PARASITISM OF BARLEY BY *USTILAGO HORDEI* (PERS.) LAGERH.: SOME QUANTITATIVE ASPECTS OF DISEASE EXPRESSION

by

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ABSTRACT

Quantitative studies were made of disease reactions in the barley-*Ustilago hordei* system. Inoculation studies involving a compatible combination of host and parasite, in which one of the mating-types was greatly diluted in relation to the other, indicated that the partial-vacuum inoculation method results in at least 100 sporidia per seed being available to form dikaryons which smut the plant. Resistance, which can be influenced by environemnt, accounts for the inoculated plants which are nonsmutted.

Two aspects of disease exist: within-plant reaction and between-plant reaction. Both being genetically determined, the two were closely correlated in all disease-producing combinations of 12 barley cultivars and 21 *U. hordei* dikaryons. Within-cultivar and within-dikaryon correlations were also found.

In over 500 plants, using a virulent pathogen dikaryon, studies were made of within-plant smutting patterns and the effects on plant growth of inoculation and smutting. Distribution of smutted heads within smutted plants was not random. Nodal tiller families tended to be either entirely smutted or entirely healthy. When families were differentially smutted, the older members most often were nonsmutted. Entire older families most often remained nonsmutted. The principal culm did not fit this age-frequency pattern. Regardless of the occurrence of smut, inoculation of plants caused reduction of tillering and changed the pattern of tillering so that inoculated plants produced lateral tillers at higher nodes of the principal culm.

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GENERAL INTRODUCTION

The covered smut disease of cultivated barley (Hordeum vulgare L.) is caused by a fungus, Ustilago hordei (Pers.) Lagerh. which is obligately parasitic (i.e., totally dependent on growth within the living host for completion of its life cycle). The principal manifestation of the disease is shown by mature barley plants whose fruits (caryopses) are replaced by masses of teliospores (sori) which have been produced by the fungus. This particular host-parasite system has been the subject of recent genetic investigation whose intent has been to learn more about host-parasite relationships in general through intensively studying a single system. Thus far, the studies have centered on the effects of single genes, either in the host or in the parasite, which have relatively large effects on disease expression. However, at this time a greater understanding of resistance other than that which allows no smutting whatsoever is especially desirable, since the use of "partial" rather than "complete" resistance may result in more effective long-term control of the disease by avoiding the intense selection pressure favoring virulent pathogen genotypes (Person, 1967). Moreover, for several reasons, this would appear to be a useful host-parasite system for the initiation of population genetics studies aimed at, among other things, a greater understanding of host-parasite coevolution. Much of what is dealt with in this thesis concerning intermediate

disease expression will be essential preliminary information if such studies are to be undertaken.

When considering any host-parasite system in terms of population genetics, it is convenient to think in terms of the relative reproductivities of the two interacting organisms. It is necessary to examine the effects of extremes of reproductive success of the host and pathogen on the success of the system, rather than on the success of the component organisms. Logically, each organism will change so as to increase its reproductivity. With an obligate parasite, however, host changes which affect parasitic compatibility must be considered along with changes in the parasite itself. In the case where the pathogen is a major selective force for the host, studies of host reproductivity must involve the pathogen. Regardless of whether or not the pathogen is limiting, studies of this kind are validly of host-parasite systems as much as they are of the component organisms. In order for a host-parasite system to survive, an equilibrium of host and pathogen reproductivities must be reached, such that neither can become completely successful and cause the elimination of the other. For the system to succeed, each component organism must suffer a reproductive decrease in order that the other might reproduce as well. That such equilibria represent the end result of host-pathogen coevolution has been discussed more completely by Person (1967).

The concept of relative reproductivity applies especially well to the barley-U. hordei system because (1) the pathogen is totally dependent on the host: only by causing disease can it reproduce sexually; and (2) the relative reproductivities of the host and pathogen are clearly reciprocally related: teliospore production only occurs at the expense of seed production and vice-versa. Although these features are also shown by many other host-parasite systems involving obligate pathogens, it is with the head-infecting smuts, where the generation time of the parasite exactly matches that of the host, that the features are shown most clearly.

It should be noted here that the existence of the barley-U. hordei system is probably more or less dependent on man and his agricultural practices. The barley-U. hordei system is not a "natural" system of parasitism, and this point must be kept in mind when discussing it in terms of evolutionary concepts such as fitness (relative reproductivity), in order to avoid drawing oversimplified conclusions.

In the past, when the natural progenitor of the barley-U. hordei system existed, mechanisms must have evolved whereby the production of both some seed and some teliospores was assured. It is with this aspect of the system (or the remnants of it) that the thesis is concerned. From the standpoint of the barley host, there are several ways in which the plants can avoid being totally smutted. First, although susceptible,

a plant can simply escape becoming infected. This is undoubtedly an important feature of natural host-parasite systems; it will not be dealt with further, except as a possible alternative in the first section of the thesis. Second, a plant can express some sort of resistance to the disease. This resistance can take more than one form. On one hand, it can be expressed in terms of a certain proportion of plants failing to develop smutted spikes. The extreme case of this is the monogenic total resistance, expressed by some barley cultivars against certain smut genotypes, where smutted spikes are not found. The percentage of plants failing to develop smut ranges from 100% for the "totally" incompatible combinations down to about 30% and sometimes as low as 15% for the most compatible ones (Ebba, 1974; Sidhu, 1972). Another way in which resistance can be expressed is through production of healthy tillers on smutted plants. Little is known about this form of resistance. It might be considered somewhat analogous to the horizontal resistance or "tolerance" found in other host-parasite systems (Robinson, 1973). This type of resistance (partial-smutting of diseased plants) results in intermediate disease levels which are neither devastating to the host nor critically detrimental to the pathogen. For convenience, the two types of resistance will hereafter be referred to as between-plant and within-plant resistance, respectively.

The purpose of these studies was to further our understanding of the disease expression of resistance in the barley-*U. hordei* system. This includes:

1) examining the artificial inoculation phenomenon to determine the basis of non-expression of disease in plants of an inoculated, susceptible cultivar;
2) determining whether or not within-plant resistance is correlated with between-plant resistance; and
3) establishing the pattern of smutting within the barley plant, as well as some of the effects of smut presence on plant growth.

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LITERATURE REVIEW

A. EARLY DEVELOPMENT WITHIN THE SUSCEPTIBLE HOST

A number of studies have been made of the development of various cereal smuts within their hosts. Seedling, as opposed to floral, infecting smuts are more likely to shed light on events occurring with U. hordei in barley, although the latter are probably not so different except in their means of initial entry and in the timing of the early events of and following infection. Intensive studies have been made on the development of loose and covered smuts of oats, Ustilago avenae (Pers.) Rostr. and U. kolleri Willie, respectively. Both, like U. hordei, are considered to be seedling infecting smuts. U. avenae is, however, also capable of some floral infection and is, therefore, considered to be an intermediate type for which infection of embryos at the time of seed germination is dependent on mycelium rather than on teliospores (Fisher and Holton, 1957). The mycelium of U. avenae proliferates either in the inner hull of the dormant seed or in the outer pericarp of the caryopsis. An attempt by Tapke (1948) to more accurately locate the fungus was a failure. In any case, the location is rather similar to the location of teliospores which are the source of infection for true seedling infectors such as U. kolleri. This difference between the two smuts has been minimized in experimental work by using identical inoculation techniques. Kolk (1930) inoculated dehulled seeds of a

susceptible oat cultivar by dusting them with dry teliospores of U. avenae. The seeds were examined for the presence of smut mycelium at various stages after planting. She found that the fungus did not reach the coleoptile until three days after planting, and it did not reach either the first vegetative leaf or the mesocotyl until four days after planting. Gage (1927), using the same fungus and host, found no mycelium in seedlings which were less than seven days old. The difference between these observations could be due to the different materials and techniques used in the two studies. In a 13-day-old seedling, Kolk observed mycelium in all parts of the coleoptile, the mesocotyl, the coleoptile node, all leaf axils and the coleoptile axillary bud. None was seen in the main tiller growing point. Working with the same system, Lutman (1910), however, did find the main tiller growing point invaded in a 13-day-old seedling which he examined. Because she had observed some mycelium in the space between the coleoptile and the first leaf sheath, Kolk felt that sheath-to-sheath inward penetration was a likely pathway. In seedlings, 13 to 30 days old, she observed general invasion of the cone of the growing point with apparently random lateral distribution of the hyphae. The mycelial concentration at the tip of the cone was somewhat lower than at the base. Sampson (1933) found a similar mycelial distribution and chronology of penetration when studying U. kolleri on a susceptible oat cultivar. On the basis of

negative evidence, she felt that passage from coleoptile to first vegetative leaf must be indirect, via the nodes, rather than direct. Mills (1966) observed that at the second leaf stage (14 to 17 days after planting) the mycelium of U.avenae was present in the growing point of susceptible oat seedlings, and that tiller primordia were invaded by mycelium from the subtending leaf sheath. Tiller buds in the axils of the coleoptiles, first leaf, and second leaf were infected after 14, 17, and 21 days, respectively. When Mills examined plants at the fourth, fifth and sixth leaf stages, he found that most apical growing points had been invaded. Western (1936) found mycelium of U. avenae in susceptible oat apical growing points within 21 days.

Some useful histological studies have been made using common bunt (Tilletia caries (De C.) Tul.) and wheat. Woolman (1930) determined that in a susceptible cultivar, coleoptile and leaf sheath invasion had occurred in some plants by seven days and primordial invasion by twelve days. Hansen (1958) saw intercellular bunt hyphae in coleoptiles of 8-day-old seedlings of a susceptible wheat cultivar. He failed, however, to find hyphae in the first leaf sheath until the seedlings were 30 to 50 days old. General invasion of all growing points did not occur until seedlings were 50 days old. In a thorough study of bunt development in two different susceptible cultivars, Swinburne (1963) found that crown nodes did not become invaded until 31 days after planting (fifth leaf stage). His

diagrams indicated that, even at this late date, little tiller bud invasion had occurred. Location of the mycelium within the plumule appears to be random. Other details of development are essentially the same as those found by Kolk (1930) for the oat smuts. Swinburne thought that the leaf sheaths were invaded by mycelium from the next oldest leaf, and that in his cultivars the growing point was first invaded by mycelium from the fourth leaf sheath. His work is somewhat atypical since the plants had not yet begun to tiller at 50 days because of the growth conditions employed.

For the floral-infecting smuts the mycelium is much more deeply embedded in the seed at planting time. Batts (1955) showed that mycelium of wheat loose smut (Ustilago tritici (Pers.) Rostr.) in susceptible wheat seeds is abundant in the scutellum, the lower embryo, and the coleorhiza. It is also beginning to permeate young nodes and internodes, but is not found in the plumule, growing point, or radicle. According to Batts and Jeater (1958), the mycelium is in such a position in dormant seeds that there is little probability of its being "left behind" during the period of rapid stem elongation. They showed that as the crown node develops to form the crown region consisting of tiller branches and corresponding nodes, internodes, and tiller crowns, the mycelium generally is able to grow into all parts of the crown. They also noted two other points: (1) that elongation of internodes proceeds from the

base upward (the lowermost being first to elongate), and (2) that no mycelium was present in internodes. Ruttle (1934) observed a general invasion of an inoculated susceptible wheat seedling 22 days old, with the surprising exception that no mycelium could be found in the plumule.

Malik and Batts (1960) established that the location of mycelium of barley loose smut (Ustilago nuda (Jens.) Rostr.) in infected susceptible barley seeds was not different from the location of wheat loose-smut mycelium in wheat seeds. No histological studies of post-germination loose-smut development in barley have been made.

Information on the development of U. hordei in barley seedlings is scant. Kozar (1967) observed in susceptible Odessa barley, which had been inoculated with teliospores, that pericarp penetration occurred as early as 48 hours after germination. Invasion of the embryos and, specifically, of the coleoptiles and node primordia was seen after 100 hours. Long, slender, relatively unbranched hyphae were found in the internodal regions of an elongated tiller. As with all other smuts, the highest concentrations of mycelium in older plants occurred in the subapical areas of the spike and in the crown region at the base of the plant.

In summary, based on results which are admittedly far from complete, other than for differences in timing, no

important differences in early post-germination development of the various cereal smuts within their hosts can be shown. This seems to be true regardless of the large difference in mode of infection between seedling infecting smuts and floral infecting smuts.

B. THE EXPRESSION OF RESISTANCE

In the previous section, a general view was provided of the pathway of infection in susceptible seedlings for several cereal smuts. Studies have also been made comparing events in resistant (showing no smut) and susceptible cultivars. Histological studies of different cereal smuts will be reviewed first, followed by macroscopic studies.

With both the covered and loose smuts in oats, resistant cultivars usually present no barrier to the initial invasion of seeds and young seedlings by the fungus. Kolk (1930) and Zade (1931) were the first to notice the invasion of seedlings of resistant cultivars by U. avenae. Sampson (1933) observed normal (susceptible) coleoptile invasion by U. kolleri in five day old oat seedlings of a resistant cultivar, after which the fungus was checked in its development. Western (1936) confirmed this, but also found that for single races of both smuts the resistant cultivar 'Markton' did not allow any penetration whatsoever. The fungi were stopped at the cuticle of the epidermis. All other resistant combinations, including several other races tested on 'Markton', allowed

invasion of coleoptiles (or even deeper tissues). These combinations differed in the length of time during which the mycelium could persist in a state which was indistinguishable from that of a susceptible combination. In most cases, pathogen arrest was not accompanied by any type of host tissue alteration. Brandwein (1937), working with several cultivars of resistant oats, observed coleoptile invasion by both smuts to be general. This indicates that the choice of material can determine what will be observed.

Infection by T. caries in resistant wheat cultivars presented a similar picture. Gaines (1923) and Crepin et al. (1937) reported that seedlings of resistant and susceptible wheat cultivars showed no difference in extent of hyphal invasion for the first two weeks of growth. Crepin et al. observed penetration into the second leaf sheath in both cases. Woolman (1930) discerned three distinct phases of pathogenesis: (1) entrance into and development within epidermal cells of the coleoptile; (2) development within deeper coleoptile tissues and leaf sheaths; and (3) entrance into nodes, internodes and growing points of the embryo. He found that stage (1) was always attained but that, in resistant material, stage (2) was attained only in some plants (with no delay) and stage (3) was never attained. Griffith et al. (1955) confirmed this observation using different cultivars and smut races.

With Ustilago nigra Tapke, the semi-loose smut of barley, Tisdale and Tapke (1924) found that prior to penetration of the coleoptile and the first leaf base the reactions of resistant and susceptible cultivars were indistinguishable.

The resistance shown toward floral infecting smuts, although similar, must be viewed in a different context. Embryo invasion of the dormant seed is the normal condition leading to a susceptible disease reaction. Ruttle (1934), Bubentzov (1941) and Vanderwalle (1946) showed that in some resistant wheat and barley cultivars, the mycelium was not present in the embryos, although it was often found elsewhere in the seeds. Bubentzov found one resistant wheat cultivar in which a minority of the embryos were infected, however. Vanderwalle (1942) and Batts and Jeater (1958) also found resistant wheat cultivars in which the embryos were as frequently invaded as those of susceptible cultivars. A thorough investigation was carried out by Popp (1951 and 1959) who compared levels of embryo infection, seedling infection and adult plant infection in both wheat and barley. He found that for wheat, most cultivars showed a high percentage of embryo infection (not generally involving the plumular apex), an intermediate percentage of seedling infection, and (if they possessed high resistance) a low percentage of adult plant infection. Thus resistance

appears to be expressed throughout the life of the plant. Other wheat cultivars (or the same cultivars inoculated with other races of the pathogen), and all of the several barley cultivars examined showed a good correlation between percent embryo and percent adult plant infection by U. tritici and U. nuda, respectively. This implies that embryo exclusion may be a relatively more important contributor to resistance in barley, as compared with wheat. Ohms and Bever (1954 and 1955) found that embryos of resistant cultivars were as frequently invaded as those of susceptible when two winter wheat cultivars were inoculated with three races of U. tritici. Gaskin and Schafer (1962) histologically examined five resistant wheat cultivars for the presence of U.tritici mycelium at various intervals following inoculation. In four of them, resistance occurred after the embryo, and, occasionally, even after the plumular apex, had been invaded. In the fifth cultivar, embryo infection did not occur.

Convincing macroscopic evidence is also available showing that smut mycelium is often present in resistant plants. Hubbard and Stanton (1934), and Stevens (1936) found that when two oat cultivers were inoculated with U. kolleri, reduced stands (the proportion of plants emerging) resulted even though the plants were apparently healthy. Brandwein (1937) did not observe this effect in greenhouse studies involving these same two cultivars. He could,

however, produce the effect by planting loose and covered smut inoculated seeds of resistant and susceptible cultivars in soil which was afterward severely compacted (Brandwein, 1938). He concluded that an interaction between infection and dehulling (which was commonly done to obtain higher levels of smutting) can significantly weaken the coleoptiles, resulting in reduced emergence. Zade (1931), Welsh (1932) and Hubbard and Stanton (1934) all observed resistant oat cultivars which exhibited reduced height, reduced yield and delayed heading when inoculated with U. kolleri and/or U. avenae. Similarly, with flag smut of wheat, Urocystis tritici Kornike, Churchward (1937-8) observed stunting and chlorosis of inoculated plants which remained unsmutted, and were classified as resistant. Blasting (= sterility) of heads of resistant oat cultivars has been observed after inoculation with both U. kolleri and U. avenae (Reed and Stanton, 1938) and with U. avenae (Halisky, 1956; Holton, 1966). Reed and Stanton also found a few teliospores in some of the blasted heads.

