Genetics

Variation in Aggressiveness Among and Within Races of *Ustilago hordei* on Barley

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ABSTRACT

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Components of aggressiveness and disease severity among 13 physiological races of *Ustilago hordei* on Hannchen, Nepal, and Odessa barley were studied to determine inter- and intrarace variability. Variation was observed among the physiological races of *U. hordei* for the aggressiveness components of peduncle compaction and extent of sorus formation in heads, leaves, and nodes. Variation within races 1, 7, 8, and 12, determined by using random sporidial crosses originating from different teliospores within a race collection inoculated onto Odessa, was observed for extent of peduncle compaction, plant dwarfing, and sorus formation in heads, leaves, and nodes. These race collections were heterozygous for gene(s) governing these traits. Races 7 and 12 were the most variable for these components and races 1 and 8 were the least.

Additional keyword: parasitic fitness.

Races of *Ustilago hordei* (Pers.) Lagerh. are differentiated based on their pattern of virulence to differential barley (*Hordeum vulgare* L.) varieties (27,28). All known races of *U. hordei* can infect and cause smutting of the ovaries in the developing spike in the universally susceptible barley cultivar, Odessa (CI934) (7,28). In addition to variation in virulence combinations among races of *U. hordei*, variation also exists in the degree of disease severity. The term *aggressiveness* has been used to describe variability in severity of disease reactions among virulent biotypes of a pathogen in a host (1). Aggressiveness also has been defined in terms of percent infection of inoculated Odessa plants (2,4). Other pathogen-induced manifestations of disease severity in the host plant, such as the ability of some isolates to sporulate in the tissues of the leaves, stems, nodes, and inflorescences of infected barley plants, have been reported (7,12,15,25). The genetic nature of some aggressiveness factors in *U. hordei* on barley has been established (3,4).

Variation in the degree of destruction of floral tissues, the extent that smut sori form within leaf and nodal tissues, plant dwarfing, and peduncle compaction associated with specific races of *U. hordei* has been noted on susceptible barley cultivars when infected by different races of *U. hordei* through numerous greenhouse trials (D. A. Gaudet, *unpublished data*). The purpose of this paper is to describe the variation in aggressiveness components peduncle compaction, plant dwarfing, and sorus formation in the heads, leaves, and nodes, and to document inter- and intrarace variation in *U. hordei* for these aggressiveness components.

MATERIALS AND METHODS

Teliospores of 13 physiological races (R1–13) of *U. hordei* (28) and an isolate (R14) generated in an inbreeding study of race 8 (20) were used as inocula for the studies of variation in aggressiveness reactions among races on three barley cultivars. The race and isolate collections were propagated and verified on differential barley hosts within 3 yr before starting the study, the reactions were compared with those published by Tapke (28), and the teliospores were stored at 4 C until used.

Three spring barley differential cultivars, Hannchen (CI531), Nepal (CI595), and Odessa (CI934), were employed. Odessa was selected because it was highly susceptible to all known races and isolates of *U. hordei* (28). Both Hannchen and Nepal were highly susceptible to eight of Tapke's (28) differential cultivars (R. L. Kiesling, *unpublished data*).

Before inoculation, dehulled barley seeds were pregerminated in 90-mm petri dishes containing two filter papers (Whatman No. 1) moistened with distilled water for 24 h at 21 C to remove dead seeds and seeds damaged by dehulling. The coleoptiles of the pregerminated seeds then were dusted thoroughly with teliospores of the *U. hordei* races and the R14 isolate. Inoculated seedlings were incubated in the dark for 48 h at 21 C, transplanted into 15-cm pots containing an autoclaved (121 C for 4 h) mixture of loam, sand, and peat (3:1:1), and grown under supplemental light (18-h day length) to full maturity in greenhouses held at 20 C. Four inoculated seedlings were planted per pot, which constituted a replicate. A split plot design with four replicates was used with three cultivars used on the whole plots and 14 races on the subplots (26).

Races 1, 7, 8, and 12 were used to determine the extent of heterozygosity in the aggressiveness components within races of *U. hordei*. Different dikaryons of the races produced a wide range of reactions on the susceptible cultivar Odessa (9). Teliospores of the races were germinated on 3% potato-dextrose agar (PDA), and the primary sporidia were isolated using a micromanipulator. The colonies were transferred to PDA, grown at 21 C for 1 wk, and stored on pieces of sterile toothpicks in sterile sealed Pyrex tubing at 1 C or in a liquid nitrogen refrigerator. The mating type of each sporidial line was determined using the modified Bauch test (16).

