Some Effects of Moderate Adult Resistance to Crown Rust of Oats

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ABSTRACT

The oat (*Avena sativa*) varieties Portage, Ajax, Minhafer, Lodi, and Rodney were consistently moderately resistant to moderately susceptible to crown rust (*Puccinia coronata* f. sp. *avenae*) when grown adjacent to buckthorn (*Rhamnus cathartica*), whereas most other commercial oat varieties including Coachman, Bonkee, and Ortley were susceptible. In 3 years of tests, the spread of crown rust from inoculated centers was much less in plots of the moderately resistant varieties than in the susceptible varieties.

In comparisons of rust development in adult plants there were fewer infections, hyphal growth was retarded, the number of days to onset of sporulation was greater, pustules were smaller, and fewer spores were produced per pustule in Portage than Coachman. This was so at low (10-15 C) and at moderate (21-26 C) temperatures with each of the two races used. The same relationship existed in seedling plants of the two varieties, but to a lesser degree. Present indications are that this moderate resistance is largely nonspecific, that it increases with the age of the plant, and that it may be of considerable value. Phytopathology 60:461-466.

Oat varieties with specific resistance to crown rust, *Puccinia coronata* f. sp. *avenae* Fraser & Ledingham, have remained useful for only several years. For this reason, interest has grown in the identification and characterization of types of generalized resistance. Generalized, nonspecific, or horizontal resistance are considered by us to be synonymous and to indicate resistance to all races of a pathogen. It seems likely that if generalized resistance to crown rust does exist it is of only a moderate degree. If a high type of generalized resistance approaching immunity exists, it probably would have been recognized and utilized long ago.

When the oat (*Avena sativa* L.) cultivars Portage, Ajax, Minhafer, Lodi, and Rodney were grown adjacent to buckthorn bushes, *Rhamnus cathartica* L., the alternate host of crown rust, and exposed to an abundance of aecial inoculum, they consistently remained moderately resistant to moderately susceptible (Table 1). Hybridization of crown rust on buckthorn results in a great diversity of crown rust genotypes (1, 3, 7, 13). The continued moderate resistance of these varieties when exposed to pathogenically diverse inoculum suggests that this resistance may be generalized.

Although the evidence that the observed moderate resistance is generalized is inconclusive, the present studies were undertaken to determine the value of this resistance in retarding the spread of crown rust in the field and to learn what its separate components are.

Materials and Methods.—Crown rust races 264, 276, 290, and 326 were used. One isolate (P-3) selected for virulence on adult Portage plants was unidentifiable, due to a questionable 2^{+}-3^{-} reaction on the crown rust differential varieties Ukraine, Trispernia, and Bondvic. If the 2^{+}-3^{-} reaction on Ukraine, Trispernia, and Bondvic is interpreted as resistant, isolate P-3 would be race 327. If the 2^{+}-3^{-} reaction is interpreted as susceptible, isolate P-3 would be identified as race 264. Thus, it is probable that the isolate could be a variant of one of those two races. Rust inocula collected from many oat plants growing adjacent to buckthorn were combined. This inoculum, designated P-B, was increased on plants of Portage for use in field studies.

Results.—*Rust spread in field plots.*—An important factor affecting a rust epidemic is the rapidity of increase of urediospores. A small degree of resistance may have a major effect on the ultimate amount of rust in a plot where there is little initial inoculum and where the epidemic must develop from the increase of inoculum within the plot. To test this hypothesis, the oat varieties Minhafer, Bonkee, Andrew (early maturing varieties), Portage, Coachman, Ajax, Garry (midseason maturing varieties), Lodi, Ortley, and Rodney (late maturing varieties) were tested in the field. Seeds were planted at the rate of 2.5 bu/acre (84.7 kg/ha) on the same date in the same field according to a randomized block design. Rows were spaced 15 cm apart. Plots were 1.83, 6.09, and 3.65 m^2 in 1964, 1965, and 1966, respectively. A small clump of plants susceptible to crown rust was planted in the center of each variety plot. These plants were later inoculated hypodermically with a urediospore-water mixture of inoculum. Varieties in the test plots were in the early boot stage when the susceptible plants were inoculated. The inoculated plants were thinned immediately before sporulation to provide an inoculum source as uniform as possible in each plot. In different seasons and for different isolates, flag (F) and second youngest (F-1) leaves of 10 inoculated plants had about 10-25 pustules/leaf in each of three replicates/variety.