On occasion, workers were able to modify resistance through cultural methods. Some smutting was obtained in otherwise "totally resistant" 'Markton' oats when Smith and Bressman (1931) cut back the plants at six to seven weeks after planting. Drought conditions produced the same effect the following year. However, Woodward and Tingey (1941) cut back barley plants at the boot stage and saw no effect

on smutting by U. hordei. Stevens (1936) and Brandwein (1937) could find no effect on yield, height, date of maturity, or amount of tillering when they inoculated several totally resistant oat cultivars with U. kolleri and U. avenae.

Griffith et al. (1955) observed an effect on early growth of both resistant and susceptible wheat cultivars following inoculation with T. caries. The plants were generally smaller than the uninoculated controls. The difference soon disappeared in the resistant cultivar. They observed a few teliospores in the centre of seeds of some late tillers of plants of the resistant cultivar, however, so it would appear that the fungus was still present even at maturity. Crepin et al. (1937) also saw similar effects on plant growth in other resistant wheat cultivars. U. nigra also caused distortion of seedlings developed from dehulled seed of both susceptible and resistant barley cultivars according to Tisdale and Tapke (1924).

Little has been recorded concerning the effects of floral infecting smuts on resistant cultivars of the host. Tingey and Tolman (1934) could find no correlation between percent of wheat stand (seedling survival) and loose smut susceptibility, indicating that if the mycelium were present in non-smutted plants its effects were too small to detect.

An unusual type of resistance involving an apparent hypersensitive reaction has been reported in wheat against U. tritici by Oort (1947) and by Ohms and Bever (1955), and in oats against both oat smuts by Western (1936). Ohms and Bever observed that, because of infected seeds and seedlings, stands were reduced. Earlier mentioned stand reductions may have been caused by this form of resistance, with dehulling being a necessary prerequisite, however. With floral infectors, dehulling was not part of the inoculation procedure. Oort (1947) most fully described the hypersensitivity which he observed. It resulted in death of either entire plants or of culms. Thus when heading occurred, a strong selection for healthy plants or plant parts had taken place. Details are lacking in the case described by Western (1936). Death of entire infected plants is not mentioned, but infected culms were seen to die.

Practically nothing is known about the presence or absence of U. hordei mycelium in inoculated plants of highly resistant barley cultivars. No histological studies of such material have been made. Faris (1924a) stated that plants which were infected with the fungus were not distinguishable from healthy plants until near maturity. Johnston (1934) observed reduced emergence of Glabron, a totally smut-free cultivar, when both hulled and dehulled

seeds were inoculated. The dehulled seeds seemed to be more severely affected. Reduced stands were also noted in two resistant barley cultivars by Schafer et al. (1962a). In this case, reduced emergence occurred only when seeds were dehulled and planted deeply. It is possible that in this and other cases of stand reduction reviewed, soil pathogens were involved.

C. THE EFFECTS OF ENVIRONMENT ON BARLEY COVERED SMUT

Most of the published information on the effects of environment on cereal smuts has been reviewed by Tapke (1948). The purpose of this section is not to give a comprehensive picture of what is known, but rather to present information on environmental effects which may relate to the results of this thesis. As Batts and Jeater (1958) state, environmental effects on one specific smut disease cannot be generalized to include other smuts. One especially cannot apply what is learned from floral-infecting to seedling-infecting smuts, and vice-versa. Here floral infectors will not be considered and, except for U. hordei, the seedling infectors will be mentioned only briefly.

Faris (1924a) demonstrated that, for susceptible genotypic combinations, uniformly high infection levels could be obtained in the greenhouse over wide ranges of temperature, soil acidity and soil moisture. He concluded

that field and greenhouse conditions nearly always fall into the favorable ranges of these variables. His method of inoculation was to dust seeds with dry teliospores. Aamodt and Johnston (1935) determined that soil heterogeneity had little effect on level of smut in 138 barley cultivars. Other studies indicate, however, that environment can be important. Briggs (1927) was unable to obtain high smut levels using the dry teliospore inoculation technique followed by planting in the field. Tapke (1938) clearly determined that the post-emergence environment could be important. He germinated seeds in the greenhouse and, at various times after germination, transferred plants to the field. When seeds were dry dusted with teliospores, the amount of smut was proportional to the length of time the seeds remained in the greenhouse. When, however, inoculation was with an aqueous suspension of teliospores (which was allowed to remain on the seeds for 24 hours before drying), no variation in smutting occurred among the different treatments. The study emphasized the importance of inoculation technique on the degree of environmental sensitivity of inoculated seedlings. The effect of depth of planting on smut level has been investigated by Taylor and Zehner (1931) and by Jones and Seif El-Nasr (1940). They found that, in general, the deeper the seeds were planted (up to 12 cm) the higher was the percent of plants

or heads smutted. The relationship was not a linear one, so that the most notable effect occurred at the deepest plantings. Both groups found that of all the smuts they studied, U. hordei was least affected by depth of planting. Woodward and Tingey (1941) failed to find any effect whatsoever when plantings 1.5 and 3.0 inches deep were compared.

Perhaps the most extreme case of an environmental effect on disease levels of covered smut of barley was reported by Ebba (1973). He observed an interaction between smut genotype and environment when identical plantings were made in British Columbia and California. On one barley cultivar a smut dikaryon which caused some smut in B.C. was avirulent in California, while another dikaryon, which caused a rather low level of smutting on another cultivar in B.C., gave a much higher level in California. It is impossible to say which environmental components are responsible. From the large number of other host and pathogen genotypes combined and studied at these two locations, it would probably be safe to say that this observation was exceptional. The only other report of such an interaction concerned T. caries and wheat. Reichert (1930) found a situation almost identical to the one described above, with the two locations being Washington State and Germany. It was of interest to note that, in each case, the local bunt genotype was the more successful of the two. Reichert also indicates that this interaction

was, in his experience, an exceptional situation.

That environment affects the degree of reaction between barley and covered smut there can be no doubt. Tapke (1952) subdivided the effects of the environment into three convenient intervals: (1) the period of inoculation--the method of artificial inoculation chosen being important; (2) the pre-emergence environment, and (3) the post-emergence environment. Schafer et al. (1962) and Thomas (1965) summarize it in roughly the same way, not, however, distinguishing between the pre- and post-emergence environments. This is probably because not enough work has been done to indicate any qualitative difference between them in terms of effects.

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PART I

THE EFFICIENCY OF PARTIAL-VACUUM INOCULATION OF HANNCHEN BARLEY WITH *USTILAGO HORDEI*

A. INTRODUCTION

As stated in the introduction the smut disease, when it is expressed in individual plants, may be shown by one or more, and frequently by all, spikes of the infected plant. With one or more diseased spikes taken as the criterion for recognizing the presence of disease in individual plants, the highest disease levels usually result in about 50 to 60 per cent of the plants showing the disease. Although Thomas (1965) and Sidhu (1972) occasionally observed disease levels as high as 80 to 85 per cent, Ebba (1974), who worked with a range of smut genotypes and barley cultivars interacting under widely varied environmental conditions, did not report disease levels higher than 60 per cent.

For any plant which shows at least one smutted spike it is evident that the infection process has been successful. A question arises concerning those plants which, following inoculation, show no external evidence of smutting. This could represent the extreme case in which, following successful infection, none of the spikes became smutted. It could also represent those cases in which infection had not been accomplished.

This study was, therefore, directed to the question of whether, in susceptible combinations, the failure to express the disease following inoculation was caused by the failure of the infection process, perhaps because of an inefficient inoculation technique, or to other causes.

The approach chosen to answer this question was quite simple. The standard method of inoculation has, in the past, involved inoculum made up of sporidia of the two necessary mating-types, A and a, which were mixed and shaken together for at least 24 hours in liquid broth (Thomas 1965). More recently, Ebba (personal communication) found that inoculum which has been mixed and allowed to stand for one or two days is no more effective than inoculum which has been mixed just prior to inoculation. From this, it may be inferred that the dikaryons,which ultimately result in infection and smutting, can be formed after the seed has been inoculated; they need not exist prior to the time of inoculation. It was thought that by carefully adjusting the relative amounts of A and a sporidia and observing the effect on level of smutting, an indication of the efficiency of the inoculation procedure would be obtained. Moreover, the best expression of this efficiency would be in terms of an "effective number" of sporidia. This would represent the average number of sporidia which, for each inoculated seed, would be potentially capable of taking part in a "successful" infection

(i.e., in an infection which actually results in disease expression). The effective number would thus relate to expression of the disease by individual plants, and would be a measure of the number of sporidia potentially capable of taking part in the infection process. It would, of course, give no indication of the actual physical location of these sporidia. The above approach is only one possibility. For instance, dilution of normal 1:1 mating-type inoculum would also undoubtedly work. It was felt that the mathematics and logic of the approach chosen were simpler, however.

The purpose of this study was to determine the effective number for a representative host-parasite genotype combination and, in so doing, to assess the efficiency of the inoculation procedure in establishing effective host-pathogen contact.

A short study was also made to determine whether known sporidial mixtures remain constant over time, or whether the initial ratios are brought closer to unity by faster growth of the minority type component of the mixture.

B. MATERIALS AND METHODS

A single smut dikaryon and a single barley cultivar were used in this study. The two smut haploids which made up the dikaryon were originally isolated by Thomas (1965)

from separate smutted barley plants found near Winnipeg, Manitoba. They have been since used extensively as the standard haploid cultures in the laboratory of Dr. Person, and are designated E3a and I4A. The two-rowed barley cultivar 'Hannchen' was chosen for two reasons: (1) under normal conditions a high percentage of plants show infection when 'Hannchen' seeds are inoculated with the standard dikaryon mentioned above; and (2) in the greenhouse, where mainly this study was carried out, more plants of this cultivar can be brought to maturity in a given amount of space than when other cultivars are used.

Seeds were treated with a dilute solution of formalin (one part formalin to 320 parts water) prior to inoculation in order to kill any contaminating smuts and to loosen the seed hulls. They were soaked in the solution for one hour, rinsed for 30 minutes in running tap water, and thoroughly dried before inoculation.

Seeds were inoculated using the partial-vacuum technique described by Tapke and Bever (1942). Smut cultures were maintained for up to three weeks on modified Vogel's (1956) complete agar medium in petri dishes. These were stored either in an incubator at 22°C or, more commonly, in a refrigerator at 4°C. Sporidia from each culture were transferred, separately, to 125 ml Erlenmeyer flasks containing 50 ml of complete broth. A drop or two of aqueous

achromycin suspension (10 mg/ml) was added to inhibit possible bacterial growth, and the flasks were shaken in a 22°C incubator for 3 to 4 days, by which time sporidial suspensions of maximum density were obtained. About 100 seeds were put into a dram vial. For normal inoculation, equal quantities of the two haploids (of opposite mating-type) were mixed and the mixture was pipetted onto the seed at a rate of 8 to 10 ml of inoculum per vial. The vials were then placed in a vacuum dessicator, without dessicant, and subjected to a partial vacuum (resulting in boiling) for 30 minutes. Upon rapid release of the vacuum, sporidia are drawn under the seed hulls. The excess liquid was then poured off, and the seeds were put into small coin envelopes. Seeds were allowed to dry for at least 24 hours in the opened envelopes well spaced on metal racks over paper towels. Planting took place within three days after inoculation.

In order to manipulate the ratios of the two smut haploids, the absolute concentrations of the 3 to 4 day old sporidial suspensions were first determined by diluting each 1:100 with sterile water and counting the sporidial concentrations with the aid of a hemocytometer. Where necessary, the more concentrated suspension was adjusted by adding sterile water to obtain equal concentrations of sporidia for the two cultures. The sporidial ratios were then realized by mixing unequal amounts of the standardized cultures in

the ratios desired to give a total of 8 to 10 ml of inoculum per vial. Ratios of 1:1, 100:1, 500:1, 1,000:1, and 10,000:1 (and their reciprocals) were used. The extreme 10,000:1 and 1:10,000 ratios were necessarily obtained by using a 1:100 dilution of the minority culture. This resulted in a very slight decrease in the sporidial density of the final inoculum.

Seeds were planted in soil benches in the greenhouse. About 50 seeds were sown in each four foot row. Rows were randomized. The plants were given 16 hours per day supplemental fluorescent lighting. In the field, where some treatments were also planted, seeds were sown at a rate of 125 seeds per 15-foot row. Disease levels, assessed in terms of the percentage of plants showing at least one smutted head, were recorded about three months after seeds were sown, when the plants had reached maturity. Readings were made without knowledge of treatment.

The effective number of sporidia was determined by using the following relationship (see discussion):

$$S = 1-P^n$$

where S = the disease level taken as a proportion of the control population (which had been inoculated with a 1:1 mixture);

P = the proportion of majority sporidia in the inoculum mixture;

n = the effective number of sporidia.

In logarithmic form the relationship becomes:

$$\log (1-S) = n \log (P)$$

or $$n = \frac{\log (1-S)}{\log (P)}$$

which is much more easily solved for n.

Standard dilution plating techniques were used to obtain samples, as single sporidial colonies, from 100:1 and 1:100 ratio mixtures of the two haploids in complete broth shake culture. From the dilution plates, five day old colonies were transferred to fresh petri plates of complete medium to form 25-colony master plates. These were replica-plated, according to the method of Dinoor and Person (1969), to complete agar plates containing sporidial lawns of E3a and I4A, the two standard mating-type testers. Samples were taken at 0, 7, 24 and 72 hours after mixing. In addition, at 24 hours, 50 ml of fresh medium was added to the cultures to initiate another burst of growth. Colony mating-types, based on the presence or absence of infection hyphae or "suchfäden" (Bauch, 1932), were recorded two days after transfer to the tester lawns.

Determination of significance was carried out, using a statistical technique which deals with the confidence interval about the difference between two proportions (see appendix). The 1:1 control was the standard against which all other treatments were compared.

C. RESULTS

Figure 1.1 shows the relationship between differing A:a ratios in the inoculum and the resulting levels of disease. The highest disease level, 30 per cent, was obtained with the 1:1 mixture of A and a sporidia. In Figure 1.1 this level is set arbitrarily at 1.0, and other levels are shown as proportions of this maximal level. Except for the 10,000:1 and 1:10,000 ratios, which are based on samples of ca. 100 plants, each point on the curve is based on a sample of at least 150 plants, and on 250 or more for the 1:1, 100:1 and 1:100 ratios. These numbers represent plants from at least three, and in some cases from four, separate experiments. For the 1:1, 100:1 and 1:100 ratios one of the experiments was carried out in the field. Results from different experiments were consistent.

The curve shown in Figure 1.1 is not symmetrical. The skewness becomes evident when the 100:1 and 1:100 ratios of A:a sporidia are compared with each other; when the 100:1 ratio favors mating type a, the disease level is significantly lower than that of the controls, and when it favors mating type A the difference in disease levels is not significant. The skewness is also made evident when the 500:1 and 1:500 ratios and the 1,000:1 and 1:1,000 ratios are compared. In all cases it is the ratio which favors mating type a that associates with the lower disease level.