The procedures for inoculation of seedlings with sporidial cultures were similar to those described for teliospore inoculations. Sporidial isolates from each of the four races were streaked onto PDA and grown for 48 h at 21 C. Sufficient inoculum from each sporidial culture was collected and physically mixed with a different compatible sporidial colony of the same race. Twenty randomly selected sporidia, each derived from a different teliospore within each race, were paired to create 10 crosses per race.

The mixture was spread at the base of the coleoptiles of pre-germinating barley seedlings. Each compatible sporidial mixture was inoculated onto 15 seedlings. Three inoculated seedlings were planted per pot, and five pots for each of the 40 crosses (10 per race) were arranged in a randomized complete block design.

At plant maturity, the following aggressiveness components were assessed: extent of smutting of the inflorescence or head sorus type rating, flag leaf, and the flag node. Other pathogen-induced morphological alterations of the host examined were plant dwarfing and compaction of the peduncle. Ratings were restricted to the primary tillers, which exhibited whole or partial smutting of the spike. The extent of sorus formation in the head was rated on a scale of 0–5 in increments of 0.5, which represented increased disease severity to the spike caused by development of smut sori in the inflorescence (Fig. 1). The extent of formation of smut sori in the flag leaf and flag node was visually estimated as a percentage of the area exhibiting sori. The extent of plant dwarfing was determined by measuring the tiller from the soil line to the flag node. The extent of peduncle compaction, a component of plant dwarfing, was rated on a scale of 0–5 (Fig. 2) in which each increment in the scale represented an increase in the proportion of the peduncle that was compacted.

For the study of races of *U. hordei* on the differential barley cultivars, analyses of variance for unequal subclass numbers (24) were carried out separately to examine the effect of races on the variables head sorus type rating, peduncle compaction rating, plant dwarfing, and percent smutting of the flag leaves and nodes for each cultivar. The factors replicates, races, pots, plants within pots, and tillers within plants were blocked as main effects in the statistical model. Races that had no response for all of the replicates were excluded from the analyses. The data for the leaf and nodal sori were transformed with $\log_{10}$ transformation because of unequal variances of the residuals. Analyses were carried out over the cultivars Odessa, Nepal, and Hannchen for races 3, 4, 10, 12, 13, and 14 by incorporating whole plots, cultivar, and subplot × race cultivar effects in the model.

For the study involving random sporidial crosses between races 1, 7, 8, and 12, a randomized block analysis was carried out for each race separately in the selfing study, and variance components for selfing effect were calculated (26) using the formula:

$$\text{Variance component (VC)} = \frac{\text{Mean squares}_{\text{treatment}} - \text{Mean squares}_{\text{error}}}{\text{Number of replicates}}$$

The percentage of the total variance component attributed to the variance component for the selfing effect within races also was determined.

RESULTS

The order in which the primary tillers were initiated was not correlated with any directional change in expression of aggressiveness components. However, infected secondary tillers arising from aboveground nodes of primary tillers tended to display lower average values for all components. Only the primary smutted tillers of a plant were included in the analyses.

The pattern of destruction of floral tissues by *U. hordei* was consistent among the different physiological races. In general, the proximal end of the spike contained fewer apparently healthy tissues than did the distal regions, particularly in heads exhibiting low head sorus ratings. A head sorus type rating of 1 was given to partially smutted spikes in all instances, and the smutted florets of partially smutted spikes were located at the base of the spike (Fig. 1).

Peduncle compaction appeared to be due to a weakening of the peduncle, which prevented extension of the head out of the leaf sheath. In severe cases, the elongating peduncle collapsed within the leaf sheath and remained wrapped within the sheath at maturity (Fig. 2).

