infected plants was possibly due to the presence of U. hordei in the healthy tillers. The fungus could be using some of the nutrients of the plant thereby resulting in failure of elongation. The isolation of U. hordei from healthy tillers would support this conclusion. Alternatively, the presence of the fungus in the plant, but not necessarily in the healthy tillers, may be causing a reduction in the availability of metabolites needed by the healthy tillers, thus resulting in failure to elongate. The critical point, so far as failure of elongation is concerned, appeared to be the time when the plant was undergoing elongation of internodes 4-5 and 5-6.

When the tillers that have smutted spikes were compared with those of non-inoculated and non-gibberellic acid treated plants, internodes 4-5 and 5-6 were again those that failed to elongate. Figure 5 shows a small overlap between internodes 4-5 of smutted and non-treated, healthy plants. The average elongation of internodes 5-6 of the smutted tillers compared more closely with the elongation of internodes 4-5 of non-inoculated non-gibberellic acid treated plants. Internodes 5-6 of

Explanation of Facing Figure

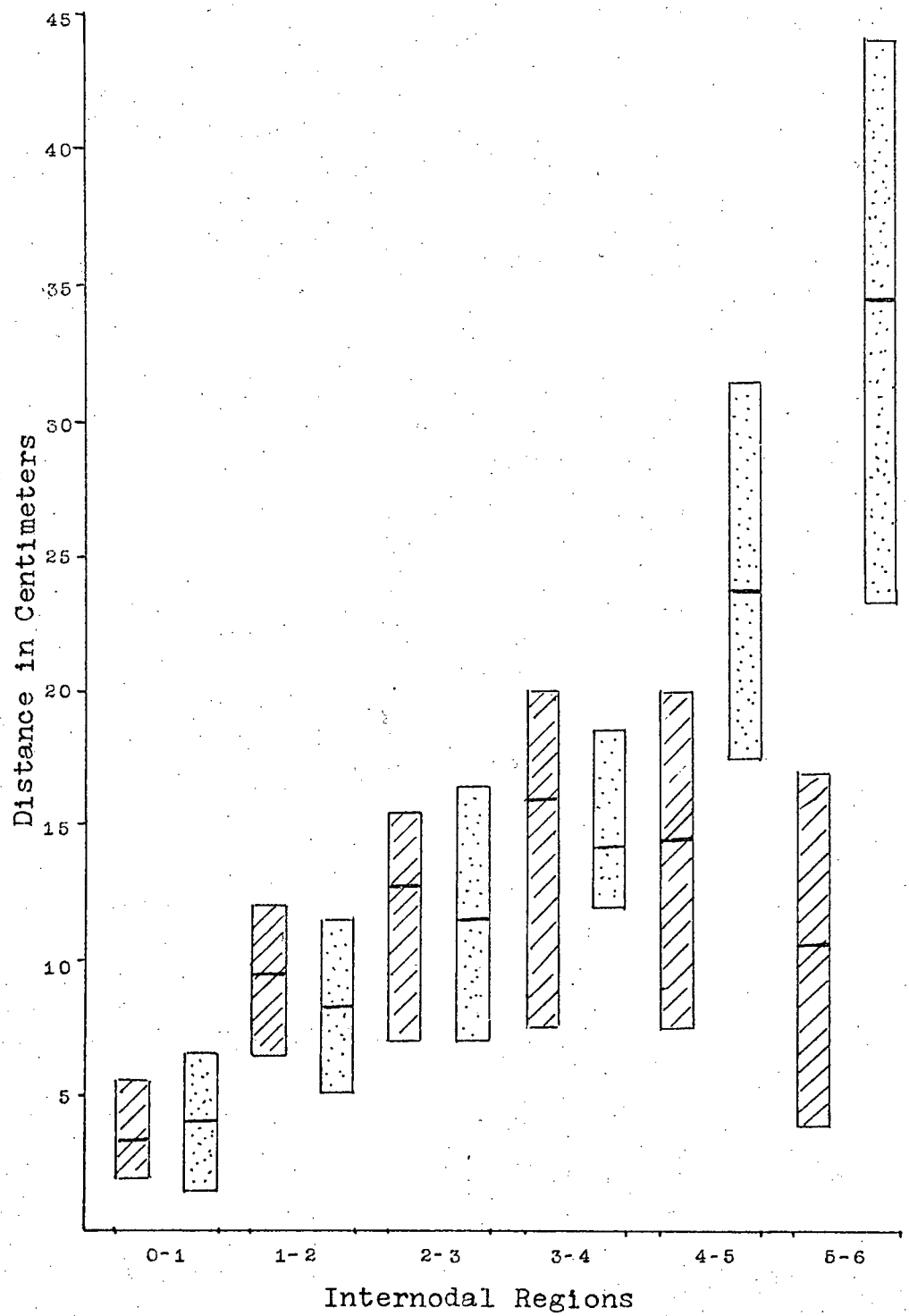
Nongibberellic Acid Treated, Smutted Plants = 

Nongibberellic Acid Treated, Control Plants = 

Average Elongation of Each Internodal Region = —

Figure 5

Comparison of Internodal Variation of Nongibberellic Acid Treated, Smutted Plants with Nongibberellic Acid Treated, Control Plants.