Varieties were in different stages of maturity when initial inoculum was first released, but (F-1) leaves were always emerged. Percentage rust (percentage of leaf area covered with rust) on varieties was estimated using the Modified-Cobb scale (4) at given distances north, south, west, and east of inoculated plants.

Conditions for epidemic development in the field were excellent in 1964 and 1965, but the weather in

1966 was hot and dry. The spread of individual isolates was usually about the same in a given variety. Therefore, rust percentages for different isolates have been combined into a single average for each variety (Table 1). The data in Table 1 are averages of rust percentages on F-1 leaves just before plants matured, when rust development was close to the maximum at distances where varietal differences were easily seen.

Varieties that were moderately resistant or moderately susceptible when grown adjacent to buckthorn were consistently less rusted by single races in plots where rust spread from inoculated plants than varieties that were susceptible (Table 1). This was true in 3 years of tests for all rust isolates at any given time or distance from initial inoculum. In the early-maturing varieties, epidemics usually developed in pure stands of Bonkee and Andrew, but not in Minhafer. In the midseason varieties, rust usually failed to become epidemic in Portage or Ajax, but did in Coachman and Garry. In the late varieties, there was usually less rust on Lodi and Rodney than on Ortley.

Factors affecting spread of rust.—The rapidity with which the number of urediospores increases depends upon the number of successful penetrations, the duration of time from penetration to sporulation, and number of spores produced. To characterize the nature of the observed moderate field resistance, experiments were made to determine the effect of plant age, temperature, and rust isolate on penetration, development of rust mycelia within the leaf, and sporulation in the varieties Portage and Coachman. These experiments were each repeated once, and the results were analyzed statistically.

TABLE 1. Percentage rust on the leaf below the flag leaf (F-1 leaf) of 10 oat varieties 30 cm from initial inoculum, just before plant maturity in 1964, 1965, and 1966

Variety	Buckthorn plot reactions[a]	Rust 1964[b]	1965[c]	1966[d]
		%	%	%
Early maturity				
Minhafer	MS	2	4	4
Bonkee	S	14	33	10
Andrew	S	9	42	11
Midseason maturity				
Portage	MR	2	2	2
Ajax	MS	12	4	1
Garry	S	8	13	3
Coachman	S	26	37	11
Late maturity				
Lodi	MS	4	27	2
Rodney	MS	6	16	1
Ortley	S	17	40	2

[a] Reaction in the Minnesota buckthorn plot for 3-5 years.
[b] Data are averages for races 276 and 290. Percentage rust (based on leaf area rusted) on 40-60 leaves/race was estimated 22 days after initial inoculum was released.
[c] Data are averages for races 264 and 326. Percentage rust on 60 leaves/race was estimated 26 days after initial inoculum was released for early and late maturing varieties, and after 21 days for midseason varieties.
[d] Data are averages for races 264 and 326 and isolates P-3 and P-B. Percentage rust on 60 leaves/isolate was estimated 24 days after initial inoculum was released for midseason and late maturing varieties, and after 18 days for early maturing varieties.

Penetration.—Seedling and adult plants of the moderately resistant Portage and the susceptible Coachman were tested to determine their resistance to penetration. The upper surfaces of the first foliage leaves of 15-day-old plants and the F-1 leaves of 50-day-old plants were inoculated by rubbing them with dry cotton swabs in which urediospores of race 264 or isolate P-3 were embedded. The plants were kept moist for 11 hr in growth chambers at 15 or 26 C. Plants were then dried for 6 hr at 10 C (lower temperature chamber) or 21 C (higher temperature chamber). After the 17-hr period in the dark, an alternating cycle of 10-15 C or 21-26 C for 8 hr of darkness and 16 hr of light was initiated, starting with the light period. Four or five leaves were randomly collected for each treatment 45 hr after the start of incubation. The excised leaves were immediately placed in a solution of alcohol-lacto-phenol-cotton-blue and processed, using the techniques described by Shipton & Brown (10). The percentages of appressoria forming substomatal vesicles and infection hyphae were determined for each treatment.