Severity of leaf sorus formation always was greatest on flag leaves and decreased in relation to earliness of leaf initiation. Figure 3 shows extensive sorus formation on the flag and
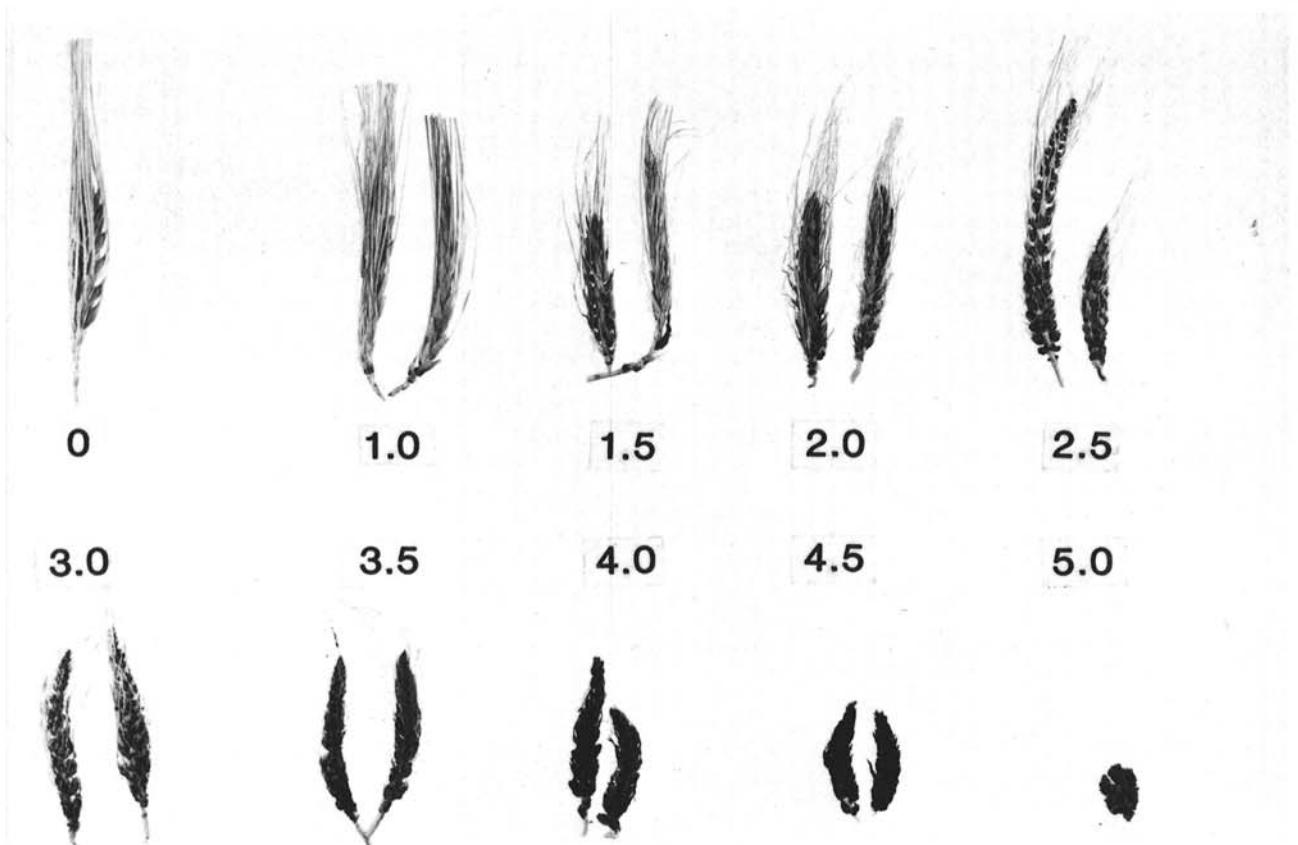

Fig. 1. Extent of sorus formation in the head rated on a scale of 0–5 increments of 0.5, which represent increased disease severity to the head caused by development of smut sori in the inflorescence.

penultimate leaves of Odessa barley plants inoculated with race 12 of *U. hordei*. Sori formed interveinally and appeared as streaks that extended partially or fully along the length of the leaf. Nodal sori ranged from the appearance of a small smut sorus erupting from the upper surface of the node to conversion of most of the nodal tissue to sori (Fig. 4). Occasionally, long sorus stripes extended down the length of the peduncle to fuse with the nodal sori. In severe reactions, sori extended to the stem and penultimate node.

There was significant variation among the 13 races and isolates of *U. hordei* in head sorus type and peduncle compaction rating but not in plant dwarfing (Table 1). There was also significant variation in leaf sorus percent among the five races that formed leaf sori; however, there were no differences among the four races that formed nodal sori (Table 1). There were no effects of the different cultivars on expression of any of the aggressiveness components except peduncle compaction ratings ($P = 0.01$). The means for the compaction ratings averaged over races were 0.88, 1.88, and 2.40 for the cultivars Hannchen, Nepal, and Odessa, respectively. There were no race $\times$ cultivar interactions for any of the aggressiveness components.

