Relation Between Two Measures of Disease Expression in Barley-Ustilago hordei Interactions

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ABSTRACT

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Disease expression was examined in 12 barley cultivars inoculated with 21 *Ustilago hordei* dikaryons. Two parameters were measured: (i) the proportion of smutted plants (among-plant disease severity) and (ii) the proportion of smutted heads on smutted plants (within-plant disease severity). Sufficient plants were inoculated to ensure that 300 plants were available for measuring among-plant disease severity and another group of 30 plants was available for measuring within-plant disease severity. Data were divided into two sets according to degree of plant tillering to eliminate the effect of a negative regression of within-plant disease severity on number of tillers per plant. Strong correlations ($r = 0.734$ and 0.767) were found for among- and within-plant disease severity, respectively. Both variables were found to be largely genetically determined. The correlation suggests identity of genes which condition each of the disease reactions, either in the host or pathogen. Some nonidentity may be indicated by cultivars and dikaryons which differ in only one of the measures, when examined in relation to a single dikaryon and cultivar, respectively.

Additional key words: disease resistance, virulence, pathogen fitness, covered smut of barley.

Various measures have been used by plant breeders and plant pathologists to describe disease severity of the various smuts and bunts of cereals. This is partly because a basic tenet of the disease process of cereal smuts has been frequently ignored, or at least considered of minor importance. This tenet is that two aspects of resistance can be identified. One type is manifested by entire plants, and is measured as the percentage of plants showing smut or bunt in at least one culm. It can be called among-plant disease severity, and is no more than the proportion of plants in which visible sporulation by the pathogen occurs. The second type of resistance involves curtailment of smut sporulation within plants known to be infected at maturity. It can be called within-plant disease severity. A convenient and simple measure of within-plant disease severity is in terms of percentage of culms showing smut on smutted plants (plants with at least one smutted spike).

In a survey of a sample of the smut literature involving 65 papers by plant breeders and pathologists, 38 of the papers contained only the plant basis of measure, and 16 contained a head basis only. In nine others, the workers attempted, in various ways, to measure more than simply plants or heads smutted; and in two others, different sections involved different methods of measurement. Thus, if this sample is representative, there has been little agreement as to which measure should be employed. Moreover, the two measures used most in the above studies are not directly analogous to the quantitatively more distinct among- and within-plant disease severity. Whereas the plant basis is a direct measure of among-plant disease severity, the head basis is not simply a measure of within-plant disease severity. A measurement of smutted heads per total heads on *all* plants is really a compounding of both disease measures. Few workers have attempted to limit their measure of percentage of smutted heads to plants known to be smutted. Some went so far as to count both percentage of plants and percentage 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 disease severity. This would be correct only if it were accurately known that smutted plants produced as many culms as nonsmutted plants, an event usually shown not to be so with various smuts and bunts (7).

Some workers called attention to the possible correlation of within- and among-plant disease severity and validly presented evidence in favor of it. Gaines (6) determined the proportion of totally bunted wheat plants, which he called *c;* the proportion of partially bunted plants, *b;* and the proportion of bunted heads on partially bunted plants, *a*. He used these values in a simple relationship to determine the overall percentage of bunted heads. He noted that the value of *a* was lower on four resistant cultivars than it was on four susceptible ones (0.25 vs. 0.67). Qualitatively, this is good evidence that a positive correlation existed in his material. Working with wheat and *U. tritici,* Oort (10) showed what appeared to be a very close correlation of within- and between-plant disease severity in a study that involved many wheat cultivars. However, his sample sizes were small, and he included no statistical analysis. His was the only study we found in which the correlation was

investigated as a main objective, and in a manner similar to that undertaken in our study.

The purpose of our 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 (i) genetically variable and (ii) correlated with severity among plants.

MATERIALS AND METHODS

Twelve barley cultivars and 21 *U. hordei* dikaryons were used. Only those genotypic combinations that showed smut in at least 5% of inoculated plants were included. The smut dikaryons included a representative of each of the 13 physiologic races described by Tapke (16). These were obtained as teliospore samples from North Dakota State University, Fargo, where they have been maintained. The other eight cultures represent as wide a range of smut genotypes as was readily available. All are from North America except the three prefixed "Et", which are from three separate collections made in Ethiopia.