The first foliage leaves of 15-day-old Portage plants were penetrated significantly less by both isolates at both temperatures than were similar leaves of the variety Coachman (Table 2). Differences between varieties in penetration of adult F-1 leaves were usually greater than of seedling leaves, and highly significant regardless of rust isolate or temperature (Table 2). Percentage penetration of adult F-1 leaves by both isolates was less at 21-26 C than at 10-15 C.

Hyphal growth rates.—Inoculation, incubation, and staining methods used to determine hyphal growth rates were identical to those used to determine percentage penetration. Four or five leaves were collected at random for all treatments 45, 93, and 141 hr after start of incubation in the 21-26 C chamber, and after 45, 93, 141, 189, and 237 hr in the 10-15 C chamber. Hyphal length was measured with an ocular micrometer (×400). To determine the distance that hyphae grew in the leaf tissue from each infection point, the limit of measurement was set at the tip of the most extended hypha visible microscopically at two opposite ends of each colony.

There was a wide range with a continuous gradation in degree of colony development for the same treatment at any given time after inoculation for both plant ages. The early-developing hyphae always grew first toward the vein closest to the point of penetration. In seedling leaves at 21-26 C, isolate P-3 grew significantly faster in Portage than in Coachman (Fig. 1-A). Race 264 grew at the same rate in both varieties. In seedling leaves at 10-15 C there were no significant differences in growth of either isolate in either variety (Fig. 1-A). Increase in colony length at 21-26 C was about three times as fast as at 10-15 C during the first 141 hr.

Many appressoria that produced infection pegs and formed substomatal vesicles in F-1 leaves of Portage apparently either died or stopped growing shortly after penetration; hyphal growth in these infections was not

TABLE 2. Percentage of appressoria of two isolates of *Puccinia coronata* that formed substomatal vesicles in first foliage leaves of seedlings or F-1 leaves (F-1 = leaf below the flag leaf) of adult Portage (P) and Coachman (C) oats after 45 hr at 10-15 C or 21-26 C

Leaf	Race	Variety	Substomatal vesicles formed			
			10-15 C	21-26 C	Avg race	Avg var.
			%	%	%	%
Primary[c]	264	Portage	74[a]	58	66 p	
		Coachman	89	77	83 r	
	P-3	Portage	79	68	74 p	70 x
		Coachman	87	85	86 r	85 y
F-1[d]	264	Portage	51[b]	14	33 p	
		Coachman	79	42	61 r	
	P-3	Portage	53	21	37 p	35 x
		Coachman	57	47	52 r	56 y

[a] Data based on 500-1,000 appressoria on at least 5-10 leaves in each of two experiments.
[b] Data for race 264 on F-1 leaves are based on 500 appressoria on at least five leaves in one experiment.
[c] Avg. for race and variety significantly different at 5% level if letters are different.
[d] Avg. for race and variety significantly different at 1% level if letters are different.

measured. Highly significant differences in hyphal growth in the two varieties occurred at both temperatures with both isolates in adult F-1 leaves (Fig. 1-B). Hyphae of both isolates grew significantly slower in F-1 leaves of Portage than in Coachman, and these differences became progressively greater with increased time for development at both temperatures. Growth of isolate P-3 hyphae in Portage was faster than race 264 in Portage, but not as fast as either isolate in Coachman. Here, as in seedling leaves, hyphae grew much faster at the higher temperature.

Development of uredia.—Inoculation, incubation, and staining techniques used to determine varietal differences in development of uredia were identical with those used in previous experiments. Leaves were randomly collected and stained after 237 hr at 10-15 C and after 141 hr at 21-26 C. The length and width of the spore mass with spores over 8 μ in diam were measured for individual uredia.

Sori of both isolates were significantly smaller in first foliage leaves of Portage than Coachman at 10-15 C but not at 21-26 C (Table 3). Varietal differences were less with isolate P-3 than with race 264 at both temperatures.

