The Dynamics of a Controlled Population of Rhynchosporium secalis, Changes in Race Composition and Frequencies

L. F. Jackson and R. K. Webster

Postgraduate Research Plant Pathologist and Professor, respectively, Department of Plant Pathology, University of California, Davis 95616.

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ABSTRACT

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The dynamic nature of the California population of *Rhynchosporium secalis* was revealed when a mixture of five isolates representing five races of widely varying pathogenicity was carried through two successive disease cycles and an intervening saprophytic stage in the greenhouse on the susceptible barley cultivar Numar. One hundred single-spore isolates obtained after the second disease cycle were characterized on 14 barley differentials and only 17 of them were found to belong to the original five races. The 83 remaining isolates represented 14 hybrid-type races. Most of these isolates differed from one or more of the original isolates in pathogenicity on only one or two of the differentials, although some of them differed on up to six of the differentials. In addition to the generation of new races, there was a shift in the fungus population toward the more simple pathogenic races and away from the more complex pathogenic races. The original isolates differed in sporulating ability in culture and on host tissue, but differences were not related to the respective pathogenic complexities of the isolates.

Additional key words: barley, disease resistance, pathogenic variability, scald of barley.

The pathogenic variability of *Rhynchosporium secalis* (Oud.) Davis and the race composition and frequencies of the fungus on barley in California have been reported (3). Since breeding programs for disease resistance cover a period of years, the pathogen population should be monitored over the same period to detect changes in the racial population and shifts in the frequencies of known races. The frequency and distribution of races in a population may change owing to the presence of specific genes for resistance in the host and to differences in the fitness of individual races. The fitness of members of a pathogen population may be estimated from their abilities to attack the host under various environmental conditions, to produce inoculum, to become disseminated, and to survive in the absence of the host either saprophytically, on alternate hosts, or in a dormant stage (6).

The present study was undertaken to determine if the composition and frequencies of races in a controlled population of *R. secalis* were stable or dynamic after two successive disease cycles and an intervening saprophytic stage in a greenhouse environment. The stability of single races after passage through a susceptible host and the sporulating ability of isolates of widely varying pathogenicity also were investigated.

MATERIALS AND METHODS

Sporulating ability of five isolates of Rhynchosporium secalis.—One isolate of each of five races of widely varying pathogenicity, races 2, 14, 61, 69, and 73, was selected for use in a sporulation assay. The isolates were grown on potato-dextrose agar (PDA) in petri plates at each of five temperatures, 9, 12, 15, 18, and 21 C. Each plate was inoculated with 1 ml of a spore suspension (25,000 spores/ml) which was spread over the agar surface in each plate with the aid of a Fischer colony-turning wheel and a triangular tool made from a glass rod. Three plates were harvested for each isolate and temperature treatment after incubation periods of 8, 12, 16, 20, 24, and 28 days. The spores were harvested in distilled water after the surface of each culture was repeatedly scraped with a wire transfer needle. Spore concentrations were determined with a hemacytometer.

The sporulating ability of each isolate on Numar, a susceptible cultivar, and on Briggs, a cultivar with considerable field resistance (C. W. Schaller, *personal communication*), also was determined. Greenhouse-grown plants were inoculated with suspensions of 1×10^5 spores/ml of each isolate. Leaves that developed lesions were collected and dried in a plant press. A cork borer was used to punch 3-mm diameter disks from the centers of lesions. Ten disks per replicate were floated on 25 ml of distilled water in a petri dish for 4 days in a 15 C incubator to induce sporulation. Spores were separated from the disks by placing the disks and water in 50-ml beakers subjected to the stirring action of a magnetic stirrer (Van Waters and Rogers, Inc., Magnestir) for 3 minutes. Spore concentrations were determined with a hemacytometer for three replicates of each cultivar-isolate combination.

Stability of single races.—Greenhouse-grown plants of the susceptible cultivar Numar were inoculated by the standard method (3) with spore suspensions of the five previously described isolates. Twelve single-spore isolates were obtained from lesions caused by each parent isolate and these were identified to race on the 14 barley differentials by the method described previously (3).