FIGURE 1.1

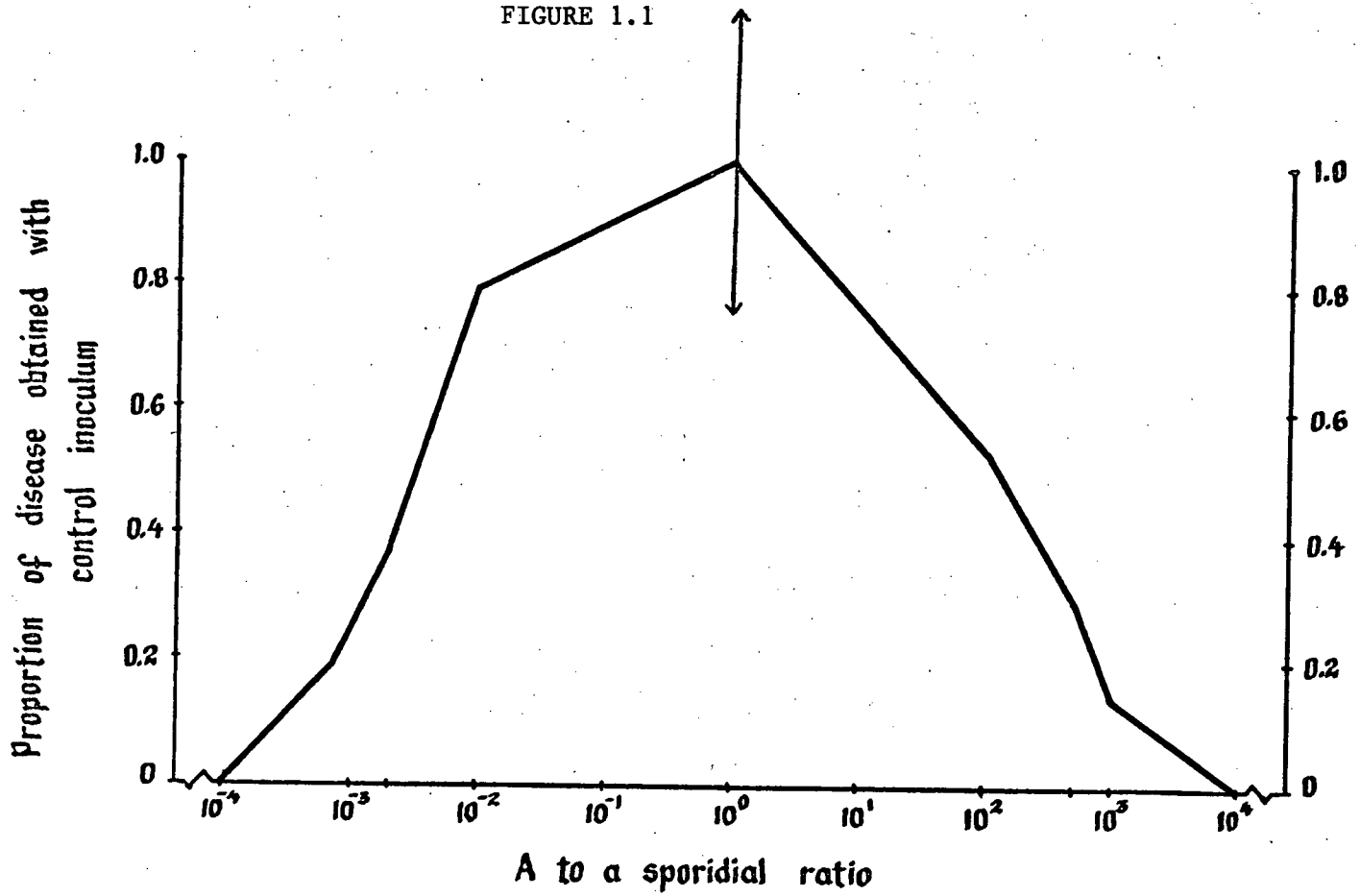

FIGURE 1.1. DISEASE LEVEL, AS MEASURED BY THE PROPORTION OF MAXIMUM POSSIBLE PERCENT OF BARLEY PLANTS SMUTTED BY USTILAGO HORDEI (AMONG THE CONTROL PLANTS--THOSE INOCULATED WITH A 1:1 SPORIDIAL MATING-TYPE RATIO), IN RELATION TO MATING-TYPE RATIO OF THE INOCULUM

Although the points in this figure represent averages of three or more experiments, the skewness was shown consistently in all the separate experiments.

At the extremes of the curve, where A:a ratios of 10,000:1 and 1:10,000 were tested, only one diseased plant was found. In accordance with the skewness, this single diseased plant resulted from inoculation with the 10,000:1 in mixture of A to a.

The number of colonies (over the total examined) which showed the mating-type of the minority culture when 100:1 and 1:100 mating-type mixtures were made and sampled at various times is presented in Table 1.2. These data show that when sporidia are mixed together unequally and maintained *in vitro*, the ratios do not change appreciably toward less disparity over a 3-day period.

D. DISCUSSION

In qualitative terms, one would expect that if the effective number of sporidia is large, so that relatively many sporidia have the potential of forming dikaryons and of taking part in a successful infection which results in smutting, then a reduction in the percentage of diseased plants should not be realized until rather disparate ratios of the two mating types are used. Likewise, if the effective number is small, the number of plants showing

TABLE 1.1

THREE SEPARATE ESTIMATES OF THE EFFECTIVE SPORIDIAL NUMBER BASED ON THREE SPORIDIAL RATIOS AND THEIR RESULTING LEVELS OF INFECTION

Sporidial ratio used*	Infection Level (proportion of control)	Calculated Effective Number
100:1	0.643	103
500:1	0.331	201
1,000:1	0.175	195

*A combination of the reciprocal ratios of A and a sporidia

TABLE 1.2

THE NUMBER OF MINORITY SPORIDIA OVER TOTAL COLONIES, AS SHOWN BY THEIR MATING-REACTIONS, OF TWO CULTURES WITH UNEQUAL SPORIDIAL RATIOS SAMPLED AT FOUR DIFFERENT TIMES AFTER MIXING

Time of Sampling (hrs.)	Sporidial Ratio	
	1 E3a:100 I4A	1 I4A:100 E3a
0	1/54	1/55
7	0/54	0/54
24	2/54	0/55
72*	0/55	0/54

*Sampled 48 hrs. after 50 ml of fresh medium was added to each culture.

showing smut should drop off relatively quickly as the ratios increase. This is because some of the seeds, which would otherwise become smutted plants if inoculated with the usual 1:1 mating-type mixture, will, at high ratios, fail to receive sporidia of the minority mating-type. Since a significant decrease in the percent of smutted plants does not occur until the ratio reaches 100 I4A to 1 E3a sporidia on the one hand, and 500 E3a to 1 I4A on the other, it would seem that the effective number must be rather large. The relationship used in its calculation is an intuitive one. From the percentage of smutted plants shown by the material inoculated with the 1:1 mating-type mixture, one subtracts the probability per seed (on the average) of missing the minority mating type, this probability being first taken to a power equal to the effective number. One would expect, then, that if the number of available sporidia is larger, the probability of not including the minority type will be smaller for a given sporidial ratio, and vice versa. The use of average probability is valid because large numbers of plants are involved in each case.

An alternate way to illustrate the expectation involved is seen in Figure 1.2, which displays the relationship between sporidial ratio and effective number when three separate expectations are considered. When a log-log

plot is used, the size of the effective number necessary to obtain each of three proportions of maximum smut; 0.95, 0.5 and 0.05, increases in a linear fashion as the sporidial ratio becomes more disparate. The three observed estimates of the effective number are also plotted according to sporidial ratio and proportion of total possible plants smutted. The effective number estimates are then obtainable visually by observing the abscissal values of the three points.

While it is possible that other more complex approaches to the determination of the effective number might yield more precise estimates, for the purpose of this study the precision afforded by this rather simple relationship is considered to be adequate.

One can only speculate about the cause of the asymmetry of decrease in smutting for corresponding reciprocal ratios. At roughly maximum asymmetry, a sporidial ratio of around 250 E3a to 1 I4A produces the same disease reduction as a ratio of 100 I4A to 1 E3a. The simplest explanation for this is that, under planting conditions, seedborne E3a sporidia do not survive as well as I4A sporidia. There is no reason to conclude that this is mating-type related, since the two haploids are known to be genetically different. Another possibility is that the two mating-types may not be physiologically

FIGURE 1.2

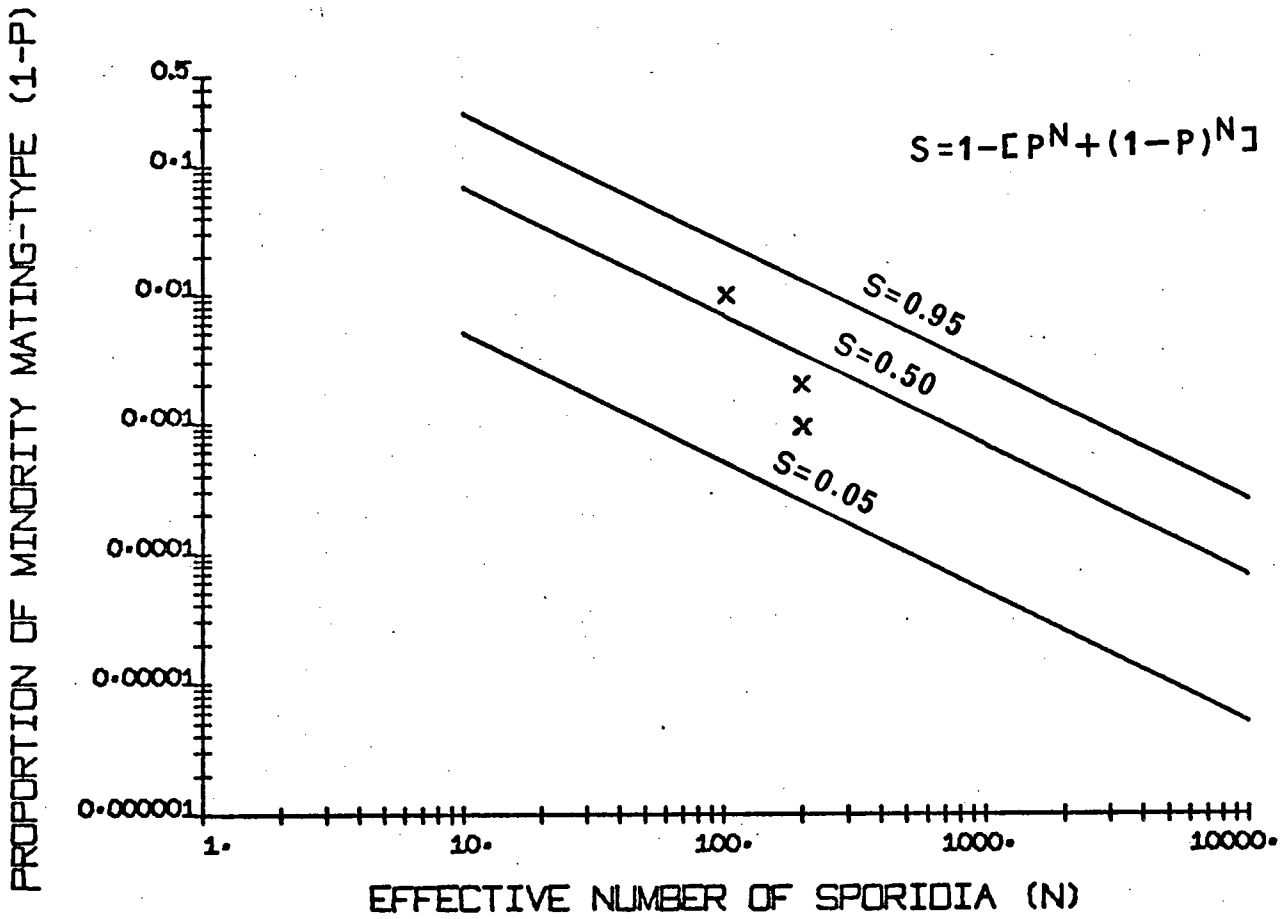

FIGURE 1.2. THEORETICAL RELATIONSHIP BETWEEN EFFECTIVE NUMBERS AND MATING-TYPE RATIOS NECESSARY TO REALIZE 0.95, 0.50 AND 0.05 OF TOTAL POSSIBLE PLANTS SMUTTED, INCLUDING PLOTTING OF THREE OBSERVED VALUES

equivalent. If, for instance, the A mating-type is the initiator of dikaryosis, this could determine the skewness.

There is another reason why the effective number estimate may be low. An assumption which is made in this work is that a minority sporidium at very disparate mating-type ratios will as frequently participate in conjugation as a minority sporidium which occurs in a less unequal ratio. While nothing is actually known for this species, work with one of the Tremellales, a group of related fungi, suggests that the amount of conjugation-inducing substance(s) produced by the minority mating-type could be insufficient when these minority sporidia are very infrequent (Bandoni, 1965). This would mean that some of these sporidia would fail to trigger the nearby majority sporidia to conjugate. The effect would be to make the calculated effective number an underestimate of the actual number of sporidia available for dikaryon formation and infection. As well, if reciprocal conjugation-inducing substances are produced by the two mating-types, the skewness of the curve could be explained by unequal production or activities of, or sensitivity to, such substances. Isogenic A and a lines will eliminate some of the above possibilities regarding skewness.

In order to make use of as many observations as possible, the corresponding reciprocal ratios were combined to calculate the effective number.

The two effective number estimates from the 500:1 and 1,000:1 ratios are in good agreement, but the one from the 100:1 ratios is somewhat lower. A weighted average of the three gives an overall estimate of 155 sporidia. From this it can be safely concluded that the effective number is at least 100.

Sampling over time from the two shake cultures of reciprocal 100:1 sporidial ratios shows clearly that the ratios do not decrease in three days time. If one assumes that something similar occurs in inoculated and planted seeds, then the ratios must remain unchanged during the first three days of germination. This is the critical period for infection by Ustilago hordei (Appel and Gassner, 1907). Results also indicate that this is true even if, after inoculation, the sporidia are able to divide several times more. These data support the validity of the concepts here employed to estimate the effective number.

The conclusion to be drawn from these experiments is that virtually all seeds are making effective contact with the fungus when the inoculum is composed of the normal 1:1 mating-type ratio. If it is conservatively estimated that 100 sporidia are potentially capable of taking part in dikaryon formation and infection (resulting in disease expression) of a single barley seed, and allowing for

variation due to sampling error (accidental deviation from the 1:1 ratio in the inoculum), the probability of one of the two mating types being totally absent is expected to be extremely small. In practice the numbers of plants which fail to show the disease are nearly always quite appreciable. This must mean that the potential for disease expression is not entirely determined by the capacity of the inoculum to produce infective dikaryons, and points to the conclusion that host resistance is also a factor. How and when such host resistance might operate is not indicated by the results of this study. Such resistance appears to be universal in barley, however, and not having been bred either into or out of the crop, it may represent a remnant of the "natural" resistance of wild barley ancestors to U. hordei.

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PART II

THE RELATIONSHIP BETWEEN TWO ASPECTS OF DISEASE EXPRESSION

A. INTRODUCTION

A basic tenet of the disease process of cereal smuts has been frequently ignored or at least considered of minor importance. It is that two aspects of resistance can be discerned. One type is manifested by entire plants and is measured as the percentage of plants showing smut in at least one culm. It can be called between-plant disease severity and is nothing more than the proportion of plants in which sporulation by the pathogen is prevented. As pointed out in the literature review, in "totally resistant" combinations of host and smut, the mycelium is not necessarily prevented either from entering the seedlings or from growing within the infected plant. Yield loss may result, even though the fungus does not sporulate; but from the standpoint of pathogen reproductivity, this is an effective form of resistance indeed. The second type of resistance involves curtailment of smutting within plants known to be infected at maturity. It can be called within-plant disease severity. It is measured in terms of per cent of culms showing smut on smutted plants (plants with at least one smutted spike).

In the past, breeders and pathologists have differed in the measure they chose to employ in describing disease severity. Table 2.1 presents a summary of the basis of measure used by many workers. The list is not exhaustive, but it is probably representative. The plant basis, as they used it, is a direct measure of the first type of resistance discussed above. The head basis referred to in the table is not simply a measure of the second type, however. Measured as smutted heads over total heads on all plants, it is really a compounding of both types of resistance. Few workers have attempted to limit their measure of percent of smutted heads to plants which were known to be smutted. Some went so far as to count both percent of plants and percent of heads smutted. One cannot safely assume, however, that simple division of the percentage based on heads by the percentage based on plants will give an unequivocal measure of within-plant severity. This would only work if it were accurately known that smutted plants produce as many culms as non-smutted plants. As Part III will show, this was usually not the case.

Correlations between percent of plants and percent of heads smutted have been shown. Clark et al. (1933) obtained a high correlation ($r = 0.741$) between the percentage of plants and of heads which were bunted, following inoculation of wheat with T. caries. They concluded that,

TABLE 2.1

THE BASIS OF MEASUREMENT OF SMUT REACTIONS USED BY MANY PATHOLOGISTS AND BREEDERS WORKING WITH CEREAL SMUTS

Reference	Plant Basis[a]	Head Basis[b]	Both
Aamodt and Johnson (1935)	x		
Batts (1955b)			x
Batts and Jeater (1958)			x
Brandwein (1940)	x		
Briggs (1926,1927)	x		
Cherewick (1958)		x	
Churchward (1937-8)	x[c]	x[c]	
Clark et al. (1933)			x
Ebba (1974)	x		
Faris (1924a, 1924b)	x		
Faris (1934)	x		
Gaines (1923)		x	
Gaskin and Schafer (1962)	x		
Griffith et al. (1955)	x		
Halisky (1956)		x	
Heald (1921)			x
Heyne and Hansing (1955)	x		
Holton (1964)		x	
Holton and Halisky (1960)	x[c]	x[c]	
Holton and Rodenhiser (1942)		x	
Hubbard and Stanton (1934)			x
Johnston (1934)	x		
Jones et al. (1940)	x		
Kolk (1930)	x		
Leukel (1936)	x		
Mather and Hansing (1960)	x		
Metcalf (1962)	x		
Metcalf and Helgason (1962)	x		
Middleman and Chapman (1941)		x	
Oort (1939)	x		
Oort (1947)			x
Person and Cherewick (1964)	x		
Poehlman (1949)		x	
Poehlman and Cloninger (1955)		x	
Popp (1951)	x		
Reed (1929, 1938)	x		
Reed and Faris (1924)	x		
Reed and Stanton (1938)	x		
Reichert (1930)	x		
Rodenhiser and Holton (1942)		x	
Ross et al. (1948)	x		

TABLE 2.1 (continued)

Reference	Plant Basis	Head Basis	Both
Ruttle (1934)			x
Sampson et al. (1927)			x
Schafer et al. (1962b)	x		
Schaller et al. (1960)		x	
Shands (1956)	x		
Sidhu and Person (1972)	x		
Smith (1932a)	x		
Smith and Bressman (1931)		x	
Swinburne (1963)	x		
Tapke (1929, 1931)			x
Tapke (1937,1945,1952,1955)		x	
Tapke (1938)	x		
Tapke and Bever (1942)		x	
Taylor and Zehner (1931)		x	
Tervet (1940,1941,1944)		x	
Thomas and Person (1965)	x		
Tingey and Tolman (1934)	x		
Tisdale and Tapke (1924)	x		
Waud and Metzger (1970)	x		
Wells (1958)	x		
Welsh (1932)	x		
Western (1936)	x		
Williams and Verma (1954)	x		
Woodward and Tingey (1941)		x	

[a]Percent of plants showing one or more smutted head.