Explanation of Facing Figure

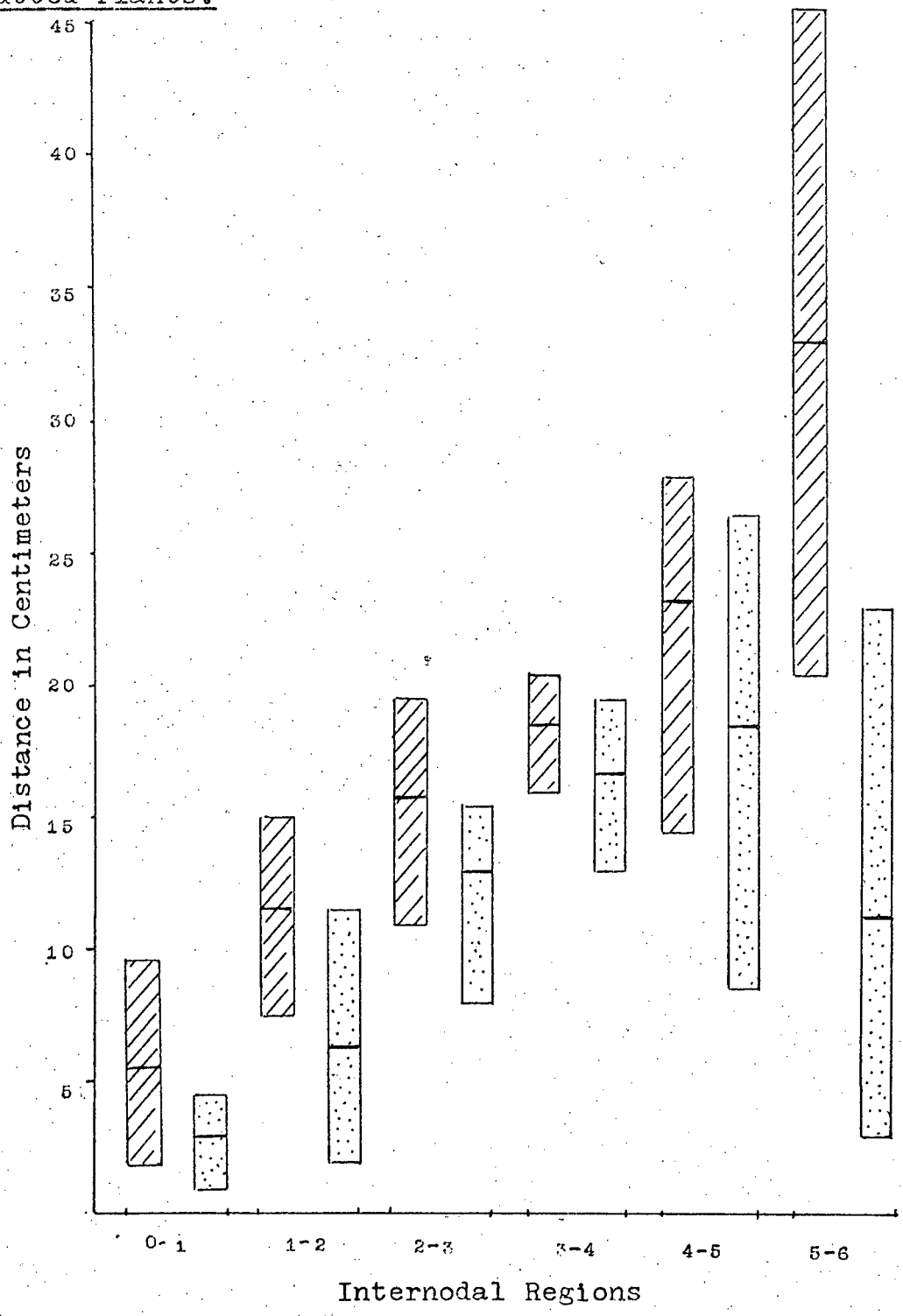
Gibberellic Acid Treated Control Plants = 

Gibberellic Acid Treated Smutted Plants = 

Average Elongation of Each Internodal Region = —

Figure 6

Comparison of Internodal Variation of Gibberellic Acid Treated, Control Plants with Gibberellic Acid Treated, Smutted Plants.