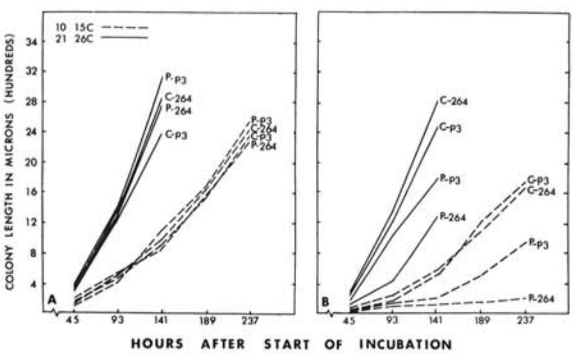

Fig. 1. Average colony length of race 264 and isolate P-3 colonies of *Puccinia coronata* f. sp. *avenae* in Portage (P) and Coachman (C) oats at different times after start of incubation at two temperatures. **A)** First foliage leaves of seedlings. **B)** F-1 leaves of adult plants.

In F-1 leaves of adults, varietal differences in size of uredia were highly significant regardless of temperature or rust isolate (Table 3). At 10-15 C, no race 264 pustules had spores 8 μ in diam or larger in Portage, and few pustules of isolate P-3 were present with 8-μ diam spores when measurements were made. Numerous and large pustules of both isolates were present on Coachman F-1 leaves at 10-15 C. Pustules of both isolates were significantly smaller in F-1 leaves of Portage than Coachman at 21-26 C, but again, differences were less with isolate P-3 (Table 3).

Urediospore production.—To determine if varieties influenced sporulation of rust races, plants were inoculated as in the previous tests, placed in a moist chamber for 18 hr at 25 C, then placed in controlled environment chambers at alternating temperatures of 10-15 C or 21-26 C for 8 hr of darkness and 16 hr of light. To measure the numbers of spores produced per pustule, leaves with close to the same number of flecks were installed in nearly horizontal troughs made of flexible, clear Mylar plastic to prevent spores from escaping. In work with seedlings, troughs were 10-13 cm long and 2 cm in diam (Fig. 2). An 8-mm open space running the length of the top allowed moisture to escape and made collection of spores relatively easy. Troughs used for adult plants were of a similar design but larger. Leaf blades were held on edge in each trough. Troughs and leaves were sloped slightly downward toward distal ends to permit collection of spores. Spores were washed off the leaves and out of the troughs with a 0.1% solution of Tween-20 (polyoxyethylene sorbitan monolaurate) in distilled water gently squirted from a plastic squeeze bottle. Leaves were next rinsed with distilled water. Spores were collected from three replicates for each treatment at 2-day intervals, starting 2 days after sporulation began. The number of spores in three 0.07-ml portions of spore suspension was counted for each collection. By knowing the number of pustules contributing to spore production and the volume of spore suspension in each collection, the number of spores produced per pustule for each treatment was calculated. The number of

TABLE 3. Average length and width of pustules of race 264 and isolate P-3 of *Puccinia coronata* in first foliage leaves of seedlings and in the leaf below the flag leaf (F-1 leaves) of Portage and Coachman oats 237 hr after start of incubation at 10-15 C and after 141 hr at 21-26 C

		Pustule size in μ			
		Length		Width	
Isolate	Temp.	Portage	Coachman	Portage	Coachman
	C	*Pustules on first foliage leaves*[a]			
264	10-15	40 p[c]	300 r	15 p	145 r
	21-26	447 p	562 p	191 p	254 p
P-3[b]	10-15	285 p	410 r	125 p	187 r
	21-26	557 p	541 p	260 p	261 p
		Pustules on F-1 leaves[d]			
264	10-15	0 p[e]	90 r	0 p	31 r
	21-26	20 p	505 r	7 p	157 r
P-3	10-15	1 p	139 r	4 p	50 r
	21-26	233 r	425 r	76 p	118 r

[a] Data are averages of 120 pustules in at least eight leaves in two experiments unless otherwise noted.
[b] Part of the data in one experiment was missing.
[c] Averages with different letters are significantly different at the 5.0% level.
[d] Data are averages of 60-120 pustules in at least 4-8 leaves in one or two experiments.
[e] Averages with different letters are significantly different at the 1.0% level.

pustules per leaf always increased as the experiments progressed. Therefore, the number of pustules contributing to sporulation was calculated as the average of the pustules present at the time of the preceding collection and at the time of each current collection for each replicate. For example, if 60 pustules were present for a replicate 4 days after start of sporulation, and if 100 pustules were present 6 days after sporulation started, 80 was the number used in the calculation of spores produced per pustule for the 6-day collection. The number of pustules contributing to sporulation for first collections was estimated at three-fourths the number present at the time of each first collection. When the highest numbers of pustules were reached for each replicate, that number was used in calculations of spores per pustule for the rest of the experiment. Leaves began to die about 16 days after start of incubation. The time of senescence was followed closely by a decline in sporulation; thus, measurement was stopped after 22 days.