When a ranking of races for all the aggressiveness components on Odessa barley are considered, races 2, 3, 4, and 12 are among the most aggressive and races 7, 10, and the biotype R14 are among the least aggressive (Table 1). The remainder fall in the intermediate range with some races producing severe reactions in some components and light reactions in others. For example, race 11 produces a low head sorus type and only traces of leaf sori but can produce extensive nodal sori. Races 2, 3, 4, and 12 consistently produced severe head sorus type ratings and leaf sorus formations on Odessa (Table 1). Race 12 affected plant height the most and caused the highest leaf sorus ratings (Table 1). Isolate R14 produced the lowest head sorus type reactions, no leaf sori, and the lowest compaction rating. The least affected plant height developed the tallest infected plants (Table 1).

Fig. 3. Odessa barley, inoculated with *Ustilago hordei,* exhibiting extensive formation of smut sori in the flag and penultimate leaves.

Fig. 2. Extent of peduncle compaction rated on a scale of 0–5 in which each increment in the scale represents an increase in the proportion of the peduncle that was compacted.

Fig. 4. Odessa barley, infected with *Ustilago hordei,* exhibiting extensive formation of smut sori in flag node and peduncle.

Variability was observed for the different aggressiveness components among the paired sporidial inoculations within teliospore collections of the four races (Table 2) ($P \leq 0.01$). Variation among the paired sporidial inoculations was observed in four out of the five aggressiveness components within races 7 and 12 but only one of the five aggressiveness components within races 1 and 8. Variability in plant dwarfing was observed within three of the four races. Trace levels of leaf and nodal sori were produced within races 1, 7, and 8. Sporidial crosses within race 12 produced the severest head sorus type rating, plant dwarfing, leaf sorus formation, and plant compaction of all of the intraracial crosses (Table 2). When the percentage of the total variation attributable to selfing within races (variance component) was considered for the aggressiveness components, race 7 appeared to be the most variable followed by race 12 (Table 2).

DISCUSSION

Cultivar effects were observed for peduncle compaction only. As there were no other race × cultivar interaction effects, the remaining discussion will be restricted to the physiological races of *U. hordei* on Odessa barley unless otherwise specified.

In this study, we observed variability for the aggressiveness components head sorus type rating, leaf and nodal sorus formation, and peduncle compaction among and within races of *U. hordei* on Odessa barley, and within races for plant dwarfing. Variation in the extent of smutting of the host spike has been reported within the genus *Ustilago* (7,27). Faris (7) reported a range in the degree of sorus formation between partially smutted ovaries and entirely smutted spikes in barley infected with *U. hordei* and variation in the intensity of smut sorus formation in the awn tissues. The nature of differential smutting of barley floral tissues is unknown. The cause of partially smutted heads

has been attributed to variation in the rate of pathogen ingress (8,10,17). If the pathogen is slow to establish in the meristematic regions of the embryonic spike, only proximal florets will be smutted, whereas if host colonization is rapid, all florets will be smutted. Rapid colonization of meristematic tissues in the embryonic spike may account for extensive sorus formation in awns and florets caused by races 2, 3, 4, and 12 of *U. hordei*.

Sorus formation in barley leaves by *U. hordei* has been reported (11,12,24). Race 6, previously reported to produce leaf sori at 24 C (11), failed to produce leaf sori at 20 C. Failure of race 6 to form leaf sori at 20 C and extensive leaf sori formation by race 12 at this temperature may suggest that different temperature optima exist among the races of *U. hordei* for the expression of this trait.

The percentage of leaf area exhibiting leaf sori decreases with earliness of leaf initiation. Therefore, the rate and extent of colonization of the meristematic regions of the leaf primordia may be an important factor in the formation of leaf sori. Kiesling (12) proposed that colonization of the sixth leaf (flag leaf minus two) occurred between days 14 and 24 after seeding and subsequent growth at 24 C. In a study of the relative growth rates of wheat leaf primordia, Williams (31) observed that the first leaves tended to develop at exponential rates, but the rate of increase declined with each successive leaf formed; the flag and penultimate leaves developed at a drastically reduced rate between 18 and 21 days after seeding. If a similar developmental scheme for leaf primordia exists in barley, a substantially longer period would be available for ingress and colonization of the flag and penultimate leaves than for the first leaves formed. Races capable of producing leaf sori may infect and become established in meristematic regions of the leaf more rapidly than those unable to form leaf sori. Environmental factors such as temperature and plant vigor also would affect the expression of this trait. The ability of most races