Explanation of Facing Figure

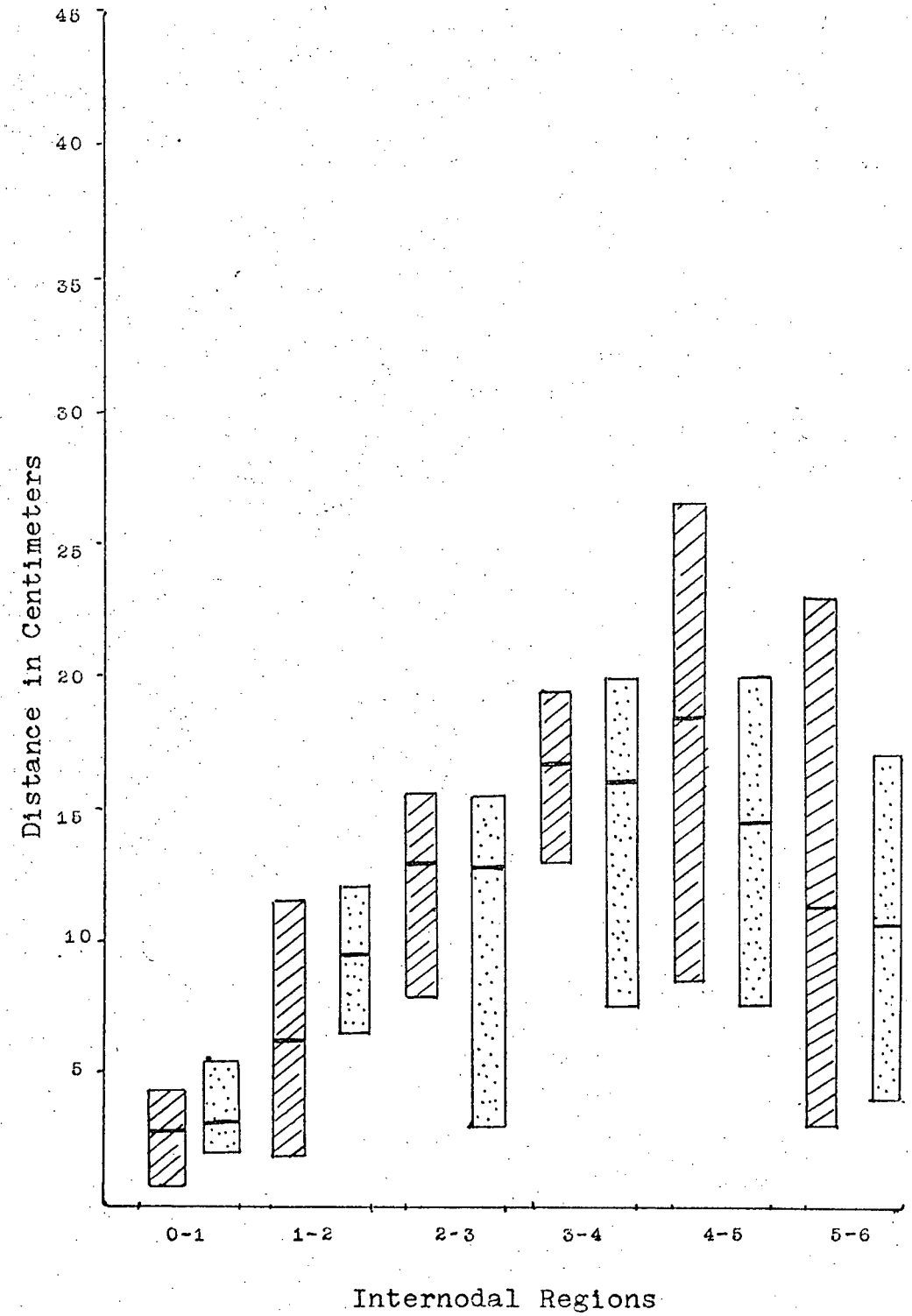
Gibberellic Acid Treated, Smutted Plants = 

Nongibberellic Acid Treated, Smutted Plants = 

Average Elongation of Each Internodal Region = —

Figure 7

Comparison of Internodal Variation of Gibberellic Acid Treated, Smutted Plants with Nongibberellic Acid Treated, Smutted Plants.



smutted tillers showed a marked reduction in elongation. The average elongation of internode 5-6 of the smutted tillers is similar to the elongation of internodes 1-2, and 2-3 of either the untreated control or the smutted tillers. One effect of the disease was to inhibit elongation of internodes 4-5 and 5-6 of barley plants.