Of the 21 dikaryons used, one was derived from the haploids E3a and I4A used in earlier work (8). The constituent haploids for the other 20 were both derived from a single teliospore. These dikaryons were thus produced through "selfing" of two of the four products of 20 tetrads derived from 20 different teliospores. The tetrads were obtained in the following way: a thin layer of modified Vogel's (18) complete agar was poured into a petri dish. Blocks of agar about 15 mm^2 were cut and each was placed on a 22 mm^2 sterile glass coverslip. Aqueous 5-ml suspensions of teliospores were prepared in test tubes. A drop of achromycin suspension (10 mg achromycin per milliliter of H_2O) was then added to these suspensions and a small drop of the teliospore suspension was transferred to the center 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 five-to-ten viable teliospores per block had germinated. A suitably germinated teliospore, with all four sporidia accessible for microdissection was selected on each block. Using a de Fonbrune micromanipulator and an upward-bent fine glass needle, the four primary sporidia were drawn from the promycelium, one to each of the four sides of the block. Their position on the promycelium was recorded directly on the coverslip, near the edge. Visible colonies formed from the four sporidia in 3-4 days. They were transferred singly to fresh plates, and, after sufficient growth, were tested for mating type (5). Small amounts of sporidia were put into screw-cap tubes containing fine textured, oven-dried silica gel for long-term storage. Two of the four cultures, of opposite mating type, of each tetrad were randomly selected to form the dikaryons used. Seed treatment and inoculation procedures have been described earlier (8).

We thought that 30 plants would be sufficient for measuring within-plant disease severity for each host-parasite combination. Only plants with three or more culms were included. To be reasonably 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 solving for n in the relationship:

$$(1-P)^n = 0.01$$

where P = the percentage of plants showing smut,
n = the number of plants needed to be 99% certain of obtaining at least one smutted plant.

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

Inoculated seeds were planted in the field in rows 4.5-m long. This provided an average of about 60 plants per row. Many combinations were planted, discontinously, in more than one block of several rows each, at one location.

Data were recorded when all spikes had emerged. Generally, the percentage of smutted plants was based on samples of at least 300 plants. Where fewer plants were available, the percentage of smutted plants was still high enough to yield statistically adequate sample sizes (13). The arcsin transformations and correlation analyses of smutted head and smutted plant percentages were done by computer.

RESULTS

Table 1 is a matrix of the 12 barley cultivars and 21 smut dikaryons, showing the disease reactions observed. The work of Tapke (16) was consulted to determine which host-parasite genotypic combinations to investigate. Any combination which, in his studies, gave less than 1.0% smut (he used a head-per-row basis) was not included in our study. Even though the practical lower limit in our work was taken at 5.0% plants smutted, in three cases where there was less than 5.0% plants smutted, sufficient data nonetheless were obtained for inclusion. Information on expected smutting for some of the other combinations was obtained from T. Ebba (*unpublished*). Five-to-eight rows (and more later if necessary) were planted if no disease information was available for a combination. Table 1, has three points which do not involve any of the 21 dikaryons; though they represent crosses of different smut 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. An inverse relationship was found between the average number of culms per plant and the percentage of smutted culms on smutted plants. This complication had to be dealt with before the correlation analyses could be properly made.

Field plots were established at Vancouver, British Columbia (B.C.), and also in the Imperial Valley of California. An average of 4.3 culms formed per smutted plant in B.C., but in California, where conditions were more favorable to barley growth, 13.8 culms formed on the average per smutted plant even though the 29 host-parasite combinations planted in California were not systematically chosen for that location. Regression analysis revealed a significant negative regression (probability of zero slope - 0.00) of frequency of smutted culms for diseased plants on average tiller production.

TABLE 1. Combinations of host barley cultivar and pathogen (*Ustilago hordei*) dikaryon, and disease reactions observed

