Factors Conditioning Dominance of Race 276 of *Puccinia coronata avenae* on *Avena sterilis* Populations in Israel

U. Brodny, I. Wahl, and J. Rotem

First two authors, Institute for Cereal Crops Improvement, Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel. Last author, Department of Plant Pathology, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel.
Portion of Ph.D. dissertation submitted to Tel Aviv University by the first author.
Accepted for publication 25 June 1987.

ABSTRACT

Brodny, U., Wahl, I., and Rotem, J. 1988. Factors conditioning dominance of race 276 of *Puccinia coronata avenae* on *Avena sterilis* populations in Israel. Phytopathology 78:135-139.

Isolates of *Puccinia coronata avenae* races 276 and 263 were collected from *Avena sterilis* in different ecological locations. Components of pathogen fitness of the isolates were compared in different temperatures in the greenhouse and in the field. The results obtained may help provide an explanation for factors that contribute to the dominance of race 276 over race 263. These results were found countrywide and over many years, despite the fact that race 263 had a wider host range in seedling populations of its main host, *A. sterilis* L., in greenhouse trials. The study revealed that isolates of race 276 showed advantages under different environmental conditions in producing higher yields of urediospores with preferential viability. Telial formation occurs later, and the period of urediospore productivity is thus prolonged.

The oat crown rust pathogen *Puccinia coronata* Cda. var. *avenae* Fraser and Ledingham develops in Israel in its natural habitat on indigenous populations of *Avena sterilis* L. and other wild *Avenae* species as well as on cultivated oats. The fungus completes the life cycle on *Rhamnus* species. Both the main *Avena* hosts and *Rhamnus* alternate hosts are indigenous to the Mediterranean region, which is the source of their genetic diversification.

P. c. avenae as an obligate parasite has coevolved with both hosts for millenia. The present state offers an appropriate setting to study various traits of the pathogen (e.g., virulence and the biotic and abiotic factors influencing their evolution).

Research in Israel during the past 30 years has shown that the crown rust race 276 has consistently dominated in all parts of the country, whereas race 263 occurs infrequently (1,14,18,19). On seedlings of differential cultivars, race 276 differs from race 263 in virulence on the differential Ukraine (CI 7007).

Despite its outstanding prevalence in nature, race 276 has been found to have a narrower host range than race 263 on *A. sterilis* seedling tests in the greenhouse. These results were obtained in previous studies in which populations of *A. sterilis* were characterized with respect to their resistance of several races of *P. coronata*. The frequency of a susceptible host response among the 574 *A. sterilis* accessions inoculated with race 263 was significantly higher than that of race 276 (U. Brodny, Z. Eyal, and I. Wahl, *unpublished*).

Studies by Katsuya and Green (5), Martens (7), Lesovoi (6), and Mikhailova and Metreveli (8) have shown that competitiveness of some cereal rust races can be influenced not only by the host but also by "adaptability to abiotic factors." This study was made to examine some of the factors that may be responsible for the dominance of race 276 over 263 in nature.

MATERIALS AND METHODS

In all experiments, 10 isolates each of race 276 and 263 were compared. The only exception was the telia study, which consisted of 24 isolates (12 for each race). Each of the isolates studied was chosen from a sample of 20–30 monouredial isolates from different locations of countrywide distribution. The locations ranged from the Upper Galilee and Golan Heights, with cold winters and abundant rainfall, in the north, to the arid Negev desert with high temperatures, in the south. Isolates were identified on a set of differential cultivars of oats according to Simons and Michel (15). The isolates investigated were of a monouredial origin. They were individually increased on seedlings of the cultivar Markton (CI 2053), which is generally susceptible to crown rust.

Seedlings of Markton were grown in plastic pots filled with a 1:1:1 (v/v) mixture of sandy loam, sand, and peat. The seeds were sown (eight per pot) with their embryonic ends down and the grooves toward the center to obtain uniform exposure to the inoculum.