[b]Percent of heads showing smut.

[c]Different measures were used in different sections of work.

as a selection criterion in plant breeding, head counts would be nearly as reliable as plant counts, in spite of the fact that the former was consistently lower than the latter in identical material. Ruttle (1934) also found a similar correlation in a single barley-U. hordei combination. Tapke (1929, 1931) recorded both head and plant smut percentages for both four wheat cultivars inoculated with U. tritici and two barley cultivars inoculated with U. nuda. The data show a strong correlation between percent of plants and percent of heads smutted. Tapke did not discuss this finding, however, in his paper. Gaines (1923) decided that percent of smutted heads was the preferable measure because, among other reasons, it gave a more realistic estimate of the impact which smut has on a crop. As will be mentioned later, he was aware of the correlation as well. Briggs (1926) and Churchward (1932-3) argued that for genetic studies the plant, not the culm, must be maintained as the unit. In their genetic studies, even though they, too, were aware of the correlation, they used the plant basis. Rather than count both head and plant smutting percentages, some workers tried to get an indicator of within-plant disease severity in another way: by recording plants as either diseased, partially diseased, or healthy. Gaines (1923) and Churchward did this with wheat-T. ceries. With oats and both smuts, Welsh (1932) used this method to evaluate

the overall resistance of six cultivars. In no case in the above works could a correlation be seen between percent of diseased plants and frequency of partially diseased plants. Since the data were not particularly abundant for this purpose in any of the studies, however, the results should be judged inconclusive rather than negative. Reed (1938) used the same sort of scoring in an extensive study involving eight oat cultivars and a total of five races of both oat smuts. He presented evidence of a negative correlation between percent of plants smutted and the number of partially smutted plants. At best, this type of scoring of smut severity gives a nonparametric or qualitative measure of within-plant disease severity, unless large numbers of observations are involved.

It is not really valid to establish a correlation between percent of smutted plants and percent of smutted heads (on all plants), because these two things are logically not independent (Steel and Torrie, 1960). They cannot be expected to be independent because the higher the frequency of smutted plants, the higher should be the frequency of smutted culms on those plants, even if within-plant disease severity does not vary. Barring a negative correlation between within- and between-plant disease severity, which is unlikely, it would be surprising if a positive correlation between the non-independent variables, that is, percent plants smutted and percent culms smutted, was not found.

There is no a priori reason, on the other hand, why the percent of smutted culms on obviously diseased plants should not be independent of percent of plants smutted. Hence, the empirical investigation of the relationship between these two variables is valid.

Some workers called attention to the possible correlation of within- and between-plant disease severity and validly presented evidence in favor of it. Although they presented no date in support of it, Batts (1955b) and Batts and Jeater (1958) concluded from earlier literature and, presumably, from their own observations, that it exists. In only two instances was the relationship properly investigated. Gaines (1923) determined the proportion of totally bunted wheat plants, which he called c, the proportion of partially bunted plants, b, and a value, a, which was the proportion of bunted heads on partially bunted plants. He then determined the overall percent of bunted heads, d, by the relationship:

$$d = ab + c$$

The important thing is that he noted that the value of a was lower on the four resistant cultivars than it was on the four susceptible cultivars, (0.67 vs. 0.25). Qualitatively, this is good evidence that a positive correlation existed in his material. Oort (1947), working with wheat and U. tritici, showed what appeared to be a very close correlation

of within- and between-plant disease severity in a study which involved many wheat cultivars. His sample sizes are rather small in most cases and he included no statistical analysis. His was the only study found in the literature in which the correlation was investigated as the main objective and in a manner similar to that undertaken in this thesis.

The purpose of this study was to determine, using as broad a sampling of barley and U. hordei genotypes as possible, whether, in this host-parasite system, the severity of disease reaction within plants is (1) genetically variable, and (2) correlated with severity between plants.

B. MATERIALS AND METHODS

Twelve barley cultivars and 21 U. hordei dikaryons were used in this study. Only those genotypic combinations which showed smut in at least five percent of inoculated plants were used. The smut dikaryons included representatives of the original 13 physiological races described by Tapke (1942). These were obtained as teliospore samples from North Dakota State University, where they have been maintained. The other eight cultures represent as wide a range of smut biotypes as it was possible readily to obtain. All are from North America except the three prefixed "Et", which

are from three separate collections made in Ethiopia.

Of the 21 dikaryons here used, one was derived from the haploids E3a and I4A which were used in section I. For the other 20 dikaryons, the constituent haploids were both derived from a single teliospore. These 20 dikaryons were thus produced through "selfing" of two of the four products of 20 tetrads derived from 20 different teliospores. The tetrads were derived in the following way: A petri dish was poured with a thin layer of complete agar. Blocks about 15 mm^2 were cut and each was placed on a 22 mm^2 sterile cover slip. Five ml aqueous suspensions of teliospores were prepared in test tubes. After adding to these suspensions a drop of achromycin suspension (10 mg achromycin per ml H_2O), a small drop of the teliospore suspension was transferred to the centre of each agar block with a Pasteur pipette. Depending on the germination rate of the teliospores used, the concentration was adjusted so that from 10-100 teliospores were included in each drop transferred. The agar blocks with their teliospores were then incubated at 22°C for about 12 hours (somewhat longer for older samples), by which time most of the 5-10 viable teliospores had germinated. On each block a suitably germinated teliospore, with all four sporidial products accessible for micro dissection, was selected. Using a deFonbrune micromanipulator and an upward-bent fine glass needle, the four primary

sporidia were drawn away from the promycelium, one to each of the four sides of the block. Their position on the promycelium was recorded directly on the cover slip, near the edge. In 3-4 days, visible colonies had formed from the four sporidia. They were transferred singly to fresh plates and, after sufficient growth, were tested for mating-type as described in Part I. Small amounts of sporidia were put into screw-cap tubes containing fine-textured, very dry silica gel for long-term storage. Two of the four cultures of each tetrad were selected at random to form the dikaryons which were used throughout the study.

Seed treatment and inoculation were as described in section I.

Considering the size of the overall experiment in relation to the amount of field space and recording time available, the minimum number of smutted plants which would be examined for within-plant severity in each genotypic combination was set at 30. Only plants with three or more culms were included. In order to be 99 percent certain of obtaining 30 plants, for those combinations where information was available concerning the amount of smut to be expected, the number of inoculated seeds needed for planting was determined by using the relationship:

$$(1 - P)^n = 0.99$$

where P = the percentage of plants showing smut;

n = the number of plants needed to be 99 percent certain of obtaining at least one smutted plant.

The net result was that more seeds were planted when, for a particular genotypic combination, it was expected that the smutting percentage would be low.

Inoculated seeds were planted in the field in 15-ft. rows. The rate of seeding varied from 90-150 seeds per row, depending on percent viability of the seed and on the amount of tillering exhibited by the cultivar at a given seeding rate. Usually a particular genotypic combination was planted in 1-2 blocks of several rows each. In California, where a planting was made during the winter months, the procedures were identical except that the rows were 20 feet long.

Data were recorded when all spikes had emerged. Generally, the percent of plants smutted was based on samples of at least 300 plants. In the few cases where fewer plants were available, the percentage of plants smutted was high enough so that sample sizes were statistically adequate nonetheless (Steel and Torrie, 1960). Smutted plants which were to be examined on a tiller basis were carefully pulled. When time was not limiting and the plants were available, 40-50 plants were examined. Plants with one and two tillers were also recorded, but for reasons to be given they were not used in determining the degree of within-plant severity. All plants in a row were pulled

and recorded before going on to the next row in order to avoid biased sampling. Incomplete smutting of spikes was also recorded on a single plant basis.

When smutted head and smutted plant percentages had been calculated, the correlation analyses were done by computer.

C. RESULTS

Table 2.2 is a matrix of the 12 barley cultivars and 21 smut dikaryons, showing the disease reactions which were observed. The work of Tapke (1945) was consulted to determine which combinations of host and parasite genotypes to investigate. As shown by his data, any combination which gave less than 1.0 per cent smut (he used a head per row basis) was not included in this study. Thus, many of the negative and low readings in the table for the 13 races and eight of the barley cultivars (his work did not include 'Conquest,' 'Gateway,' 'Keystone' or 'Vantage') are the findings of Tapke. Also, while the practical lower limit in the present work was taken as 5 per cent plants smutted, in three cases where there was less than 5 per cent plants smutted, sufficient data were nonetheless obtained so that they could be included. Information on expected smutting for some of the other combinations was obtained through personal communication with Ebba. If no information was available about a

combination, 5-8 rows (and more later if necessary) were planted. In the table, there are three points which are not part of the matrix. While they represent crosses of different races or collections, they are not crosses of the same haploid products as shown elsewhere in the table. Thus their possible genetic significance is not discussed.

TABLE 2.2

COMBINATIONS OF HOST CULTIVAR AND PATHOGEN DIKARYON, AND DISEASE REACTIONS OBSERVED

Barley Cultivar	Ustilago hordei dikaryon[a]						
	R1	R2	R3	R4	R5	R6	R7
Conquest	-	-	-	2.0[b] 19.0	-	-	-
Excelsior	-	L	50.6 87.4	L	-	-	-
Gateway	L	-	-	-	-	-	-
Hannchen	53.2 83.3	L	-	20.7 66.3	-	L	-
Himalaya	-	-	-	45.8 89.8	-	L	-
Keystone	L	-	-	3.9[b] 22.1	-	-	L
Lion	- 27.0 71.2	34.6 80.8	-	12.6 67.5	18.9[b] 53.7	-	
Nepal	-	55.5 95.5	57.5 96.7	56.2 91.9	-	-	-
Odessa	31.5[b] 64.4	47.1 72.6	59.5 94.6	42.3 69.0	37.2 75.2	22.9[b] 70.4	25.0[b] 52.8
Pannier	-	-	L	39.9 79.0	-	-	L
Trebi	7.6 30.6	L	L	6.8 31.0	12.1 44.7	L	-
Vantage	-	-	-	-	-	-	-

TABLE 2.2 (continued)

Barley Cultivar	Ustilago hordei dikaryon						
	R8	R9	R10	R11	R12	R13	Et1
Conquest	-	L	6.8[b] 18.1	L	L	L	27.6 71.4
Excelsior	-	26.6 70.8	-	-	L	46.8 83.6	-
Gateway	-	-	-	-	-	L	-
Hannchen	-	28.2 78.3	48.4 82.5	58.6 81.0	32.3 71.0	35.2 69.9	37.0 85.9
Himalaya	-	-	27.0 86.1	-	32.3 72.3	9.0 64.4	-
Keystone	-	6.0 42.1	5.3[b] 24.2	-	-	10.3 24.8	53.0 70.5
Lion	-	4.6 65.3	24.1 73.1	26.7 71.4	-	18.1 76.0	5.9 70.0
Nepal	L	30.7 56.0	49.3 83.2	-	61.5 93.4	54.1 88.4	40.7 84.1
Odessa	23.6[b] 59.1	53.4 96.6	53.5 71.9	54.8 77.9	44.6 86.6	55.8 80.7	17.4[b] 35.7
Pannier	-	L	29.4 95.7	-	L	-	-
Trebi	-	-	7.6 40.2	44.3 69.5	L	6.8[b] 13.8	2.8 32.2
Vantage	-	24.0 60.0	46.1 60.5	58.7 66.8	-	42.1 81.0	44.6 77.4

TABLE 2.2 (continued)

Barley Cultivar	Ustilago hordei dikaryon							Additional
	Et2	Et3	Uh6	Uh12	v1v2	v3	EXI	
Conquest	-	-	-	-	23.0 55.0	12.6[b] 33.9	-	
Excelsior	-	-	56.3 85.6	-	-	-	-	(V3XUh-6) 13.4[b] 76.4
Gateway	-	-	-	-	L	-	L	
Hannchen	7.9 72.1	-	-	67.1[b] 81.4	32.0 85.7	14.7[b] 46.0	24.0[b] 46.2	
Himalaya	-	-	-	-	24.0 91.0	18.6[b] 76.8	-	
Keystone	2.7[b] 28.6	-	-	-	10.3 53.5	8.5 52.1	-	
Lion	L	7.4 55.1	20.6 83.2	19.8 75.2	36.0 76.2	11.1[b] 46.6	8.0[b] 42.4	(Uh-6xUh-12) 61.7[b] 67.7
Nepal	L	26.8 53.9	47.3 95.2	-	-	-	-	
Odessa	30.3 81.9	19.7[b] 48.2	40.3 85.7	62.2 95.6	45.5 86.9	16.0[b] 53.4	32.8[b] 48.1	
Pannier	-	-	30.1 77.5	-	29.7 85.3	-	-	
Trebi	5.9 55.6	-	11.2 53.9	23.2 55.6	36.5 61.6	16.5[b] 46.9	10.8[b] 50.2	(R5xR11) 48.1[b] 85.1
Vantage	14.8 53.0	-	-	31.5[b] 66.1	27.8 72.6	-	16.6[b] 42.8	

[a]Numerical values indicate sufficient data for analysis. Upper value is percent of plants smutted; lower is percent of smutted culms on diseased plants. - = no smut observed. L = less than 5% plants smutted; insufficient data.

[b]High tillering plants

Before the correlation analysis could be properly done, a complication involving variation in plant tillering had to be first dealt with. An inverse relationship between the average number of culms per plant and the percent of smutted culms on smutted plants was discovered. For example, Nepal inoculated with Uh6 was planted both at the University of British Columbia and in California. The latter planting was on highly fertilized land and other conditions there were also conducive to extremely high tillering. At British Columbia, where smutted plants produced an average of 4.26 culms, 95.2 percent of the culms were smutted. In California, where smutted plants produced an average of 12.8 culms, a significantly lower 71.1 percent of the culms were smutted. There was no significant difference in percent of plants smutted at the two locations. A similar find was made when Lion and Uh6 were planted at both locations; only here the percentage of plants smutted was actually significantly higher in California. Whether the data for a combination were obtained at British Columbia or in California was largely random. Yet the mean percent of smutted culms on diseased plants for the 29 combinations under high-tillering conditions was 44.3, while that of the 82 low-tillering combinations was 59.4 percent. Analysis of all 111 points revealed a significant negative regression of frequency of culms on diseased plants on average tiller production. When

low- and high-tillering plantings were analyzed separately, however, there was no significant regression in either grouping, (an average of nine or more culms per plant being considered high-tillering). Division of data into two separate analyses reduced the variation of average culms per plant with each data set, thus minimizing the effect of this variable on within-plant disease severity. This was felt to be the simplest and most effective way to avoid this complication. For the large number of low-tillering plantings, the variation in average number of culms was low because only plants with three or more culms were considered. The mean was often not much higher than three culms per plant. The variation in average amount of tillering was greater for the high-tillering plantings, but a regression was, nonetheless, not found.

Figures 2.1 and 2.2 are the within- and between-plant disease severity scatter diagrams for low- and high-tillering plantings, respectively. In determining the r values, the arcsine transformation was used (Steel and Torrie, 1960). In both analyses, the variances of x and y were very close (expressed as percentages). Hence scatter diagrams involving units of standard deviation were thought unnecessary. As shall be brought out in the discussion, a strong genetic component in the variance of within-plant disease severity is indicated.