Barley cultivar	*Ustilago hordei* dikaryon[a]																				
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	Et1	Et2	Et3	Uh6	Uh12	vlv2	v3	EX1	Additional
Conquest				2.0[b]					L	6.8[b]	L	L	L	27.6					23.0	12.6[b]		
				19.0						18.1				71.4					55.0	33.9		
																						(V3 × Uh-6)
Excelsior		L	50.6	L					26.6			L	46.8				56.3					13.4[b]
			87.4						70.8				83.6				85.6					76.4
Gateway	L												L						L		L	
Hannchen	53.2	L		20.7		L			28.2	48.4	58.6	32.3	35.2	37.0	7.9			67.1[b]	32.0	14.7[b]	24.0[b]	
	83.3			66.3					78.3	82.5	81.0	71.0	69.9	85.9	72.1			81.4	85.7	46.0	46.2	
Himalaya				45.8		L				27.0		32.3	9.0						24.0	18.6[b]		
				89.8						86.1		72.3	64.4						91.0	76.8		
Keystone	L			3.9[b]			L		6.0	5.3[b]			10.3	53.0	2.7[b]				10.3	8.5		
				22.1					42.1	24.2			24.8	70.5	28.6				53.5	52.1		
																						(Uh-6 × Uh-12)
Lion		27.0	34.6		12.6	18.9[b]			4.6	24.1	26.7		18.1	5.9	L	7.4	20.6	19.8	36.0	11.1[b]	8.0[b]	61.7[b]
		71.2	80.8		67.5	53.7			65.3	73.1	71.4		76.0	70.0		55.1	83.2	75.2	76.2	46.6	42.4	67.7
Nepal		55.5	57.5	56.2				L	30.7	49.3		61.5	54.1	40.7	L	26.8	47.3					
		95.5	96.7	91.9					56.0	83.2		93.4	88.4	84.1		53.9	95.2					
Odessa	31.5[b]	47.1	59.5	42.3	37.2	22.9[b]	25.0[b]	23.6[b]	53.4	53.5	54.8	44.6	55.8	17.4[b]	30.3	19.7[b]	40.3	62.2	45.5	16.0[b]	32.8[b]	
	64.4	72.6	94.6	69.0	75.2	70.4	52.8	59.1	96.6	71.9	77.9	86.6	80.7	35.7	81.9	48.2	85.7	95.6	86.9	53.4	48.1	
Pannier			L	39.9			L		L	29.4		L					30.1		29.7			
				79.0						95.7							77.5		85.3			
																						(R5 × R11)
Trebi	7.6	L	L	6.8	12.1	L				7.6	44.3	L	6.8[b]	2.8	5.9		11.2	23.2	36.5	16.5[b]	10.8[b]	48.1[b]
	30.6			31.0	44.7					40.2	69.5		13.8	32.2	55.6		53.9	55.6	61.6	46.9	50.2	85.1
Vantage									24.0	46.1	58.7		42.1	44.6	14.8			31.5[b]	27.8		16.6[b]	
									60.0	60.5	66.8		81.0	77.4	53.0			66.1	72.6		42.8	

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

[b]Planted under high-tillering conditions.

Hence, the more tillers that were produced by smutted plants, the lower the frequency of smutted culms on those plants, and vice-versa. This relationship was also evident in two host-parasite combinations each planted at both locations. When low-tillering (B.C.) and high-tillering (California) plantings were analyzed separately, however, there was no significant regression in either grouping (probability of zero slope = 1.00 and 0.32, respectively). Because there were two locations, variation in average culms per plant was bimodal for all 111 host-parasite combinations, and division of data into two sets greatly reduced this variation within each set and eliminated the bimodality. Division of data was thus thought to be the most effective way to avoid this complication.