Seven-day-old seedlings were inoculated using Schein's quantitative inoculator (11). The inoculum consisted of a suspension of 3 mg urediospores/ml of distilled water containing 0.01% Tween 20 as a surfactant. The density of the deposited inoculum was equivalent to 150 urediospores per square centimeter of leaf surface. In addition to the leaves, petroleum jelly-coated microscope slides were sprayed, and the number of urediospores per square centimeter of the slide was determined by counting under the microscope. The inoculated plants were kept for 24 hr in a moist chamber at 18–20 C and then placed in growth cabinets at 15, 20, and 25 C (±1.5) with a 12-hr photoperiod (fluorescent incandescent light intensity of 120 SI cm^{-2}/sec^{-1} at plant height). Plants chosen for inoculation in different treatments in each experiment and for all isolates were arranged in a randomized complete block design. Each trial was done in three replications and repeated twice.

In studies of competitiveness of races 276 and 263 in mixture, both races were represented by all 10 isolates in equal amounts, as determined by weight. The experiment was made in three replications and repeated twice. The 10 isolates of each race were divided in two groups with different components. All groups contained one isolate each of race 276 and 263, both from the same location. Each set of four pots (32 Markton seedlings) was inoculated with a 1:1 mixture of spore suspension of races 276 and 263 containing five isolates as described above. After the formation of open uredia, urediospores were harvested 14 days after seedling inoculation and used for inoculating another set of seedlings following the same procedure.

Urediospore harvest was repeated three times, and subsequent inoculation cycles were repeated four times in succession. A total of 400 uredial pustules was randomly sampled in each generation for the ensuing inoculation. Concurrent with Markton seedlings, seedlings of the differential Ukraine were also inoculated. These

served as "indicator plants" (7) because of their susceptibility to race 276 and resistance to race 263. Pustules denoting susceptibility were of infection type 3-4, and those expressing resistance were of infection type 0-1. Both types of pustules were counted on the infected seedling leaves of Ukraine at the end of each of the four generations.

Urediospores with more than 95% germinability were used in trials designed for evaluating infectivity of urediospores after exposure to different degrees of relative humidity (RH). Such urediospores belonging to races 276 and 263 (3 mg urediospores per sample) were placed in closed containers (six containers per treatment) in which RH of 0, 45, and 85% was maintained. All containers were kept at 28 ± 1 C. Different RH values were obtained by regulating water vapor pressure with dry P_2O_5 or saturated solutions of $Ca(NO_3)_2$ and $ZnSO_4$, respectively, according to Schein and Rotem (12). After 6, 15, and 21 days of exposure, urediospores were inoculated on one-leaf Markton seedlings to assess their infectivity, as expressed by the amount of uredia formed on 1 cm^2 of leaf area. This experiment consisted of six replications for each isolate, where each container served as a replication. Each replication consisted of 16 replicants. The number of uredia was counted on 3-cm segments of the center of each leaf after a 14-day incubation period. Also, the width of the segment was determined, and the average number of uredia per square centimeter was calculated. The percentage of infectivity was determined in each treatment by comparing it to plants inoculated with fresh urediospores under the same conditions.

Receptivity data were recorded 14 days after inoculation on seedlings inoculated with isolates of race 263 or 276 at 15, 20, and 25 C. The mean total of uredia produced per square centimeter of leaf area for each isolate treatment was calculated. Each trial consisted of six replications for each isolate, each with 20 replicants.

Precocity of telia formation by races 276 and 263 was investigated by inoculation with 12 isolates of each race. Ten pots with eight plants per pot were inoculated with each isolate. The inoculated plants were kept at 25 C. Fourteen days after inoculation, the number of developing uredia was counted every other day, and the number of telia formed on each date was determined. Finally, the number of telia-producing pustules, in relation to the total number of pustules, was calculated for a period of 28 days following inoculation.

In studies of urediospore productivity, sporulation data were collected every 2 days for 8 days (four harvests), beginning 9 days after inoculation. Urediospores were removed into a 5-ml aqueous 1% saline solution by using vacuum suction. Spores were collected from all pustules on a 3-cm segment of adaxial leaf surface. Each experiment had six replications for each isolate, each one containing five-leaf segments and 6–10 pustules per leaf segment. The number of urediospores in each sample was determined with a haemacytometer, and the cumulative number of urediospores harmfulness on the parasite."