Race composition and frequencies in a controlled

population.—Thirty-six flats of plants of the susceptible cultivar Numar were inoculated at the one and one-half- to three-leaf stage with a composite spore suspension from the five previously described isolates. The composite inoculum consisted of 150-ml spore suspensions (3×10^5 spores/ml) of each isolate. Inoculation and incubation were by the standard methods (3). After 2 weeks, infected leaves were collected and air-dried. Leaves were cut into 2.5-cm segments, placed on wire screens over water in plastic crispers, and incubated at 15 C for 4 days to induce sporulation. The leaf segments were comminuted in water in a Waring Blendor. The resulting spore suspension was filtered through a double layer of cheesecloth and used to inoculate 4-week-old plants of the susceptible cultivar Numar grown in 20 10-cm diameter pots, five plants per pot. One-hundred single spores were isolated from lesions that developed on individual plants and the resulting isolates were identified to race on the 14 barley differentials as before.

RESULTS

Sporulating ability of five isolates of Rhynchosporium secalis.—The sporulating ability of each isolate on PDA varied with temperature and length of incubation. In general, isolates reached maximum sporulation after longer incubation periods at the lower temperatures and after shorter incubation periods at the higher temperatures. Maximum sporulation was reached at 15 C after 20 days by isolate 146-1 (race 14), at 9 C after 28 days by isolate 156-1 (race 61), at 9 C after 24 days by isolate 132-1 (race 73), at 12 C after 28 days by isolate 150-1 (race 69). and at 9 C after 24 days by isolate 155-1 (race 2). Ranking of the isolates on the basis of total sporulation on PDA over all temperatures and incubation periods was 146-1 = 156-1, 132-1 = 150-1, 155-1, which corresponded to an order for races of 14 = 61, 73 = 69, 2. Differences were significant at the 1% level [LSD (P = 0.01) = 4908.88].

The isolates differed in ability to sporulate on host tissue. The ranking of isolates on the basis of sporulation on the susceptible cultivar Numar was different from that on the field-resistant cultivar Briggs, and in both cases the ranks overlapped (Table 1). Isolates 132-1 and 150-1 (races 73 and 69) sporulated better on Numar than on Briggs, isolate 155-1 (race 2) sporulated better on Briggs than on Numar, and isolates 146-1 and 156-1 (races 14 and 61) sporulated equally well on both cultivars (Table 1).

Stability of single races.—Twelve single-spore isolates from each of four parent isolates that had been passed through the susceptible cultivar Numar reacted like their respective parent isolates when characterized on the set of

TABLE 2. Race composition and frequencies in a controlled population of *Rhynchosporium secalis* on barley cultivars after two successive disease cycles

Race designation	Percent of isolates
2[a]	9
9	6
14[a]	4
17	31
25	6
26	2
27	2
( )[b]	2
( )[c]	2
( )[d]	5
( )[e]	4
58	4
61[a]	2
( )[f]	1
69[a]	2
( )[g]	13
72	4
73[a]	0
74	1

[a]Races used in original mixture.
[b,c,d,e,f,g]Previously unidentified races.
[b]Differs from race 17 in reaction on barley cultivar C.I. 2376.
[c]Differs from race 26 in reaction on barley cultivar Trebi.
[d]Differs from race 25 in reaction on barley cultivar C.I. 2376.
[e]Differs from race 56 in reaction on barley cultivar Atlas 46.
[f]Differs from race 69 in reaction on barley cultivars Hudson, Atlas 46, and Turk.
[g]Differs from race 72 in reaction on barley cultivar Atlas 46.