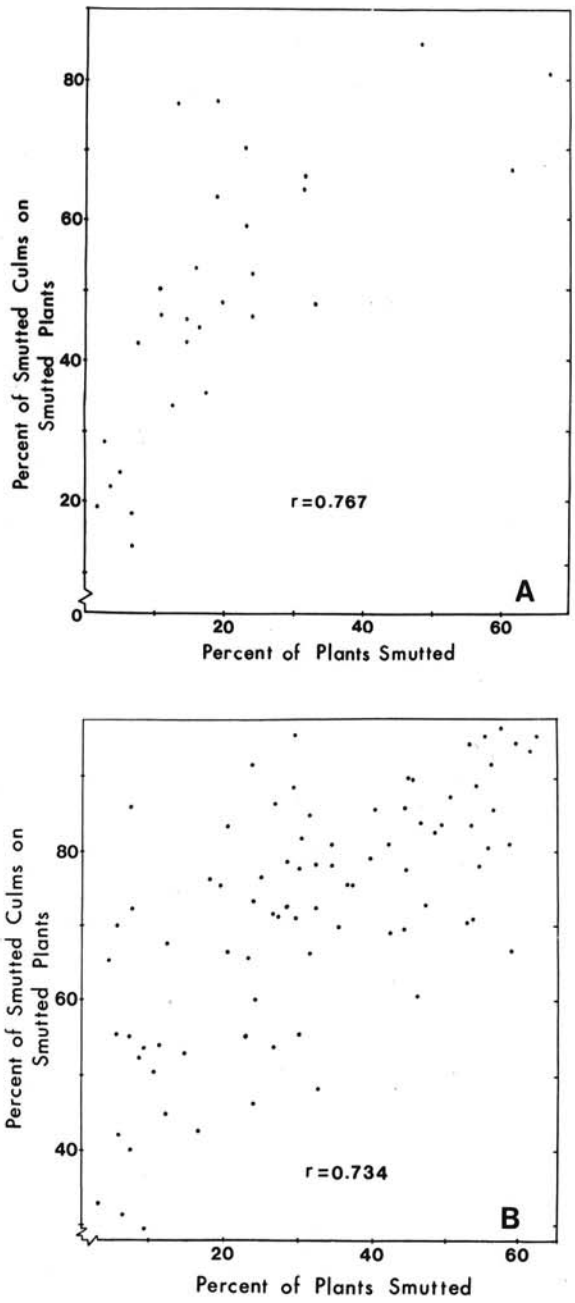

Fig. 1-(A, B). Scatter diagrams illustrating the correlation of among-plant disease severity as measured by percentage plants smutted, shown on the abscissa, and within-plant disease severity as measured by percentage smutted culms on smutted plants, shown on the ordinate, for **A)** 29 barley-*Ustilago hordei* genotypic combinations planted under high-tillering (California) conditons and **B)** 82 genotypic combinations planted under low-tillering (British Columbia) conditions.

Figure 1 presents the within- and among-plant disease severity scatter diagrams for high- (A) and low-tillering (B) plants, respectively. The variances of x and y were very similar to one another in each analysis. Scatter diagrams involving units of standard deviation were thus judged as unnecessary.

Separate within-cultivar and within-dikaryon correlation analyses were made on low-tillering plantings of five cultivars and three dikaryons. 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. Five of the eight correlations were significant. Of the three not significant, two of them, "v1v2" and "Odessa" might be explained by small sample size and lack of variance, respectively.

DISCUSSION

The inverse relation between the average number of culms produced by the plants and within-plant disease severity has been noted by others, either directly or indirectly. Woodward and Tingey (20) observed that higher levels of smutting were obtained with barley-*U. hordei* on less fertile soil than were obtained on more fertile soil. Since they measured smutted heads, they probably were observing the increased within-plant disease severity that accompanied a decrease in tillering on poorer soil. Milan (9) observed that the rate of sowing, which also influences tillering, did not affect the percentage of inoculated wheat plants smutted by *U. tritici,* but had a direct effect on the percentage of culms smutted. Batts and Jeater (1) suggest that each infected plant contains a small amount of mycelium and thus, the

TABLE 2. Common-cultivar and common-dikaryon correlations of between- and within-plant disease reactions for barley-*Ustilago hordei* genotypic combinations[a]

Common cultivar or dikaryon	Number of points[b]	Value of r[c]
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

[a]All plants were grown at Vancouver under low-tillering conditions.

[b]Number of dikaryons or cultivars associated with the indicated cultivar or dikaryon.

[c]An asterisk indicates a significant correlation, $P = 0.05$.

more tillers produced the smaller the proportion of tillers that become smutted. They suggest further that the amount of mycelium varies from embryo to embryo, so 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 these authors neither supports nor refutes their hypothesis concerning both limited and variable amounts of mycelium in embryos. The hypotheses are nonetheless simple and plausible.

Regression analysis of the effect of tillering on within-plant disease severity was made only to determine whether this complication was important enough to require removal. The analysis was weakened by the fact that the independent variable, within-plant disease severity, was not normally distributed. After division of data, however, the distribution in each case appeared closer to normal. Thus, the divided data probably more clearly meet the assumptions for valid regression analysis.

Correlations between the percentage of plants and the percentage of heads smutted or bunted have been shown in various ways for several smuts and bunts (2, 3, 4, 11, 12, 14, 15, 19). Where this was intentionally done, a logical error was made. Correlation is not statistically valid where two dependent variables are concerned. Naturally a higher frequency of smutted plants would yield a higher frequency of smutted heads, in this case, even if within-plant disease severity was invariate. Barring the unlikelihood of a negative correlation for within- and among-plant disease severity, it would be surprising if a positive correlation between the two nonindependent variables (percentage of plants smutted and percentage of heads smutted) was not found. At the same time, there is no obvious reason for the percentage of smutted culms on obviously disease plants to be dependent of the percentage of plants smutted. Thus, the empirical investigation of the relation between these two variables is valid.