On first foliage leaves of seedlings, first urediospores of both isolates were released from both varieties about 141 hr after start of incubation at 21-26 C and after about 237 hr at 10-15 C. On F-1 leaves of adults, the time to sporulation differed with the variety, rust isolate, and temperature. In Coachman adult F-1 leaves, sporulation started about the same time as in seedlings. In Portage adults, race 264 sporulated about 12 hr later than in seedlings at 21-26 C, and 2 days later at 10-15 C. Isolate P-3 in Portage F-1 leaves sporulated about the same time as in seedlings at 21-26 C, but was about 12 hr slower at 10-15 C. At both host ages, the

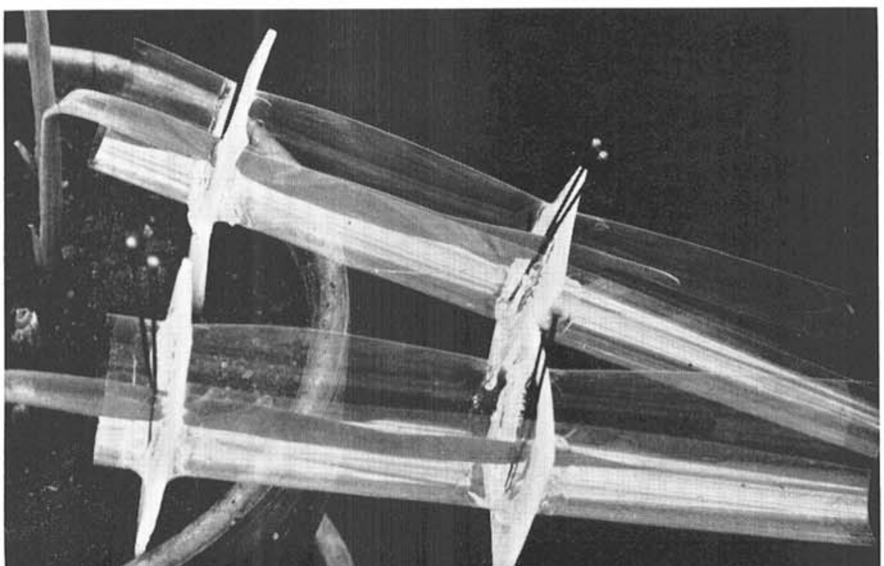

Fig. 2. Mylar plastic troughs used to collect spores from first foliage leaves of oat seedlings.

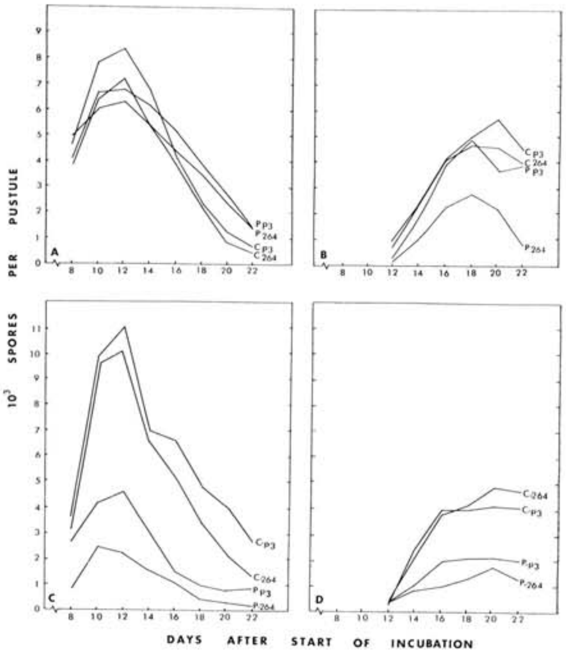

Fig. 3. Spores produced per pustule by race 264 and isolate P-3 on Portage (P) and Coachman (C) oats; **A)** on first foliage leaves of seedlings at 21-26 C; **B)** at 10-15 C; **C)** on F-1 leaves of adults at 21-26 C; **D)** at 10-15 C.

number of primary sporulating pustules increased faster at 21-26 C than at 10-15 C, and more secondary pustules were produced in a shorter time at the higher temperature.