Data for all experiments were analyzed in an analysis of variance, and the Waller-Duncan k-ratio t test (P=0.05) was used to determine differences among isolates (16).

In addition to the greenhouse experiments, field studies were made in an 80 × 25-m field, divided into 12 (5 × 5 m) replicate plots (each with seven rows) with a barrier of the cultivar Saia (CI 7010) 10 m long on all sides of each plot. This cultivar has a broad spectrum of resistance to crown rust in Israel.

The field experiments were made during two growing seasons at the Bet Dagan Agricultural Experimental Station, The Volcani Center. In each season, five plots were inoculated with five different isolates of race 276, and five other plots were similarly inoculated with different isolates of race 263. The remaining two plots were not inoculated and served as controls.

The plots in this field were planted with a mixture of the cultivar Markton and a line of *A. sterilis* susceptible to both races. In the center of each plot, 10 plants used as spreaders were inoculated with either race 276 or 263 by injecting a urediospore suspension into the stem. Disease development caused by the respective races was evaluated at intervals of 10, 16, and 23 days following inoculation by measuring the percentage of leaves covered by uredia. The plants examined were sampled uniformly from each plot (7–8 plants from each of seven rows, yielding a total of 50 plants) according to the disease assessment scale procedures devised by James (4) and by identification of races via methods described previously. Each plant was tagged and readings made on the flag leaf and one leaf down. In addition, the concentration of spores of each race released in each plot was estimated with the aid of a Rotorod spore sampler type U (Ted Brown Associates, Los Altos Hills, CA). The part catching the spores in this trap is a U-shaped rod, dipped in rubber cement thinner which is sticky when slightly dried. This rod is attached to a small electric motor operated by a 12-V battery. Because the motor runs at 2,400 rpm, the rods sample approximately 120 L of air per minute. The duration of each catch was 2 hr. The efficiency of using this spore sampler for sampling fungus spores in the atmosphere has been demonstrated by Edmonds (3).

Spores were counted with a microscope equipped with an epicondenser using vertical illumination on a grid divided into 25 squares entered under the lens (110×). The counts were made along both arms of the U-shaped rod in each microscopic field. The number of spores per liter of air was calculated according to the formula described by Ted Brown Associates (17).

RESULTS

Shift in race populations. The races 276 and 263 differed in their abilities to survive in mixture when inoculated on a susceptible cultivar in a greenhouse at a temperature of 20 C. Race 276 increased its initial level when mixed with race 263. After the third generation (each generation being 14 days), there were significant differences in the number of uredia between the two races. In the first experiment, initial inoculations were made using five pairs of isolates of the races 276 and 263. In this experiment, race 276 consisted of 238 uredia out of a total of 400 sampled (59.4%) after four generations; in the second experiment, which included initial inoculations of five other pairs of isolates of the races tested, race 276 consisted of 250 uredia out of 400 samples (62.5%) after four generations (Table 1).

Temperature—effect on urediospore infectivity and productivity. Infectivity of urediospores maintained for 6–21 days at various RH values at 28 C was higher in the case of race 276. Although survival rates decreased with an increase in storage period and RH values, infectivity for race 276 was significantly higher than that of race 263 (Table 2).

After 21 days of storage at 0% RH, the infectivity of race 276 was 14.4%, compared with 6.5% for race 263. A similar direction in the resulting infectivity was obtained at 45% RH (7.3 and 3.1% for race 276 and 263, respectively) and at 85% RH (2.4 and 0%, respectively) (Table 2).

At all temperatures tested, race 276 produced more pustules per

TABLE 1. Number of uredia produced by mixtures of races 263 and 276 grown for four uredial generations on the variety Markton at 20 C

Experiment no.	Generation[a] no.	Uredia (no.)[b] 276	263	χ^2 values (1:1)[c]
I	1	207	193	0.49
	2	209	191	0.81
	3	220	180	4.00
	4	238	162	14.44
II	1	202	198	0.04
	2	216	184	2.56
	3	234	166	11.56
	4	250	150	25.00

[a] One generation means a cycle from inoculation to sporulation at an interval of 14 days.