The measures used of within- and among-plant disease severity were chosen primarily because they are simple. A refinement of the within-plant measure involves the fact that one culm of each smutted plant must be diseased and is not free to vary. Excluding these culms from the percentage calculation would have resulted in disease percentages lower than those expressed. Because both correlations are high, such a refinement would not affect the results.

Cultivars such as Odessa, which possesses little among- or within-plant resistance to any of the dikaryons, and Keystone, which generally exhibits a high degree of both types of resistance, undoubtedly contribute greatly to the correlation. The within-cultivar analyses, however, revealed that the correlation is not limited to among-cultivar comparisons. Even though cultivars that possess a generally high or low level of both types of resistance easily can be picked out, dikaryons do not form such distinct groups. A dikaryon was not found, for instance, with a relatively high among- and within-plant disease-producing ability on all, or even most, cultivars. This leads to the conclusion that, initially at least, studies on the inheritance of host resistance would yield more clear-cut results than studies of the inheritance of pathogen virulence.

The variation shown in within-plant disease reaction is largely genetical. The range of sampling variation is about ±5%. Environmental variation, based on observed smutting differences between locations for identical combinations, is less important than genetic variation. Several such combinations were planted in blocks at different locations in the field at Vancouver, or even in different years, yet variation due to these environmental differences, when combined with sampling error, ranged only about ± 10%. Therefore, the substantial remaining variation in within-plant disease reactions must be genetic. An F-test of total- vs. experimental variances confirmed this statistically. Earlier studies have established that among-plant disease reactions are largely genetically determined (17). The close correlation of this variable to within-plant disease reactions can itself be taken as evidence for the genetic determination of the within-plant disease reaction.

One might expect that the relation between the two disease-severity measures could be explained in part through a simple Poisson relationship. This assumes that number of culms smutted per plant reflects "dose" of smut that the plant receives. If the mean number of events is determined, according to the Poisson equation, by the proportion of plants receiving no smut, then an expected frequency of one-dose plants (those with one culm smutted), two-dose plants (those with two culms smutted) etc. can be calculated. When the actual numbers of observed plants in these classes is then found, a good observed:expected fit might indicate that within- and between-plant disease severity are separate facets of the same infection events. However, when several host-parasite combinations that showed high and low disease reactions were thus analyzed (J. V. Groth, *unpublished*), good fits were not obtained. Undoubtedly, an explanation based on Poisson expectations is a gross oversimplification, for several reasons. Reactions showing high within- and among-plant disease levels were particularly discrepant. The two most frequent classes of reaction in such combinations were those showing either no smut or smut in most culms. The intermediate class (i.e., plant with only one culm smutted) was infrequent. The Poisson expectation, in these cases, would give the intermediate class a higher frequency than any other smutted class, by a wide margin.

Thus, there seems to be two distinct thresholds, or stages, through which the fungus must pass in order to produce teliospores. If the first threshold is surmounted, the fungus can cause smutting in at least one tiller of the plant, as measured by among-plant disease severity. If it crosses the second threshold, more culms will be smutted, as measured by within-plant disease severity. One can best think of this second event as taking place separately in each culm. The fungus does not get beyond the first threshold; i.e., it does not succeed in sporulating in many plants. In plants in which the smut surmounts this barrier, it encounters a second barrier as it ramifies through the tissues. Moreover, as the first threshold becomes more difficult to surmount in more resistant cultivars, so does the second. 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 inoculation of the seed. Both are measured and defined in terms of probability.

The strong correlation between the two measures of

disease severity suggests a general identity of genes which govern the two types of disease reaction. Such genes could be in the host, the parasite, or both. Combinations which differ only in their within-plant disease reactions are noteworthy because they may indicate specific cases of nonidentity of genes governing the two types of resistance. Alternatively, cultivars or dikaryons might differ in environmental sensitivity, although this seems less probable.

Because the correlation of the two disease reactions is close, it becomes less likely that contrasting phenotypes for genetic studies will be found with only one of the high disease reaction types. In particular, there seems to be a lack of host-parasite combinations that show high among-plant- and low within-plant disease reactions. Two possibilities are thus left for genetic study: cultivars with high within- and among-plant disease reactions can be crossed with those showing low within- and low among-plant disease reactions; or crosses involving varieties that differ only in their within-plant disease reactions can be made. Several examples of each of these can be picked out. The same two classes of phenotypic contrast, examples of which are also available, could be used in genetic studies of virulence in the pathogen.

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