FIGURE 2.1

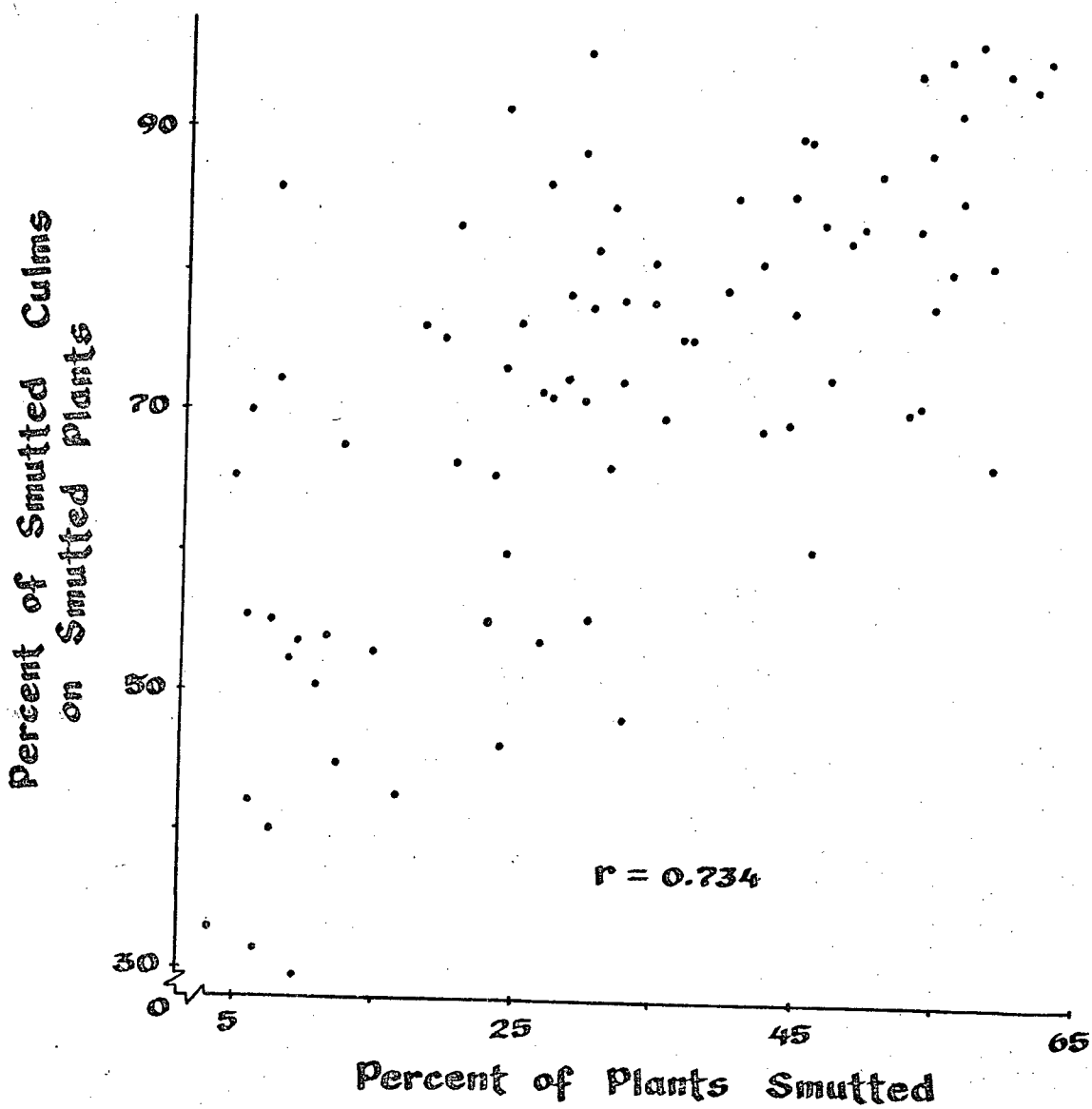

FIGURE 2.1. CORRELATION SCATTER DIAGRAM FOR 82 BARLEY-*USTILAGO HORDEI* GENOTYPIC COMBINATIONS PLANTED UNDER LOW-TILLERING CONDITIONS

FIGURE 2.2

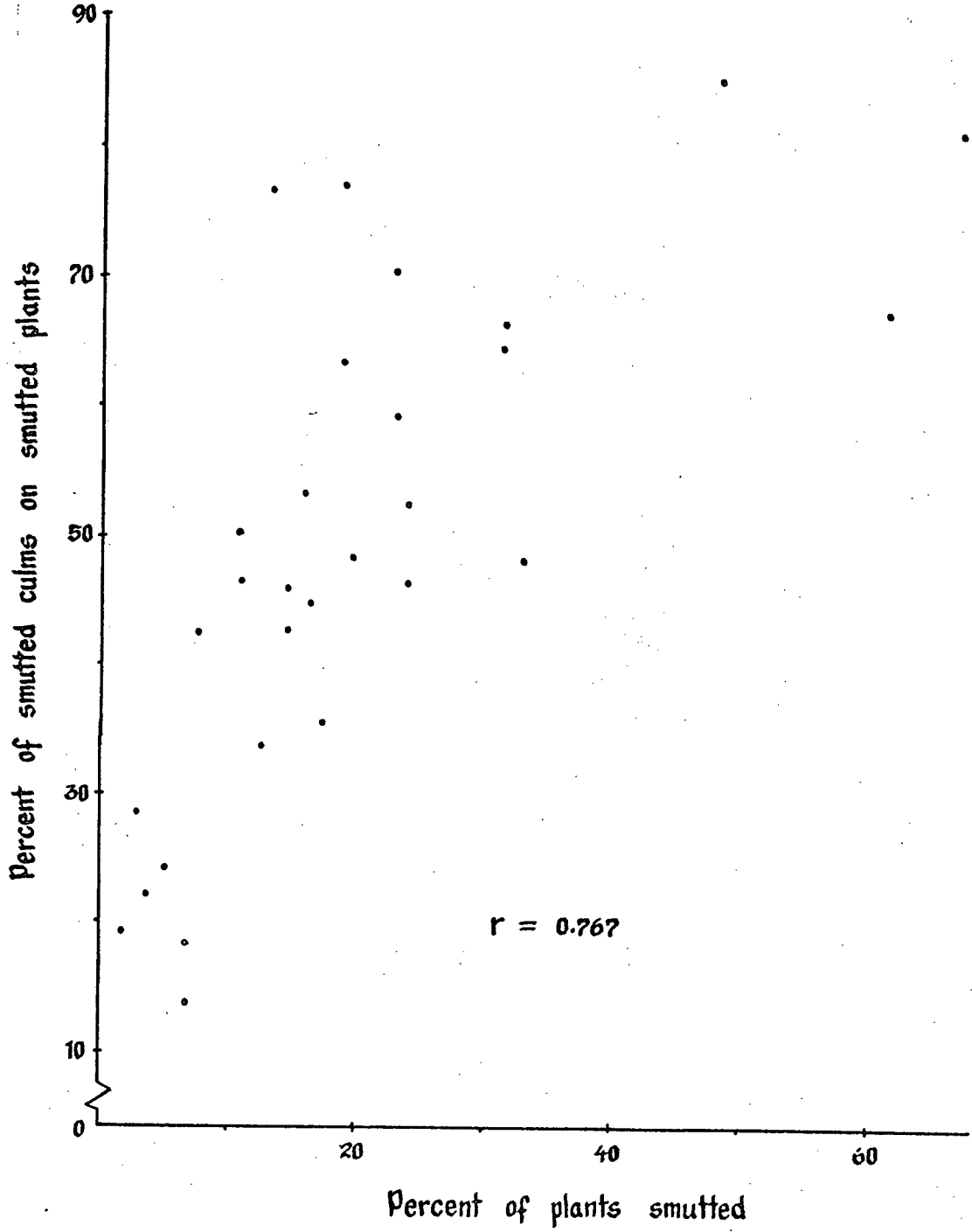

FIGURE 2.2. CORRELATION SCATTER DIAGRAM FOR 29 BARLEY-USTILAGO HORDEI GENOTYPIC COMBINATIONS PLANTED UNDER HIGH-TILLERING CONDITIONS

Separate within-cultivar and within-dikaryon correlations were made on low-tillering plantings of five cultivars and three dikaryons. Upon examination, it appeared that the other combinations did not possess sufficient information (points) or sufficient variation of one or both variables to warrant an analysis. The results are summarized in Table 2.3.

TABLE 2.3

SOME WITHIN-CULTIVAR AND WITHIN-DIKARYON CORRELATION ANALYSES INVOLVING LOW-TILLERING PLANTINGS ONLY

Common Cultivar or Dikaryon	Number of Points	Value of r
Hannchen	10	0.580
Lion	12	0.669*
Nepal	10	0.911*
Odessa	13	0.450
Trebi	10	0.815*
Race 9	7	0.758*
Race 13	8	0.762*
v1v2	9	0.500

*Significant correlation at the 5% level.

Five of the eight correlations were significant. Of the three which were not, v1v2 and Odessa can be explained by small sample size and lack or variance, respectively. It is likely that, had sufficient information been available, the correlation would have been found in all within-cultivar and within-dikaryon groupings. In conclusion, there is a strong correlation between disease severity within a barley plant and among barley plants, as measured by amount of smutting.

D. DISCUSSION

In comparing the present data for the 13 original races interacting with the eight cultivars studied by Tapke (1945) with his results, a fairly good qualitative agreement was obtained. Because the present results were recorded on a plant basis while those of Tapke were on a head basis, the present percentages were nearly always much higher. There were some exceptions, however. All of the combinations involving Trebi gave percentages lower than those obtained by Tapke. This would lead one to suspect that the two seed lots were different. As well, this study revealed that some of the races also had changed. All detected changes were in terms of reductions or losses in virulence. Gains in virulence, if they occurred, went undetected simply because combinations which Tapke found gave little or no smut

were not included in this study. Losses or reductions in virulence occurred in seven different combinations involving either race 6 (two cultivars), race 7 (three cultivars), or race 11 (two cultivars). All other races were, qualitatively at least, unchanged. Because of poor smutting in the first test, combinations involving races 6 and 7 were generally tested a second time, using products from different teliospores in each test. Some quantitative differences in virulence existed between the two sets of teliospore products used in the two tests; these could have been due to environment. Gross reductions or losses of virulence were consistently found in both dikaryons of the two races. Based on this admittedly small sampling, it may be suggested that the race samples are qualitatively uniform. One can also conclude that, in view of the notorious heterogeneity and instability of smut races (Cherewick, 1958) and the difficulty of maintaining such races over a long period of time, the race samples obtained from North Dakota were remarkably similar to those identified and collected by Tapke in the early 1940s. Detailed comparisons of quantitative results were not made, primarily because of the different measures of smutting used in the two studies. Even under casual examination, the expected "correlation" between percent of plants smutted, as measured in the present study, and the percent of all heads smutted, as measured by Tapke, was quite evident, however.

The inverse relationship between within-plant disease severity and the average number of culms produced by the plants has been indirectly and directly mentioned by others. Woodward and Tingey (1941) observed that higher levels of smutting were obtained with barley-U. hordei on less fertile soil than were obtained on more fertile soil. Since this measurement of the disease was based on smutted heads, it can be assumed that they were probably observing the increased within-plant disease severity which accompanied the decrease in average tiller production on poorer soil. Milan (1939) observed that the rate of sowing, which also can be expected to influence the tillering of plants, did not affect the percent of inoculated wheat plants smutted by U. tritici but did directly affect the percentage of culms smutted. A possible explanation by Batts and Jeater (1958) is that each infected plant contains a limited amount of mycelium. Thus, if many tillers are produced by the plant, a smaller proportion of them will become smutted. They suggested also that the amount of mycelium varies from embryo to embryo, with the result that some plants will show a higher frequency of smutted heads than others, even if other conditions are equal. Unfortunately, the histological work presented by Batts and Jeater does not really support their hypothesis concerning both limited and variable amounts of mycelium in embryos. Nonetheless, their hypothesis provides a simple

and plausible explanation for both Milan's and the present data (differences in mode of infection of the two smuts notwithstanding). If Batts had been able to study resistance in U. tritici, as was his intention (Batts and Jeater, 1958) he would undoubtedly have provided much additional evidence either for or against his hypothesis.

Regression analysis for the effect of average tiller production on within-plant disease severity was used in this work only as a means to determine whether the data would effectively eliminate variation in average tillering as a complicating factor in the between- and within-plant correlation analysis. Some problems which detract from the regression analysis will be discussed briefly. For a regression analysis, one of the important initial assumptions is that the independent variable is normally distributed (Steel and Torrie, 1960). The distribution of average tiller numbers was decidedly bimodal in this regression due to the two very different planting environments. The possibility can be suggested that some other environmental factor could be contributing to the regression. However, from the earlier work discussed above, which supports the inverse cause and effect relationship between the two variables, in situations where gross environmental differences are not present, it is considered likely that the analysis is valid as presented. The second problem is that since at least one

culm of each smutted plant has to be smutted, one culm is not free to vary. By including these culms in the numerators and denominators, one obtains a slightly higher within-plant disease percentage than would be obtained if they were excluded. As the data exist, exclusion would tend to decrease the slope of the regression, but the decrease would be slight. The significance of the regression is sufficiently large (probability of the slope being 0 = less than 0.00001), however, that the change would be negligible. Hence, the simple approach of using unaltered percentages is just as reliable as the more complex approach would be. This was also the case in the correlation analysis.

Although previous workers, mentioned in the introduction, mostly chose means of measuring within-plant disease severity which differed from those used in this study, and although most of the earlier studies were relatively small, the conclusions of this and the earlier studies are in general agreement. Ruttle (1934) mentioned one barley-U. hordei combination in which 30 percent of plants were smutted but in which only 17 percent of all heads were smutted. At this intermediate percentage of smutting (of plants) such a result would be in accordance with the correlation derived in this study. On diseased plants a portion of the culms would be healthy. Indeed, this was found by Ruttle. Also contributing to the disparity between the two measures was

the fact that smutted plants produced fewer tillers. Oort (1939) presented some evidence that for the loose smuts of barley and wheat partial smutting is perhaps less frequent than for covered smut of barley. In Oort's study the inoculated and diseased wheat and barley plants were nearly always totally smutted. Only 3.9 percent of diseased wheat plants and 4.0 percent of diseased barley plants had healthy culms, and these were nearly always in the minority. Even though the percentage of smutted plants was high (over 70 percent) this would, in comparison with high disease combinations in the present study, have to be considered a small degree of partial smutting.

Cultivars like Odessa, which possessed little between- or within-plant resistance to any of the dikaryons, and Keystone, which in general possessed a high level of both types of resistance, undoubtedly contributed greatly to the correlation. The within-cultivar analyses, however, revealed that the correlation is not limited to between-cultivar comparisons. While cultivars which possess a generally high or low level of the two types of resistance can easily be picked out, dikaryons do not form such distinct groups. A dikaryon was not found, for instance, which possessed a relatively high between- and within-plant disease-producing ability on all or most cultivars. This leads to the conclusion that, initially at least, studies on the inheritance of host resistance would be more productive.

The variability shown for 111 combinations in within-plant disease reaction is, to a large extent, genetically determined. Sampling error is of the magnitude of ± 5 percent. Environmental effects cannot be ignored, but they are almost certainly of less importance than genetic determinants. Earlier studies have established that between-plant disease reactions are largely genetically determined, so that the close correlation of between- and within-plant reactions can itself be taken as evidence for the genetic determination of within-plant disease reaction. The experimental results also attest to the minor role played by environment. Odessa, which at British Columbia was consistently heavily smutted, was planted in blocks which were more or less randomly located over a two year period. Some blocks were planted in very poor soil; yet smutting was always high on both a within- and between-plant basis. Blocks of various cultivars and dikaryons were frequently split into two or more sub-blocks. Sometimes these were planted in two different years. Yet, at UBC, environmental effects on disease were always minimal (up to about ±5 percent) when sub-block results were compared.

Because the correlation was rather close, it is not likely that contrasting combinations for genetic studies will be found each of which possesses only one of the high disease reaction types. In particular, there seems to be a lack of combinations which give high between-plant reactions and low

within-plant reactions. Two possibilities are left for further study: varieties with high-within and high-between reactions can be crossed with those with low-within and low-between reactions; or crosses involving varieties which differ only in their within-plant reactions can be studied. For example, to study the inheritance of resistance, Trebi could be crossed with Nepal and the progeny tested with either R4 or Et1, to which genotypes the parent cultivars give contrasting reactions for both resistance types. Good examples of potential parent cultivars which seem to differ in their within-plant reactions only are Keystone and Lion with R9, and Trebi and Lion with R6. To study the inheritance of virulence in the pathogen, R4 could be crossed with R11 and the progeny tested on Trebi, to which the parents give contrasting between-plant and within-plant disease reactions. R1 and Et2, which differ in their within-plant virulence only, could be crossed and the progeny tested on Trebi.

Combinations which differ only in their within-plant reactions are noteworthy because they may indicate possible non-identity of genes governing the two types of resistance. Alternatively, differences in environmental sensitivity of cultivars or dikaryons could be an important, although less likely, explanation. Background polygenes might also be important. A proper experimental design would distinguish between these possibilities in crossing studies.

PART III

THE PATTERN OF SMUTTING AND EFFECT ON PLANT GROWTH BY *USTILAGO HORDEI* IN 'HANNCHEN' BARLEY

A. INTRODUCTION

Faris (1924) and Johnston (1934), in their studies of the barley- *U. hordei* system, both reported that, following inoculation of barley with *U. hordei*, the first heads to appear in a group of plants were usually healthy, and that diseased heads usually appear at a later date. Johnston concluded that diseased culms were slower to mature than healthy regardless of whether the latter were from healthy or smutted plants. Faris reported that it was culms from healthy plants which one observed first. In neither case was the explanation supported by observational data. An alternate, or at least contributory, possibility is that older culms of smutted plants more frequently remain healthy. In part, this work was undertaken to test this possibility--that is whether, within a diseased barley plant, the diseased and healthy tillers are produced randomly, or in an age-related pattern. The work also concerns the development of barley plants and how this may be affected by *U. hordei*. Since partial resistance probably is, as parts I and II would indicate, an important contributor to host-parasite accord, it was felt that studies furthering

our understanding of the details of such resistance would be most worthwhile.