[b] 400 Pustules were sampled randomly in each generation to detect significant differences between proportions of the two tested races at $P<0.05$.

[c] The expected χ-square value, $P = 0.01$, is 6.63.

leaf area unit and more urediospores per pustule. At 15 C, 8.1 and 3.9 pustules per square centimeter and at 20 C, 8.5 and 4.0 pustules per square centimeter were produced for races 276 and 263, respectively (Table 3). In addition, race 276 produced more urediospores per pustule than race 263. At 15 C, race 276 yielded 27.9% more uredispores than race 263 (29,552 vs. 23,105); at 20 C, 40.5% more (29,973 vs. 21,327); and at 25 C, 14.9% more (21,225 vs. 18,461) (Table 3).

Duration of uredia development and initiation of telia formation. Telia formation became evident after different incubation periods at 25 C for various isolates of both races. Telia of race 276 appeared significantly later than those of race 263 (Fig. 1A). Of the 12 isolates of race 263 tested, three gave rise to telia formation 14 days after inoculation. On four other isolates, the telia appeared 16 days after inoculation; on three others, after 18 days; and on two, after 21 days. In the isolates for race 276 tested, telia started to appear 21 days after inoculation (Fig. 1A). On the 27th day after inoculation, the average leaf area covered by telia for race 263 was 52%, compared to 29% for race 276 (Fig. 1B).

Epidemiological field experiment. Disease severity of plants in field plots infected with race 276 was higher than with race 263 at each disease assessment period throughout the experiment. Twenty-three days after inoculation, disease severity of plants in field plots infected with race 276 was significantly higher than those infected with race 263 (15 vs. 7%) (Fig. 2). Similar results were found in the number of urediospores trapped in the air. In field plots infected with race 276, 35 days after inoculation, the cumulative spore concentration per liter of air was 550×10^3, compared to 320×10^3 for race 263 (Fig. 3).

TABLE 2. Infectivity of urediospores of *Puccinia coronata avenae* race 276 and 263 after storage in various conditions of relative humidity and time at 28 C[a]

	Percentage of infection[b] after exposure to the following relative humidities (RH)[c]					
	RH 0%		RH 45%		RH 85%	
Days of storage	Race 276	Race 263	Race 276	Race 263	Race 276	Race 263
6	92.9	87.1	60.8*	48.4*	37.2	30.2
15	71.3*	40.9*	33.7*	23.7*	26.1*	17.6*
21	14.4*	6.5*	7.3*	3.1*	2.4*	0.0*

[a] Relative humidities of 0, 45, and 85% were obtained by maintaining inoculum in closed containers over dry P_2O_5 and saturated solutions of $Ca(NO_3)_2$ and $ZnSO_4$, respectively. Data are averages of six replications with 16 replicants in each.

[b] Percentage of infection was determined in each treatment by comparing it to plants inoculated with fresh spores.

[c] Asterisks indicate significant differences between the two races ($P = 0.05$), as determined by the Waller-Duncan k-ratio t test.

TABLE 3. Number of pustules per square centimeter (cm^2) leaf and cumulative number of urediospores per pustule produced on leaves of Markton inoculated with races 276 and 263 of *Puccinia coronata avenae*

Temperature	Pustules per cm^2/leaf[a,b]		Cumulative no. of urediospores[b,c] produced per pustule	
(C)	Race 276	Race 263	Race 276	Race 263
15	8.1 a	3.9 b	29552 a	23105 c
20	8.5 a	4.0 b	29973 a	21327 b
25	4.9 bc	3.6 b	21225 b	18461 d

[a] Each race was represented by 10 isolates; the given values are means of six replicates of each of the 10 isolates, each replicate containing 20 replicants.

[b] Means within a column followed by different letters differ significantly ($P = 0.05$) according to the Waller-Duncan k-ratio t test.