TABLE 1. Effect of 13 physiological races of *Ustilago hordei* on Odessa barley for the aggressiveness components head sorus type rating, plant dwarfing, peduncle compaction rating, and percent leaf and nodal sori

Race	Number	Head sorus type			Plant dwarfing			Peduncle compaction			Leaf sori[a] (%)			Nodal sori[a] (%)		
		Mean	SE	Rank	Mean	SE	Rank	Mean	SE	Rank	Mean	SE	Rank	Mean	SE	Rank
R1	18	3.2	0.15	5	73.5	4.6	6	1.2	0.2	10	0	0	6	0	0	5
R2	12	3.8	0.18	2	70.9	5.6	4	3.1	0.3	5	1.5	0.3	4	0.67	0.09	3
R3	18	3.7	0.15	3	77.3	4.6	10	3.7	0.2	1	3.2	0.8	3	0.05	0.07	4
R4	6	3.5	0.23	4	73.8	7.9	7	3.0	0.4	6	3.5	0.9	2	0	0	5
R5	8	2.8	0.20	9	68.0	6.9	2	2.2	0.3	7	0	0	6	0	0	5
R6	9	3.1	0.18	6	70.5	6.5	3	3.2	0.3	4	0	0	6	0.77	0.10	2
R7	15	2.7	0.13	10	81.1	5.0	11	2.1	0.2	8	0	0	6	0	0	5
R8	11	3.0	0.15	7	71.8	5.8	5	3.6	0.3	2	0	0	6	0	0	5
R10	11	2.9	0.15	8	82.9	5.8	12	1.5	0.3	9	0	0	6	0	0	5
R11	11	3.0	0.15	7	76.6	5.6	9	3.4	0.3	3	0.2	0.1	5	2.10	0.38	1
R12	4	4.0	0.25	1	65.0	9.7	1	3.2	0.5	4	22.0	5.8	1	0	0	5
R13	2	3.0	0.36	7	76.0	13.8	8	1.0	0.7	11	0	0	6	0	0	5
R14	9	2.2	0.17	11	97.2	6.5	13	0.2	0.3	12	0	0	6	0	0	5
P		<0.01			>0.05			<0.001			<0.01			>0.05		

[a]Geometric means and approximate standard errors are given for these variables.

TABLE 2. Effect of sporidial crosses within races 1, 7, 8, and 12 of *Ustilago hordei* on Odessa barley for the aggressiveness components head sorus type rating, plant dwarfing, peduncle compaction rating, and percent leaf and nodal sorus formation

Race	Number	Head sorus type rating			Plant dwarfing			Compaction rating			Leaf sori (%)			Nodal sori (%)		
		Mean	VC[a]	%[b]	Mean	VC	%	Mean	VC	%	Mean	VC	%	Mean	VC	%
R1	8	3.05	0.01	4	67.6	20.4	10	1.94	0.01	1	0.02	0	···	0.02	0	···
R7	10	2.81[c]	0.05	12	67.0[c]	31.4	17	1.80[c]	0.57	24	0.04	0	···	0.45[c]	0	···
R8	8	2.77[c]	0	···	67.6	25.1	9	1.41	0.09	5	0	0	···	0	0	···
R12	10	4.6[d]	0	···	56.8[e]	16.5	8	3.07[c]	0.16	12	22.5[c]	0.04	10	0.04	0	···

[a]VC = Estimate of variance component for the selfing effect.
[b]Percentage of the total variance component attributed to the variance components of the selfing effect.
[c]$P < 0.001$.
[d]$P < 0.05$.
[e]$P < 0.01$.

to produce small amounts of leaf sori under some conditions supports the hypothesis that leaf sorus production is related to rate of pathogen development in the host. However, it should follow that those races that form leaf sori also would produce the highest level of infection. This is not necessarily true as race 1 was the most aggressive and race 12 was considered nonaggressive among the races of *U. hordei* according to Emara (4). Therefore, other genetic factors that affect the ability of races to colonize and sporulate in infected leaves may exist.

Few races were capable of producing nodal sori. Nodal sori form in the parenchymatous regions of the node (15), and isolates of *U. hordei* may induce hyperplasia and hypertrophy in nodal tissues (25).