Very little information is available concerning the exact location of healthy and diseased culms in partially smutted or bunted cereals and plants. Churchward (1937-38) found that the resistant wheat cultivar Hope showed a low percentage of late tiller bunting when inoculated with T. caries. Usually the fifth or sixth tiller was diseased. Reed (1938) found that the oat species Avena brevis, when inoculated with race 1 of U. kolleri, showed smutted lateral tillers and healthy spikes of principal culms in nearly all of the 20 to 30 per cent of diseased plants.

Neither of these workers paid attention to exact tiller relationships. At best they casually distinguished the principal culm from the smaller lateral culms. No other work was found which in any way further showed smutting patterns in cereals.

The pattern of barley growth is similar to that of the more thoroughly studied wheat. According to Hector (1936), the two are so similar that the information on growth patterns in wheat also applies to barley. A brief account of the pattern of growth in barley, given by Sarvella et al. (1962), compares closely with the description for wheat given by Percival (1921). In general, the

pattern of tillering can be called monopodial, with a central unbranched culm (the principal or apical culm) from several of whose lower nodes lateral culms (primary laterals) arise. Each of the primary lateral culms is itself capable of producing (secondary) laterals. Except for the limited number of nodes on each culm, the growth pattern is, theoretically, indeterminate. Plants can produce 100 culms if the environment is unusually favorable. In reality, however, plants usually do not proliferate to this extent. A more detailed description of growth patterns will be presented in the results section.

B. MATERIALS AND METHODS

This work was carried out entirely in the greenhouse. The barley cultivar 'Hannchen' was chosen because of its high tillering capacity under greenhouse conditions. The single smut dikaryon composed of haploids E3a and I4A was used throughout. Seed treatment and inoculation were as described in part I. Uninoculated seeds were treated exactly as those inoculated, except that sterile complete broth (followed by a distilled water rinse) was substituted for inoculum. During the 3 to 4 months of growing time, pots and flats were rotated and rearranged regularly.

When 2 to 3 culms had appeared on the plants, they were tagged in order to establish a record of the tiller relationships. They were again tagged at a later date, when more culms had appeared. Without such tagging, it was nearly impossible to determine, with certainty, the tillering patterns of mature plants. One by 1.5 inch white marking tags with strings were used. To prevent tags from deteriorating, after the information on tiller nodal origin and sequence of appearance had been recorded on them in pencil, they were dipped in paraffin. It was found that even if a tagged culm died, its exact position could still be determined.

When all culms had headed, their position, sequence and fate (headed, smutted or dead) were recorded. Information relating to single plants was in each case transferred to a schematic plant diagram to aid in subsequent analysis. Pots, which were found to be more suitable, were used exclusively in the next three plantings. Three plants were grown in each pot. One of the three plants in each pot was uninoculated in two of the plantings. These plants served as controls when observing possible effects of latent infection in nonsmutted but inoculated plants. To obtain profusely tillering plants, in the last planting, inoculated seeds were planted singly in forty pots and highly fertilized.

Statistical techniques used to analyze these data included the confidence interval about the difference of proportions, the simple t test, the paired t test and the Chi-square test.

C. RESULTS

In these studies, a total of 528 barley plants were labelled and recorded. Noninoculated plants totalled 83. Of the 425 inoculated plants, 209, or 47.0 per cent became smutted. The 209 smutted plants produced a total of 1,198 culms, for a mean of 5.73 culms per plant. Of these, 927, or 77.4 per cent, were smutted.

One of the primary objectives of the study was to determine how the nonsmutted culms were distributed in smutted plants. To do this, a thorough understanding was needed of how a barley plant grows. Figure 3.1 shows a photograph of a rather typical month-old barley plant with six young culms. Below it there is a line drawing of the plant, naming the normal and modified leaves which subtend the tillers. The terminology is also introduced in the diagram. A diagrammatic sketch of a hypothetical plant, in which the internodes have been greatly lengthened and all leaves are excluded, can be seen in the appendix. These diagrams were useful in analyzing the data. In reality, the

region of tillering, commonly called the crown region, is greatly compressed, as the photograph would indicate.

Some qualitative statements can be made about the general pattern of growth of 'Hannchen' barley under the experimental conditions that were employed. Plants invariably produced the principal culm (1S) as the first and oldest culm. If, for some reason, it died very early, the next oldest culm assumed its role. If a lateral culm was separated early from a plant, it also acted as a principal culm, that is to say, the lateral's lower nodes produced lateral culms to a much greater extent than it would have done had it remained attached to the original plant. Only one primary lateral was produced from each node in this cultivar. With rare exception the lateral culm arising in the axil of a lower leaf was older than one arising from the axil of a higher leaf, so that, for example, the 2L culm appeared before the 3L culm in almost all plants. This can be seen in the photograph (Figure 3.1), where the 1L, 2L and 3L laterals are clearly in order of descending age. In labelling, the numerical subscript indicated the order of appearance of primary lateral culms (a primary lateral being defined as the first lateral produced from a node of the principal culm). The exceptions to this order were often in the form of late primary laterals from lower leaves, usually the CN or 1L nodes. These nearly

FIGURE 3.1

FIGURE 3.1. ILLUSTRATION OF THE PATTERN OF EARLY TILLER PROLIFERATION IN A ONE-MONTH OLD PLANT OF 'HANNCHEN' BARLEY

FIGURE 3.1

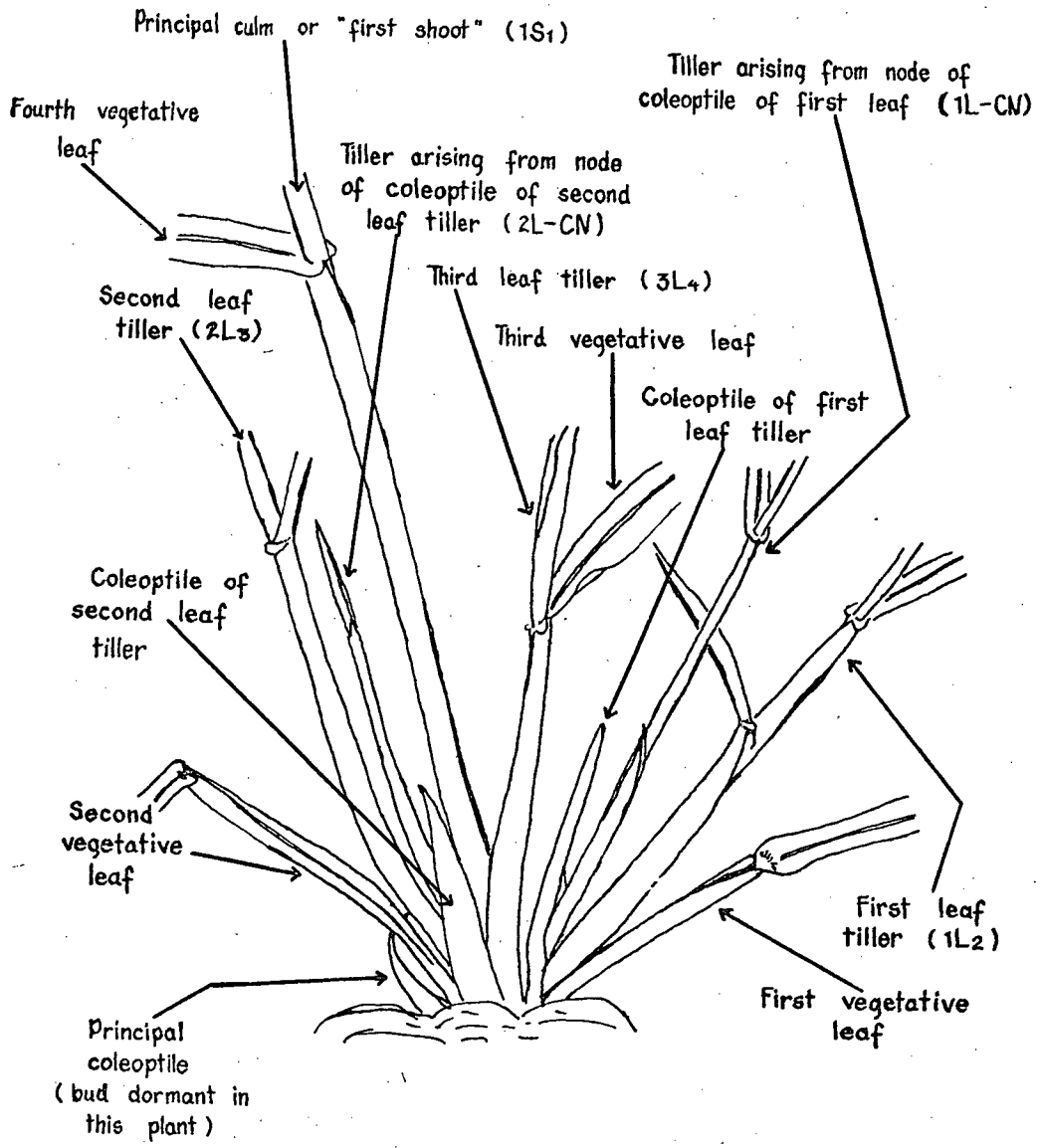

FIGURE 3.1. CONTINUED

always died before heading. In only one case was an exceptional order of appearance of primary lateral culms seen on a partially smutted (and hence important) plant. Because this may not reflect relative tiller bud age in the very young seedling, this plant was not included in the analyses. Plants produced laterals from the coleoptile node up to as high as the eighth vegetative leaf node. In general, however, about 3 to 4 nodes were involved. The nodes most commonly employed were 1L through 5L. Throughout the rest of this thesis a primary lateral together with all of its lateral culms will be referred to as a tiller family. Attention was paid to the order of appearance of culms within a tiller family, where the sequence did not vary from what would be expected (e.g. 2L followed by 2L-CN followed by 2L-1L). No attempt was made to compare relative ages of secondary laterals arising from different primary laterals (for instance 1L-CN with 2L-CN). At all levels of tiller production there was occasional absence of expected tillers (e.g. the 2L lateral did not appear). In many cases this was probably due to injury to the young bud. Quantitative data relating to lateral tiller production will be presented later for inoculated and noninoculated plants.

1. The Distribution of Smutted and Healthy Heads in Smutted Plants

Some simple statistical tests were devised in order to allow definitive statements to be made regarding the

randomness or nonrandomness of the location of healthy heads in smutted plants. The first of these tested whether or not the distribution of smutted and nonsmutted heads among tiller families was random. Table 3.1 presents the analyses, separately, for two, three, and four or more (bulked) tiller families from all smutted plants. The procedure was to classify each family according to whether all culms were smutted, or all were healthy, or whether some were smutted and some healthy (mixed). From these results the values of p, the probability of a culm in that family group being smutted, and q, the probability of a culm remaining healthy, were obtained for each family size. If randomness of smut distribution were the case, the proportions of families showing the various degrees of smutting should fit the expanded binomial $(p + q)^n$, where n is the number of culms in the family. Thus for two-tiller families, the expected frequency of those with both heads smutted is p^2, that of mixed is 2pq, and that of those with both heads healthy is q^2. For tiller families in which the number of culms was three or more, attention was not paid to the composition of the mixed families or to the relationship of culms within the families. By applying a Chi-square test for goodness-of-fit, it can be readily seen that the observed numbers do not fit the expected. In each case the observed frequency of mixed families is too low while that of the other two classes is too high. Hence there is a

TABLE 3.1

OBSERVED AND EXPECTED NUMBERS OF VARIOUSLY SMUTTED TWO, THREE AND FOUR OR MORE TILLER FAMILIES, AND THE APPLICATION OF A CHI-SQUARE TEST FOR RANDOMNESS OF SMUTTING AMONG FAMILIES, USING BINOMIAL EXPANSIONS TO CALCULATE EXPECTED VALUES

Grouping	All Smutted	Mixed	All Healthy	Total
Two tiller families				
Observed	81	9	25	115
Calculated values of:[a] p = 0.74 q = 0.26				
Expected (if randomly distributed)	(p^2) 63	(2pq) 44	(q^2) 8	115
Probability that smutted heads are randomly distributed = <0.001				
Three tiller families				
Observed	37	5	14	56
Calculated values of: p = 0.71 q = 0.29				
Expected (if randomly distributed)	(p^3) 21	$(3p^2q+3pq^2)$ 34	(q^3) 1	56
Probability that smutted heads are randomly distributed = <0.001				
Four or more tiller families (average 6.2 tillers)				
Observed	33	5	9	47
Calculated values of: p = 0.65 q = 0.35				
Expected (if randomly distributed)	(p^6) 4	$(1-p^6-q^6)$ 43	(q^6) 0	47
Probability that smutted heads are randomly distributed = <0.001				

[a] p = the probability, based on the observed data, of a single culm being smutted.
q = the probability of a single culm remaining healthy.

strong tendency for tiller families to be composed entirely of either smutted or healthy spikes.

Attention was next paid only to the 19 tiller families which were mixed. They were classified according to whether the nonsmutted spike(s) were the oldest, youngest, or neither. If smutting is randomly distributed in relation to age, the ratio of oldest healthy to youngest healthy tillers should be 1:1. The test is shown in Table 3.2. Sample sizes were small, so the Yates correction factor for continuity was applied in carrying out the Chi-square test (Stansfield, 1969). A significant difference exists between the observed and the expected ratios. The conclusion is that there is a clear tendency for older spikes within mixed smutted families to remain healthy.

TABLE 3.2

TEST FOR RANDOMNESS OF POSITION OF NONSMUTTED TILLER(S) WITHIN SMUTTED TILLER FAMILIES, IN TERMS OF AGE (ORDER OF APPEARANCE) OF THE TILLERS

	Position of nonsmutted tiller or tillers		
	Oldest	Neither	Youngest
Observed	15	0	4
Expected (if randomly distributed)	8.5		8.5

Probability that oldest:youngest ratio fits 1:1 = 0.015*

*Applying Yates small sample correction for continuity.

Also investigated was the relationship, if any, between age (position) of a tiller family and its probability of being smutted. A test, similar to the one explained above, was applied to smutting among families. A tiller family was considered smutted if any of its spikes were smutted. Nonsmutted families on all smutted plants were classified according to their age relative to the smutted families. A nonsmutted family (or a series of them) could thus be classified as older or younger than the family or families showing smut, or of intermediate age, or neither, depending on when the primary laterals of the families had appeared. Table 3.3 gives the details of the Chi-square tests of how well the ratio of oldest to youngest nonsmutted family position fits the expected 1:1 ratio (based on the assumption that there is no relationship between family age and smutting probability). Two different results are tested--one ignoring the principal culm, which cannot be regarded as a tiller family, and one including the 1S as the oldest culm (and hence considering only plants in which 1S survived). When only lateral tiller families are counted it is clear that there is a highly significant difference between the actual oldest:youngest ratio obtained and the 1:1 expected. Thus there is a strong tendency for older tiller familes to remain healthy in partially smutted plants. When the 1S culm is included, however, the tendency can no longer be seen using the simple test, as the second part of Table 3.3 shows. A more complete picture can be

TABLE 3.3

TEST FOR RANDOMNESS OF POSITION OF NONSMUTTED TILLER FAMILES WITHIN THE SMUTTED BARLEY PLANT, IN TERMS OF AGE (ORDER OF APPEARANCE) OF THE FAMILIES

	Position of nonsmutted family or families		
	Oldest	Intermediate	Youngest
Later tillers only			
Observed	55	8	26
Expected (if randomly distributed)	40.5		40.5
Probability that oldest:youngest ratio fits 1:1 = 0.001			
Principal tiller included (as oldest tiller)			
Observed	31	30	25
Expected (if randomly distributed)	28		28
Probability that oldest:youngest ratio fits 1:1 = 0.45			

seen in Table 3.4. Here tiller families, along with the principal culm, are presented by position and fate. The data of this Table are shown graphically in Figure 3.2, where tiller families are classified and the percentage in each class which showed smut are given. The 1S culm is the only plant part which does not fit the pattern of decreasing frequency of smutting with age. Data for the 1S culms and, to a lesser extent, for the CN and 1L families, are affected by the relatively high death rates; had these culms all survived to

heading, the percentages of smut may have been changed. The graph and table show that the CN and 1L positions (bulked) were smutted significantly less frequently than the 2L position, which was in turn smutted significantly less frequently than the 3L, 4L or (bulked) 5L and up positions. The latter three were not significantly different.

One other notable point emerges in Table 3.4. The frequency of tiller family death seemed to be directly proportional to their age. This fact was noticed casually during labelling and harvesting. Later emerging culms seemed to be "favored" by the plant. Not only were they more likely to survive, but they grew faster. The very late culms, sometimes seen, especially in response to late heavy watering, were the fastest growing of all. Because these arose spuriously, they were not included in the analyses.