Spore production per pustule on the first foliage leaves of seedlings for each 2-day collection period is shown in Fig. 3-A, B. The comparable data for F-1 leaves of adults are shown in Fig. 3-C, D. Varietal differences in spore production were not statistically significant on seedlings at 21-26 C (Fig. 3-A), but at 10-15 C, significantly fewer spores per pustule were produced by both isolates on Portage than on Coachman (Fig. 3-B). On F-1 leaves of adult plants at 21-26 C, race 264 produced about four times as many spores per pustule on Coachman as on Portage, and isolate P-3 produced nearly three times as many (Fig. 3-C). Race 264 produced more than three times as many spores per pustule on Coachman than on Portage, and isolate P-3 produced about twice as many on F-1 leaves at 10-15 C (Fig. 3-D).

Temperature affected onset of sporulation, number of spores produced per pustule, and total spores produced in seedlings and in adults. Spore production started earlier and peaks were reached 6 to 8 days sooner at 21-26 C than at 10-15 C. More spores were produced at the higher temperature than at the lower.

Seedling and adult plants of Coachman were roughly comparable in numbers of spores produced by either isolate at a given temperature. This was not true in Portage, as adult resistance reduced sporulation by both isolates at both temperatures.

Discussion.—Although varieties differed but little in apparent resistance when grown near buckthorn bushes, such differences resulted in considerable effect on the spread of rust in fields of single varieties (Table 1). In fields of single varieties, epidemics often develop from relatively few infected plants. Under these conditions, small varietal differences, apparently because of their effect on the amount of infection, the incubation period, and spore production, are sufficient to reduce the development of epidemics in the field.

Isolate P-3 and race 264 hyphae grew at about the same rate in first foliage leaves of Portage and Coachman (Fig. 1-A). Pustules in seedlings were usually larger in Coachman than in Portage (Table 3). The number of hyphae and haustoria per unit area were not measured, but it was obvious that after several days of incubation, rust colonies in Coachman usually had more areas of densely staining mycelium than those in Portage. Evidently, resistance was already being expressed in 21- to 25-day-old Portage plants, probably affecting the time of sporulation.

Many workers have observed that the crown rust fungus sporulates sooner at higher temperatures (2, 5, 7, 8, 11, 14). In all host-parasite combinations, hyphae grew faster and symptoms appeared earlier at 21-26 C than at 10-15 C.

The usefulness of specific resistance is typically short lived; hence the search for other types of resistance. According to van Der Plank (12), generalized or horizontal resistance to a pathogen is expressed by fewer successful infections, slower developing infections, fewer spores produced, and a shorter duration of spore production. Specific or vertical resistance is expressed by hypersensitivity of the host to attack by the pathogen. It was not determined in these studies if resistance in varieties adjacent to buckthorn is generalized or specific, but there is evidence to support both possibilities. The fact that Portage was effective in reducing penetration, hyphal growth, and spore production is good evidence for generalized resistance. Also, Portage and other varieties have consistently appeared more resistant than others to all genotypes of the crown rust pathogen to which they have been tested. This includes several years of tests adjacent to buckthorn where, presumably, a wide range in rust genotypes is present. Several of the varieties tested can also resist attack by genotypes of the rust pathogen present in other areas of the USA (6, 9).

Generalized or horizontal resistance, according to van der Plank (12), is apparently uniformly effective against all genotypes of a pathogen. Varieties more resistant when grown near buckthorn were not uniformly effective against all races in field and greenhouse tests. For instance, isolate P-3 was more virulent than any other isolate on Portage. In addition, Portage possesses seedling resistance to many races of crown rust.

The resistance to crown rust in Portage may be due to a combination of specific and generalized genes for adult resistance. In this case, specific genes for resistance could supplement genes for nonspecific or generalized resistance resulting in the observed differential reactions to different genotypes of crown rust.

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