TABLE 1. Sporulation of five isolates of *Rhynchosporium secalis* on the barley cultivars Numar and Briggs

R. secalis			Mean spore production	Level of	Homogeneous
Isolate	Race	Cultivar	(thousands)	Significance[a,b]	subgroups[a]
150-1	69	Numar	3084.00	1%	x
		Briggs	1818.33		y
146-1	14	Numar	2328.23	N.S.	xy
		Briggs	2420.67		xy
132-1	73	Numar	1896.67	5%	y
		Briggs	963.00		z
156-1	61	Numar	1873.67	N.S.	y
		Briggs	2148.67		xy
155-1	2	Numar	994.33	1%	z
		Briggs	2714.67		x

[a]LSD (P = 0.05) = 808.18
[b]LSD (P = 0.01) = 1101.92

differentials. However, four of the 12 single-spore isolates from isolate 155-1 differed from that parent isolate in pathogenicity to two or three of the 14 barley differentials. Two of the isolates had become pathogenic to Kitchen and Steudelli, one had become pathogenic to Kitchen and La Mesita, and one had become pathogenic to Kitchen, Osiris, and Steudelli.

Race composition and frequencies in a controlled population.—The 100 single-spore isolates represented a greater degree of pathogenic variability than did the five isolates of the original mixture. Eighteen races were identified among the 100 isolates, including 14 hybrid races that differed from each of the five races of the original mixture. Isolates of the five original races together made up only 17% of the sample. Race 17, a hybrid, dominated the sample and accounted for 31% of the isolates [race 17, which differs from race 2 in reaction on Kitchen and Steudelli, was one of the races obtained when an isolate (155-1) of race 2 was passed through the susceptible cultivar Numar]. Another hybrid race, for which no designation had previously been assigned, accounted for 13% of the isolates. It differed from race 73 in reaction on Atlas 46 and C.I. 5831, and from race 69 in reaction on Atlas 46 and Hudson. The remaining hybrid races, including five races not previously encountered in California, each represented from 1 to 6% of the sample (Table 2). Sixty-one of the 83 isolates that composed the hybrid races represented changes in pathogenicity from one or more of the original races to only one or two differentials, whereas the remainder represented changes in pathogenicity to as many as six differentials.

DISCUSSION

The five isolates used in this study, which were known to differ in pathogenicity, also were shown to differ in sporulating ability with regard to maximum spore production, optimum temperature for sporulation, length of incubation period required for maximum sporulation, and sporulation on host tissue. The relative sporulating abilities of the isolates on PDA was not necessarily indicative of their sporulating abilities in host tissue. Neither their sporulating ability in host tissue nor their sporulating ability in culture appeared to be related to their pathogenic complexity. The relative sporulating abilities are thus considered to be attributes of isolates rather than of races. Habgood (1) noted extensive variation in sporulation between single-spore subcultures from single-spore isolates and postulated control by cytoplasmic factors.

The generation of new races in a controlled population of the fungus that was monitored for two disease cycles indicated the dynamic nature of the pathogen population. The fungus behaved as an interacting population with access to a large gene pool for pathogenicity through which different races arose by as yet unknown mechanisms of recombination and segregation. The presence of mechanisms for asexual variation also is indicated by the generation of new races among single isolates. Hansen and Magnus (2) identified changes in pathogenicity in single-spore isolates from four of five parent cultures of *R. secalis* in Norway. In screening for disease resistance, therefore, the utilization of a mixture of isolates representative of the range of pathogenicity available to the fungus, instead of single pathogenic races, is mandatory.

Transcending the asexual variation expressed as the generation of new races in a mixed population of *R. secalis* after two disease cycles was a shift in the population towards the simpler pathogenic races. The observed shift could be regarded as evidence in support of van der Plank's stabilizing selection theory (9), acceptance of which contradicts conclusions drawn from observed frequencies of races of *R. secalis* in California (3). Fitness characters that enable an organism to increase its frequency in populations have been suggested to be independent of those controlling pathogenicity or virulence (4, 5, 8, 10). In studies solely with the conidial stage of the northern corn leaf blight fungus, *Trichometasphaeria turcica,* in which mixtures of simple and complex races were evaluated for differential survival ability on a simple corn inbred, the simple race predominated in some mixtures and the complex race predominated in other mixtures (6, 7, 8), which indicated the independence of pathogenicity and fitness. In view of the fact that opposite conclusions regarding the operation of stabilizing selection could be drawn from two studies of *R. secalis* populations, fitness properties are considered to be characteristic of isolates rather than of races that particular isolates happen to represent.

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