TABLE 3.4

THE PRODUCTION BY SMUTTED PLANTS AND FATES OF TILLER FAMILIES, ANALYZED BY NODAL POSITION

Nodal Position	Tiller family fate (%)			Percent of Surviving Fam. Smutted*	Sample Size
	Headed	Smutted	Dead		
1S	14.6	50.2	35.2	80.4^{bc}	213
CN and 1L	29.0	46.0	25.0	61.3^{a}	124
2L	17.6	61.7	20.7	77.8^{b}	188
3L	10.3	68.5	21.2	86.9^{c}	184
4L	14.8	74.7	10.5	83.4^{c}	162
5L and above	12.5	72.2	15.2	85.3^{c}	184

*Values not sharing the same letter are significantly different (p=0.05). Statistical comparisons are made vertically.

FIGURE 3.2

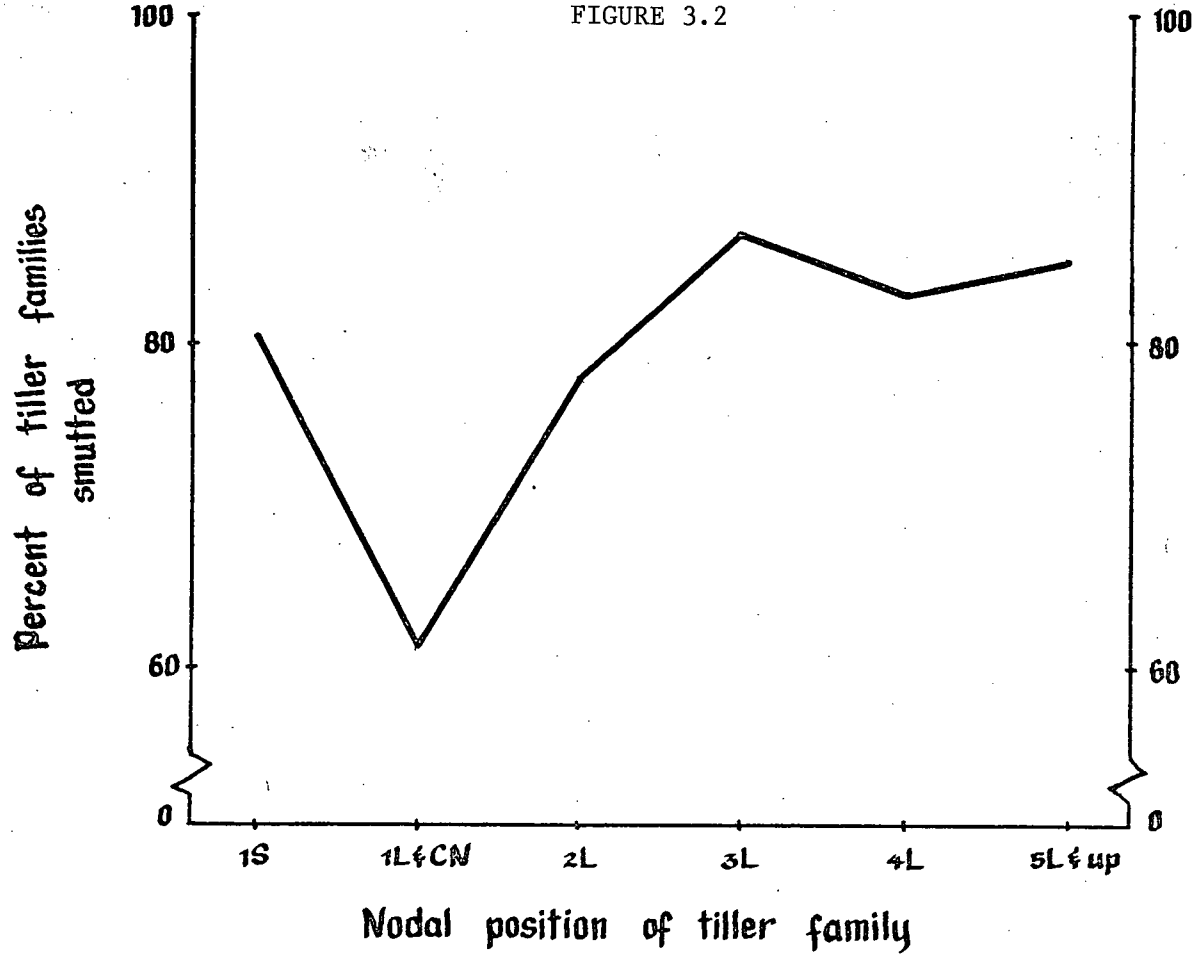

FIGURE 3.2. FREQUENCY OF COVERED SMUT OCCURRENCE IN TILLER FAMILIES OF BARLEY PLANTS IN RELATION TO RELATIVE AGE OF THE TILLER FAMILY (AS INDICATED BY POSITION)

2. The Effect of Inoculation and Smutting on Plant Growth

The main effects of inoculation and smutting on plant growth were examined: stunting, effects on tillering, and effects on the pattern of plant growth.

Three weeks after planting, seedlings of the 1973-4 greenhouse experiment season were examined for stunting. Out of 206 inoculated seedlings, 25 exhibited some stunting (plants short with narrow, dark green leaves). None of 83 uninoculated plants was stunted. Of the 25 stunted plants, 16 became smutted and nine did not. This sample is too small for statistical treatment. One can, however, conclude that while stunting is probably smut-related, no clear relationship between stunting and subsequent smutting was seen (i.e., stunted plants were not always smutted).

Because of rather large among-pot variation observed in 1972-3, it was thought that plants of different treatments should be paired, each pair being from the same pot, in order to measure the effects, if any, of inoculation and smutting on tillering. The analysis is presented in Table 3.5. Whether or not plants showed smut, there was a significant reduction in the number of tillers per inoculated plant, both initiated and surviving (to maturity), when such plants were compared with uninoculated plants. There was no

TABLE 3.5

MEAN NUMBERS OF CULMS INITIATED PER PLANT, AND THEIR FATES (HEADED, SMUTTED OR DEAD), WHEN PLANTS WERE UNINOCULATED, INOCULATED BUT FAILED TO BECOME SMUTTED, OR INOCULATED AND SMUTTED

Treatment Comparisons	Number of paired Observations	Mean number of culms per plant*		
		Headed	Dead	Total
Noninoculated	68	5.56	2.93	8.49
Inoculated nonsmutted		4.91	2.47	7.83
Inoculated nonsmutted	63	4.97[a]	2.13	7.10[b]
Smutted		4.63[a]	2.81	7.44[b]
Noninoculated	48	6.33	2.87	9.21
Smutted		4.10	2.35	6.46

*Means not sharing the same superscript are significantly different (P=0.05, paired t analysis). Statistical comparisons involved paired means only.

significant difference, in total tillers initiated or surviving, between smutted and nonsmutted inoculated plants. The situation was different for dead tillers. Noninoculated plants had significantly more dead tillers than either smutted or nonsmutted inoculated plants. This is probably a reflection of the overall higher tiller production of noninoculated plants. Smutted plants had significantly more dead tillers than did nonsmutted inoculated plants.

To examine the effect of inoculation and smutting on the pattern of plant growth, smutted, nonsmutted inoculated and noninoculated plants were analyzed with regard to the percentages of plants attempting to produce tiller families from each node. The data, along with the statistical analyses, are presented in Table 3.6. The date are plotted, to show the differences more clearly, in Figure 3.3. Smutted and nonsmutted inoculated plants differed in their growth patterns only insofar as smutted plants attempted tiller family production with a significantly higher frequency at the CN and 6L nodes. This may relate to the higher death rate of culms in smutted plants, whereby dead culms are replaced by new ones. A marked effect of smutting and inoculation on the pattern of plant growth is seen when the noninoculated plants were compared with the other two groups. In the figure, it is clear that the noninoculated plants produced lateral tillers from lower nodes than either nonsmutted inoculated or smutted plants. In other words, inoculation had the effect of decreasing lateral tiller production at the 1L node and increasing it at the 5L and 6L nodes. The CN node did not conform to this effect, although it might have been expected to do so.

A final observation should be noted here. Smutted plants exhibited a marked delay in emergence of heads. In each planting the healthy plants were the first to be

TABLE 3.6

COMPARISONS OF GROWTH PATTERNS (PERCENT OF LATERAL TILLER PRODUCTION FROM DIFFERENT NODES OF THE PRINCIPAL CULM) OF 209 SMUTTED, 194 NONSMUTTED INOCULATED, AND 83 NONINOCULATED 'HANNCHEN' BARLEY PLANTS

Nodal Position	Percent of plants attempting tillering from node*		
	Smutted	Nonsmutted Inoculated	Noninoculated
CN	25.8[a]	13.4**	21.7[a]
1L	35.9[b]	44.8[b]	90.4
2L	91.9**	93.3**	98.8**
3L	87.6**	93.3**	96.4**
4L	78.5[c]	84.0[c]	62.6
5L	56.0[d]	46.4[d]	13.2
6L	28.2	12.9	3.6**

*Statistical comparisons are made horizontally. Values not sharing the same superscript letter are significantly different (p=0.05).

**Sample size too small for statistical analysis.

recorded and harvested, while those which appeared last were invariably smutted plants. Heads of nonsmutted inoculated plants did not emerge noticeably later than those of non-inoculated plants.

FIGURE 3.3

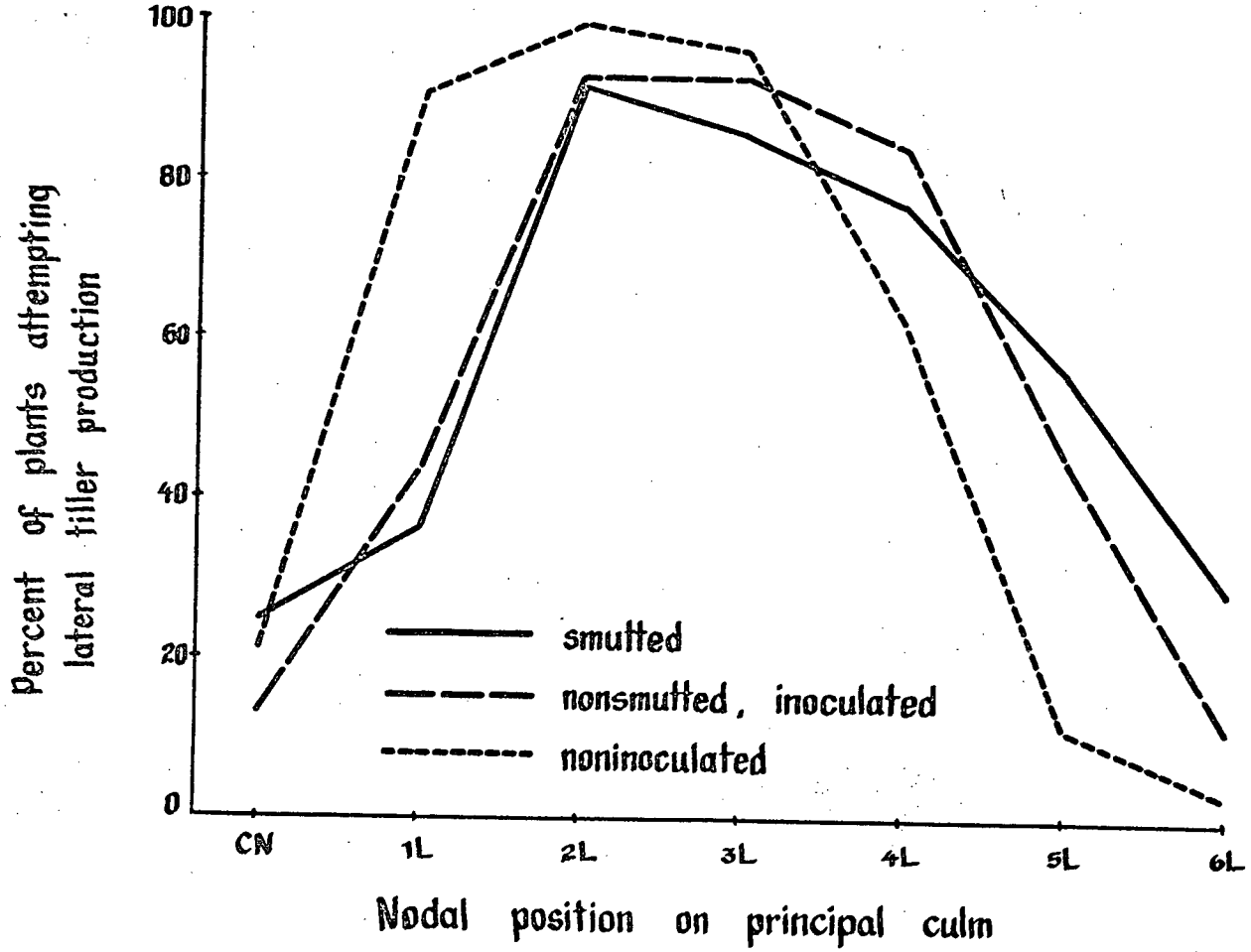

FIGURE 3.3. THE PERCENT OF SMUTTED, NONSMUTTED INOCULATED AND NONINOCULATED BARLEY PLANTS INITIATING LATERAL TILLER PRODUCTION ACCORDING TO NODAL POSITION ON THE PRINCIPAL CULM

D. DISCUSSION

Since very little has been written concerning growth of the barley plant, the information on growth of 'Hannchen' barley was learned almost entirely through first-hand observation. The paucity of information about barley may be due to the belief that growth of barley is quite similar to that of wheat or oats, two much more thoroughly studied cereals. The assumption can also be made that plumular development in the dormant seed of barley is similar to that of wheat, as Hector (1936) states in his chapter on barley. In wheat, both Percival (1921) and McCall (1934) found that two bud primordia are present in the dormant seed. One of these is the apical bud, which will give rise to the 1S culm, and the other is in the axil of the coleoptile and will develop into the CN culm. Bonnett (1935) very briefly described the same condition in seeds of both 2- and 6-rowed barleys. Percival found that it was not until 10 to 15 days after germination that the bud primordia in the axils of the oldest vegetative leaves could be detected, lowermost first. In conclusion, the above studies indicate that there is a clear difference in development among bud primordia at the time of initiation of smut infection. Whether this pattern of early development varies appreciably has, unfortunately, never been determined. Histological studies have necessarily involved only a small number of embryos, so that the rare

case of atypical development, if it exists, would have gone undetected. As Percival has pointed out, however, one can expect normal biological variation in this and, indeed, as the present studies show, in all aspects of barley growth.

1. The Distribution of Smutted and Healthy Heads in Smutted Plants

Smutted heads did not occur randomly in partially smutted plants. Members of a tiller family tended to either be all healthy or all smutted. Further, at both the within-family and between-family level, older culms remained healthy more frequently than did younger culms. It should be emphasized that these were statistical tendencies only. Exceptions were not rare.

A phenomenon similar to the above occurred within individual spikes. Occasionally, spikes were found which contained both smut teliospores and healthy seeds. Invariably, the healthy seeds were found on the upper part of the spike and the smut on the lower part. The same observation was recorded by Faris (1924a). Bennett and Finch (1971) determined that meiosis in barley begins at the top of the spike and proceeds downward, so that upper seeds are slightly older. Thus the within-spike smutting pattern is the same as that seen at the within- and between-tiller family level. The only other report found of within-spike partial disease was by Holton and Rodenhiser (1942). They

observed partially diseased heads of wheat infected with T. caries in which sound seed and bunt balls were both randomly located.

As indicated in the literature review, the pathway which smuts take in spreading to various parts of the crown has not been conclusively determined. Here, too, it is likely that considerable variability exists, so that the mycelium does not necessarily have to move precisely through the same tissues in each seedling. Histological evidence pointing to any specific or frequent route, taken by the fungus in spreading to various parts of the crown, is weak. Also weak is evidence for a difference in distribution of mycelium within the crown which would explain the tendency for older culms to remain healthy. In both cases, the weakness of the evidence stems from the fact that only small numbers of infected embryos have been examined. From the limited evidence which is available it appears that the smut hyphae enter the crown from below, so that older leaf sheaths and nodes are infected first, and that the mycelium is more abundant in the lower part of the crown than in the upper. This available evidence does not provide a ready explanation of the observed distribution of smutted culms. On the contrary, if the location of the hyphae within the crown were the determining factor, one would expect the older culms to be more frequently smutted. In conclusion, histological work on the pathway of infection and the distribution

of smut mycelium does little to explain the pattern of smutting which was most frequently observed. In the less frequent cases, where younger culms were nonsmutted, the infection pathway and/or the distribution pattern of mycelium within the crown is a possible explanation.

In a few plants, the oldest and youngest primary lateral culms were smutted while one or more intermediate tiller families were healthy. This may be the result of multiple infections (infection of a seed by more than one dikaryon). Such multiple infections have been conclusively demonstrated in oats infected with U. kolleri by Person and Cherewick (1964). Although not determining smutting patterns based on tiller relationships, they showed that different dikaryons can occur in different culms of the same plant. The similarity of U. kolleri to U. hordei is such that it would seem quite likely that such multiple infections occur in the latter also. In U. kolleri genotypic mosaics indicate that variation is possible in how and/or when smut hyphae spread throughout the crown region. A thorough study of the multiple infection phenomenon in barley-U. hordei would well complement the present studies.