Plant dwarfing has been observed in barley infected with *U. hordei* (7,27) and in oats infected with *U. avenae* (Pers.) Rostr. (29,30). Variation in plant dwarfing among different races of common and dwarf bunt also has been reported (8,22,23). Although there were no differences among the 13 physiological races and isolates of *U. hordei* in their ability to dwarf barley plants using teliospore inoculation, differences were observed among and within the four races using sporidial crosses. Teliospore inoculation may have masked the variation of plant dwarfing among races because all dikaryons would have been formed randomly in different inoculated plants. Consequently, some of the genotypic variability for plant dwarfing would have been allocated to the experimental error. Backcross lines involving race 12 that cause severe plant dwarfing have been recovered (14).

Most races were capable of causing some peduncle deformation or compaction under greenhouse conditions. Compaction of the peduncle by some isolates of *U. hordei* has been reported previously (27). Preliminary histological studies have demonstrated that the barley peduncles become compacted because there is insufficient thickening of the sclerenchyma fibers in the hypodermal region of the stem to provide structural support for the elongating peduncles (D. A. Gaudet, *unpublished*). Variation in extent of peduncle compaction caused by races of *U. hordei* among barley cultivars could be attributed to genotypic variation in peduncle strength.

The relative aggressiveness ranking presented for races of *U. hordei* did not correspond to that presented by Emara (4), who considered percentage of infection on Odessa under field conditions. Percent infection of *U. hordei* on barley is highly sensitive to environmental conditions (5).

Variability in aggressiveness and disease severity components within races of *U. hordei* has not been investigated extensively. These results demonstrated that the genotypic variability existed within parental races 1, 7, 8, and 12 for a number of aggressiveness components. Significant variation for these aggressiveness components in the random matings of sporidia within races would be indicative of heterozygosity for these traits in the collections of *U. hordei*. Races 7 and 12 were most variable compared with races 1 and 8. Similar variation in aggressiveness (percentage of susceptible barley plants smutted) has not been observed within natural populations of *U. hordei* (2) but was observed in experimentally produced heterozygotes (3). Because percent infection is the primary determinant of inoculum production, it justifiably should be considered the main component of aggressiveness. However, other quantitative and qualitative aspects of the host-parasite interaction considered in this study also reflect the severity of the pathogen development in the barley plant.

Aggressiveness describes the variability in disease severity reactions among virulent biotypes of a pathogen in a host (1). Aggressiveness is considered a component or attribute of parasitic fitness that describes the relative ability of a parasitic genotype to persist successfully over time in a population (18,19). Head sorus type rating and smutting of the leaves and nodes reflect increased sporulation in host tissue for *U. hordei* in barley. Increased severity of head sorus formation would result initially in enhanced teliospore production; however, high head sorus ratings were associated with decreased head length and, consequently, decreased teliospore production. In recent studies of Odessa barley inoculated with crosses involving race 12, plants exhibiting extensive leaf sori often failed to produce heads (14). In this instance, high aggressiveness could lead to a reduction in reproductive capacity of the pathogen and, therefore, a reduction in the overall level of parasitic fitness. Peduncle compaction and plant dwarfing are probably due to increased physiological activity by aggressive races within the developing barley tiller. This phenomenon may be a mechanism by which the pathogen has achieved a balance with its host and prevents overaggressive races from becoming predominant in the pathogen population. Therefore, overaggressive isolates do not threaten the survival of either the host or the pathogen itself.

Variability in aggressiveness among and within races or biotypes of *U. hordei* reflects a genetically mediated capacity within the pathogen to induce morphological changes in the barley host. The polygenic nature of the inheritance mechanism(s) governing the expression of these aggressiveness components has been reported (6,9). Person et al (21) determined that high infection levels of *U. hordei* on barley were governed by a single virulence gene but segregation of polygenes modified expression of the virulence gene to produce a range in percent infection.

The effect of increasing severity in the aggressiveness components considered in this study on the overall parasitic fitness of *U. hordei* is unknown. Race 6, the most prevalent race of *U. hordei* in North America (13,28), produces extensive leaf sori at higher temperatures (11,24), severe peduncle compaction, and an intermediate head sorus type rating. Race 12, the most aggressive race according to the criteria under consideration in this study, has a limited distribution (28). Both races 6 and 12 are considered nonaggressive by Emara (4) when percentage of infection is considered as the only criterion for aggressiveness.

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