The application of gibberellic acid to diseased plants failed to cause elongation of tillers that are normally shortened by the presence of U. hordei infection. A comparison of diseased tillers with those of gibberellic acid treated non-inoculated control plants (Figure 6) showed that there was a reduction in tiller length of all internodes, indicating that there was no increased internodal elongation of smutted gibberellic acid treated tillers as compared with either the control plants or the diseased tillers of the infected non-gibberellic acid treated plants (Figure 7). When a tiller was infected, the presence of U. hordei seemed to prevent increased internodal elongation that would normally take place after treatment with gibberellic acid.

The presence of gibberellic acid produced an elongation of internodes 0-1, 1-2, 2-3 and 3-4, but had no effect on the internodes 4-5 and 5-6. Diseased tillers showed normal elongation (as compared with the control) of internodes 0-1, 1-2, 2-3 and 3-4. Internodes 4-5 and 5-6 showed a failure of elongation as compared with the

control. Diseased tillers that were treated with gibberellic acid showed no effect. A possible explanation for this result was that the presence of the disease and gibberellic acid both interfered with the normal process of internodal elongation, and that the presence of the disease (whose effect was to inhibit elongation) is not counteracted by gibberellic acid (whose effect was to promote elongation).

C. The Percentage of Diseased Tillers Following Two Different Inoculation Procedures with Susceptible and Resistant Barley Varieties.

The application of a mixture of the two haploid strains (I_4A and E_3a) to the barley seeds at or just before their germination was the usual method of inoculation. The "standard" dikaryon generated by this mixture produced the disease in the barley varieties Himalaya and Vantage, but not in the varieties Excelsior and Conquest. Whether the failure of disease induction on the latter two varieties was the result of failure of infection or of failure at some later stage in the parasitic phase of the life cycle is unknown. Table XX shows the results of experiments which were designed to elucidate this point. Following inoculation, by injection of the growing plants, the variety Conquest showed the disease, suggesting that the block to disease development on the variety Conquest occurred early. Perhaps this blockage occurred at the time of initial penetration of the dikaryon, or prior to the establishment of the "crown" node, or later, at the time of development of tillers from the crown node. The establishment and maintenance of the dikaryon in the meristematic tissues of the host was necessary at all the preceding developmental stages as failure to do so at any one stage would result in the dikaryon being left behind, and the spike

Table XX

Seed Variety — Presence Of Smut In Various Varieties Of Barley

Seed Variety	Infected seed		Injected plant		Control
	Number of tillers infected	Total number of tillers	Number of tillers infected	Total number of tillers	
Himalaya	25	42	15	58	-
Excelsior	0	68	0	61	-
Vantage	41	55	35	67	-
Conquest	0	70	6	62	-

primordia would then be able to develop free of the disease. The time at which the failure occurred, and the reasons for it, remain unknown.

The tillers of Excelsior developed at a much earlier stage in plant growth than did those of the other varieties studied. The early development and rapid growth of the tiller and spike primordia may prevent entrance of the fungus.

SUMMARY

A. Multiple Infection

- the resistance of the newly-germinated seedling of H. vulgare to U. hordei and the resistance to infection of subsequently formed tillers are not correlated and are probably not controlled by the same gene or genes.

- haploid sporidia with several genetic markers were obtained from infected plants, indicating recombination as a result of hyphal fusion followed by mitotic recombination or as a result of some other event, (for example transformation), which has not been identified.

B. The Effects of Gibberellic Acid on Inoculated and Uninoculated Barley Plants

- the application of gibberellic acid increases the overall tiller height of healthy plants by effecting the elongation of the internodal regions 0-1, 1-2, 2-3, and 3-4.

- gibberellic acid does not effect elongation of the internodal regions 4-5 and 5-6.

- healthy tillers of diseased plants are uneffected by the application of gibberellic acid.

- diseased tillers are usually shorter than healthy tillers - the application of gibberellic acid does not effect the tiller length of diseased tillers.

C. The Percentage of Diseased Tillers Following Two Different Inoculation Procedures with Susceptible and Resistant Barley Varieties

- the injection of U. hordei (compatible mating types) into the developing spike of a normally resistant strain of H. vulgare resulted in the production of smut, indicating that blockage to normal infection occurs at the time of penetration of the newly germinated seedling, at the level of tiller primordia development, or at the time of spike primordia development.

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