A possible basis for the observed pattern of smutting has been mentioned by other workers. Sampson (1932) observed a barrier of lignified cells between the nodal tissues

containing U. kolleri mycelium and the apical growing point in resistant oat plants. She concluded that if the plant could produce this barrier before the fungus reached the growing point, the culm or culms would not become smutted. She suggested that susceptible plants were unable to lay down this barrier in time, because the fungus was growing rapidly, and that growth of the mycelium in totally resistant cultivars was strongly retarded. Regardless of whether or not lignification is the critical event, the hypothesis that degree of smutting depends on relative rates of growth of the embryo and the smut mycelium is promising and popular, particularly in explaining total resistance. Ohms and Bever (1955) found that the mechanism of resistance to U. tritici in one wheat cultivar seemed to be simply that the seedlings outgrew the smut mycelium. The same can be said for other studies, mentioned in the literature review, where early embryo infection of totally resistant cultivars was as high as that of susceptible cultivars. Swinburne (1963) believed that for wheat infected by T. caries, once the hyphae reached a growing point, smut was always expressed in that culm. In other words, resistance could occur any time before the smut had reached the growing point, but not after. The critical period, then, appears to be that period of growth prior to development of some barrier to hyphal growth, such as lignification or elongation of the internodes

of a culm. If the mycelium does not get beyond the area of the barrier, it will be left behind. This appears to be the key to the smutting pattern most frequently observed. The barrier would be expected to be produced first in the older culms, allowing a shorter period from germination during which the smut mycelium must reach the growing point, when compared with younger culms. The rarity of mixed tiller families might be explained by a combination of relative similarity of age and close proximity of primordia of tiller family members.

The principal culms did not fit into the same age: smut frequency pattern as the lateral culms, and the above hypothesis cannot apply to them. While there can be no doubt that the 1S culm is the oldest culm on the plant, it is not the least frequently smutted, by any means. Hence, the observation that healthy culms appear before smutted in a group of inoculated plants is probably not due to the smutting-age relationship of culms. It seems to occupy a special place with regard to smutting. The apical growing point could perhaps be considered the "target" of smut hyphae spreading throughout the crown region. The most that can be said here is that it occupies a unique position with regard to the age: smutting frequency relationship, when compared with lateral culms.

Some workers have tried to relate size of seed and rapidity of germination to smut susceptibility in determining if, from the standpoint of the plant, the length of the critical period is important. Results vary. Tervet (1944) found that rapid, vigorously germinating oat seeds were less susceptible to loose and covered smut than less rapidly germinating seeds of each of several cultivars. Reed and Faris (1924) had found this not to be the case, however, using the same host and pathogens. Brandwein (1938) could find no correlation between covered smut suceptibility and the germination and growth rate of susceptible and resistant oat cultivars. Faris (1934) actually found bunting percentages to be higher on rapidly germinating seeds of several wheat cultivars inoculated with Tilletia Foetida (Wall.) Livo and T. caries; but Smith (1932a) could find no relation between growth rate and bunt susceptibility in the wheat cultivars he examined. These inconsistencies might best be explained by differences in experimental procedures and by the fact that variation in seed size, vigor of germination or growth rates are complex phenomena about which generalization is risky, particularly when one tries to relate them to a concept equally complex, such as smut susceptibility.

Increased smut susceptibility caused by such treatments as deep sowing and cooler growing temperatures, which tend to slow germination and plant growth rate, can also be

explained on the basis that they allow a longer critical period. Swinburne (1963) expressed this idea for wheat-T. caries, and Jones and Seif-El-Nasr (1939), with regard to planting depth, for several cereal smuts, including U. hordei.

2. The Effect of Inoculation and Smutting on Plant Growth

Seeds which had been inoculated grew into plants with fewer surviving culms than those which had not, regardless of whether or not these plants became smutted. This represents, as far as the author is aware, the first report of any effect of inoculation on nonsmutted plants when a compatible host-pathogen combination is involved. It is also the first report, of any kind, of such an effect in the barley-U. hordei system.

Reports of effects on germination or growth of nonsmutted inoculated plants when the host possessed total or near total resistance to smutting were covered in the literature review. Rather surprisingly, the effects observed in this work were much more subtle than any of those reported. Neither apparent blasting, seedling stand reductions, nor increased tiller mortality were observed in nonsmutted inoculated plants in the present work. The early finding that nonsmutted "immune" cultivars were in reality being rather severely affected by smut caused some

alarm. Of course, it was also realized that if such cultivars were widely planted, the smut would, barring large population shifts toward virulence on the resistant cultivars, soon be eliminated because of low reproductivity.

The only other effects observed on nonsmutted inoculated 'Hannchen' barley plants were the infrequent stunting of seedlings and the shift in the pattern of tillering. All of these effects could be caused by smut mycelium being present in the plant only at the seedling stage. Due to space limitations, plants were competing for root space and light, and differences between treatments were probably magnified by this inter-treatment plant competition. Alternatively, if it could be shown that nonsmutted inoculated plants were, overall, less vigorous than noninoculated plants, it would support the premise that the mycelium is present in the plant tissue for a relatively long time, perhaps to maturity. One can safely conclude that in this particular genotypic combination, smut mycelium is present for at least a short period in inoculated seedlings of ultimately "healthy" plants. The situation is not as had been found in some resistant combinations of host and smut (discussed in the literature review) where the pathogen was unable to penetrate beyond the pericarp. While effects on plants were not investigated in these latter cases, it is doubtful that the pathogen could have had a significant effect on plant growth

when exclusion was so effective. Of course, as the same workers who demonstrated such resistance showed, one cannot generalize about the details of resistance in the host-parasite system based on what was found in a single combination, particularly when that combination exhibits a different (i.e., partial) sort of resistance than that which has been commonly studied. Resistance to U. hordei by near-exclusion from the seed may occur in barley. It has yet to be demonstrated, however.

Compared with histological observation of seedlings, the present work is admittedly a more indirect way of demonstrating the presence of smut mycelium in nonsmutted plants. It has the advantage that much larger numbers of plants can be observed. Detailed histological studies of smut infection all suffer from the major criticism that conclusions are based on a small, often inadequate, number of observations. Nevertheless, histological studies would complement the present studies, and vice-versa.

Smutted plants exhibited other symptoms which nonsmutted inoculated plants did not. In addition to reduced tillering and occasional stunting of young seedlings, smutted plants also exhibited some chlorotic streaking and distortion of upper leaves of smutted culms (but never of healthy culms). In one of the spring plantings, stripe

smutting was common in the upper 1 to 2 leaves of smutted culms. High temperature probably induced this symptom (Schafer et al., 1962). That smutted plants should be affected in ways other than the smutting of florets is well documented. Here, too, generalization is impossible. Reports of effects on tillering, for example, are found for nearly all cereal smuts. Some workers reported reductions in tillering (Welsh, 1932; Mather and Hansing, 1960; Gaunt and Manners, 1971). Sampson (1927) and Holton and Rodenhiser (1942) found increased tillering with wheat-T. caries. Although no supporting data were presented, Ruttle (1934) observed reduced tillering by smutted plants of one barley cultivar inoculated with U. hordei. It must be remembered that in all of these experiments, nonsmutted plants were also inoculated. Whether they were infected or not one cannot say, since inoculation techniques varied. In the present work, however, there was no significant difference in number of tillers per plant reaching maturity when nonsmutted inoculated and smutted plants were compared. This contrasts with Ruttle's findings.

Although smutted plants produced an unusually high number of culms which later died before reaching maturity, this phenomenon is not confined to smutted, or even to inoculated, plants. It seems to be a normal characteristic of plant growth. Even under the best of conditions in the

field, such dead culms were commonly observed. Percival (1921) noted them in wheat and speculated that they were probably the result of insufficient adventitious roots coupled with dry growing conditions. It is not clear why smutting should have the effect of increasing their frequency. Possibly it represents a physiologic change caused by the developing sori, which deprives some of the culms of water or nutrients.

One can only speculate on the reason for the change in pattern of plant development shown in Figure 3.3. It is further evidence for the presence of smut mycelium in nonsmutted inoculated plants, and it points up how much more similar smutted and nonsmutted inoculated plants are when compared to noninoculated plants. It appears that the presence of the smut inhibited tiller production at the lowermost nodes, which then caused the plants to produce their lateral tillers from higher nodes. This is clear despite the fact that inoculated plants initiated fewer culms than noninoculated plants. Whether this inhibition is due to actual death of primordia or to some other (more subtle) factor is not clear.

GENERAL CONCLUSIONS

A huge literature has developed over many years concerning resistance in cereals to smuts and bunts. Because of the overriding economic considerations, total or near-total resistance has been dealt with almost exclusively; but from the standpoint of host-parasite homeostasis, partial or incomplete resistance, such as that with which this thesis deals, is at least as important and probably more so. One must be careful in trying to extend the considerable knowledge about total resistance to include partial resistance. This work has not revealed any great differences, however.

Parts I and III give two separate lines of evidence which show that in 'Hannchen' barley inoculated with a dikaryon to which it is, in the standard thinking, "susceptible," nonsmutted inoculated plants have not escaped infection but, rather, have remained unsmutted as the result of a type of active resistance. This is shown in Part I, which demonstrates the extreme efficiency with which the inoculation procedure insures that smut will be in contact with the germinating seed. Part III shows that, as in the majority of total or near-total types of resistance which have been investigated in several host-smut systems, U. hordei affects the growth of nonsmutted plants, indicating that the mycelium is present in at least a

proportion of the seedlings for an undetermined length of time.

Part II presents, for the first time in any of the cereal smuts, clear statistical evidence that there are two separate components to smut resistance which are closely correlated. This knowledge will be essential in population genetics studies, where all important effects which add to or subtract from a gene's fitness must be understood and accounted for. In particular, this information shows that in fitness studies the measurement based on percentage of infected plants, which has in the past been most often employed, will be inadequate since it ignores within-plant disease reactions.

In Part II, for reasons given there, it was necessary to exclude nonsmutted inoculated plants when obtaining a measure of within-plant disease severity. At this point, it would be useful to consider them once again, along with the partially and totally smutted plants, because Parts I and III indicate that these nonsmutted plants, as well as the partially smutted plants, contribute to disease curtailment via host resistance. There seem to be two distinct thresholds, or stages, through which the fungus must pass in order to produce teliospores. If it gets past the first it will be able to cause some smutting in the plant. This event is measured in terms of between plant

disease severity. If it crosses the second threshold the fungus will frequently smut many or all culms of the plant. One can best think of this event as taking place separately in each culm. For combinations which display a high degree of resistance the distinction between these two thresholds is not clear. For these combinations the nonsmutted plants occurred in the highest frequency, followed by plants with only a minority of culms smutted; the most infrequent classes were those with plants having most or all culms smutted. This continuous and smooth gradation is evidence for a similarity of mechanisms involved in within- and between-plant resistance. However, the gradation is not smooth for combinations in which disease severity is high. Here the most frequent classes were the two extremes. Plants were usually either totally nonsmutted or totally smutted. If the findings with 'Hannchen' and ExI can be applied to other combinations, and there is no reason why they cannot, then it is clear that the fungus is present for a time in a proportion, possibly in all, of the inoculated seedlings. In many plants the fungus does not get beyond the first threshold, i.e., it does not succeed in sporulating. In plants in which the smut does surmount this barrier, it usually also succeeds in surmounting the second barrier, so that all culms are smutted; but in a minority of culms the fungus is stopped at the second threshold. All levels of resistance are found and, in

intermediate combinations, the second threshold becomes relatively more important. Thus the high incidence of nonsmutted plants, even in high disease combinations, shows that two separate events occur. From the standpoint of the host, one can think of the individual culm as having two separate (but not independent) chances of remaining healthy after the seedling is inoculated. They are both measured and defined in terms of probability. How and where the two thresholds operate is not hinted at in this or previous work.

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APPENDIX A

Statistical comparison of binomial ratios

Two binomial ratios can be statistically compared using a confidence interval about the difference of their population means (Li, 1964). The shorthand form, using 1.96, the z value for 0.95 alpha, is:

$$C.I. = D \pm 1.96\sqrt{\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2}}$$

where:

D = The difference between the observed ratios which are to be compared (an estimate of their population difference).

p_1 = The proportion of sample one observations of a given outcome.

p_2 = The proportion of sample two observations of a given outcome.

n_1 = The number of sample one observations.

n_2 = The number of sample two observations.

In applying this test, if the difference between the sample proportions, D, is greater than the calculated confidence interval (in one direction), there is a significant difference between the two ratios. If no difference exists, the interval about D, of course, contains zero.

According to Steel and Torrie (1960), certain minimum sample sizes must be observed in order for the statistics p_1 and p_2 to accurately estimate the

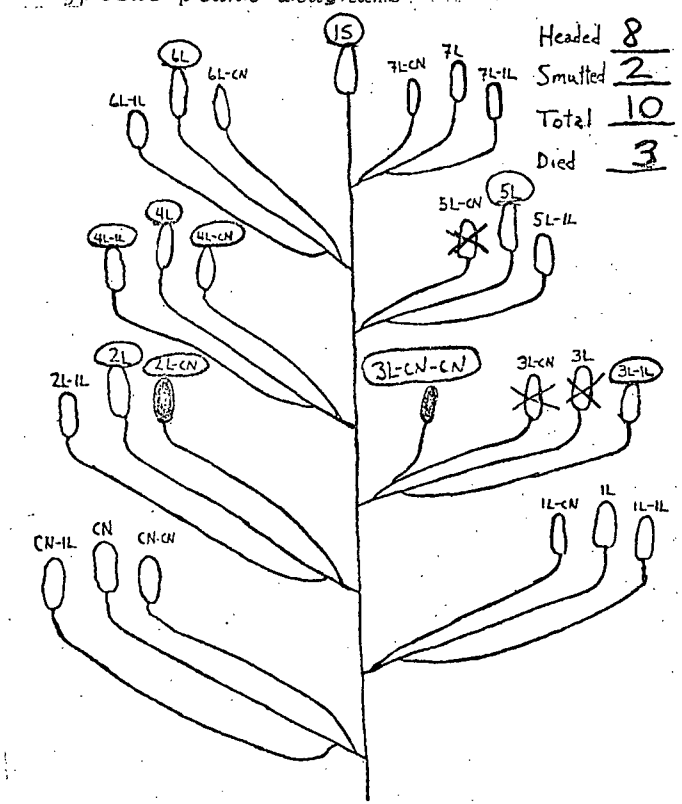
corresponding population parameters as for a normal distribution. This is shown in table A.1.

TABLE A.1

BINOMIAL SAMPLE SIZES FOR NORMAL APPROXIMATION TO APPLY

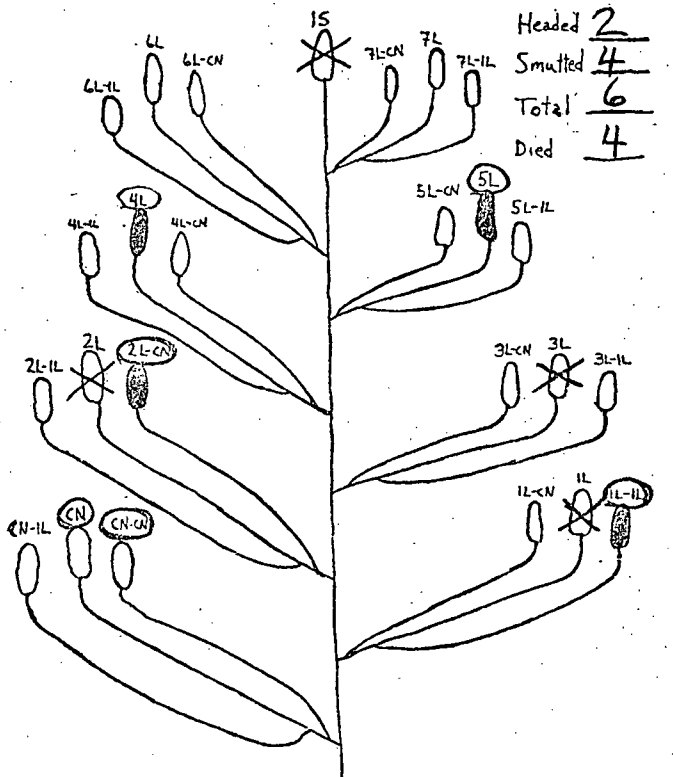
p	Number observed in smaller class (np)	Sample size (n)
0.5	15	30
0.4	20	50
0.3	24	80
0.2	40	200
0.1	60	600
0.05	70	1400

Typical plant diagrams*:



Headed 8
Smutted 2
Total 10
Died 3

Plnt. no. 39-1 Remarks



Headed 2
Smutted 4
Total 6
Died 4

Plnt. no. 14-5 Remarks

* Tillers not marked in any way were not produced by the plant; circled, head unfilled = healthy culm; circled, head filled = smutted culm; crossed out = dead.

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AWARDS

1963	Chapman Foundation Forestry Scholarship.
1965	Member Xi Sigma Pi Honorary Forestry Fraternity.
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