[c] Data are averages of six replicates of each of the 10 tested isolates. In each replicate, urediospores were collected from all pustules occurring on a 3-cm segment of leaf surface from five leaves (6–10 pustules per leaf). Spores were collected four times, at 2-day intervals, beginning 9 days after inoculation.

DISCUSSION

Crown rust race surveys made annually in Israel have ascertained that race 276 of *P. c. avenae* has been consistently dominant countrywide in populations of the indigenous, ubiquitous wild species *A. sterilis*. In contrast, race 263 has been found to be distinctly less common (1,14,18,19). The performance of both races on seedlings of standard differentials for race identification is similar except for the virulence of race 276 on the differential Ukraine, which is resistant to race 263.

The objective of this study was to explain some of the factors that may have been responsible for the preferential competitive ability (or aggressiveness) of race 276 in relation to race 263. There is a broad consensus that prevalence of a pathogen in a plant population is conditioned not only by its virulence but also by its survival ability and reproductive success (5,6,8–10).

Roane et al (10) have stressed that a "distinction must be made between genes for virulence and those for ability to grow and reproduce rapidly." Similarly, on the basis of extensive studies on diseases of cereal crops, Sebesta (13) has concluded that the "virulence of a race on a variety does not prove significant harmfulness on the parasite."

Studies by U. Brodny, Z. Eyal, and I. Wahl (*unpublished*) reject the possibility of a differential screening effect of *A. sterilis*

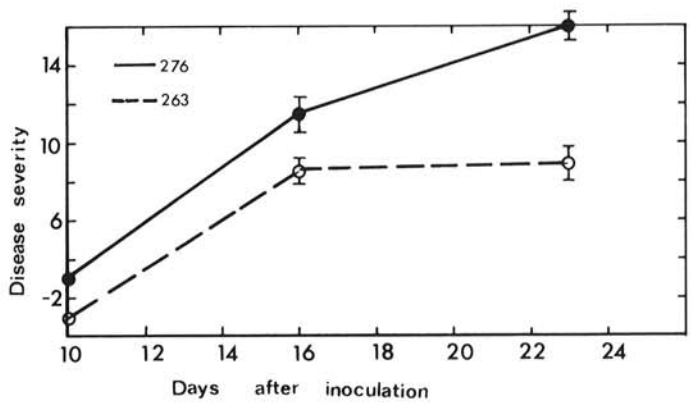

Fig. 1. Development of telia in races 263 and 276 of *Puccinia coronata avenae* at 25 C. **A,** Time of telia formation of the two races. Twelve isolates of each race were tested. **B,** The respective percentage of coverage of leaves by telia of races 276 and 263. Each data point is an average of 20 plants with two replicates. Vertical bars indicate ± SE of the mean.

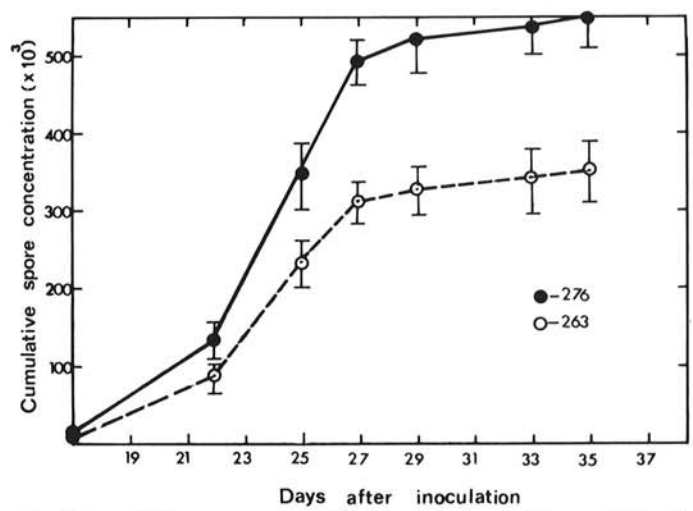

Fig. 2. Rate of disease progress in field plots inoculated with races 276 and 263 of *Puccinia coronata avenae*. Each data point is an average of five plots in two experiments. Vertical bars indicate ± SE of the mean.

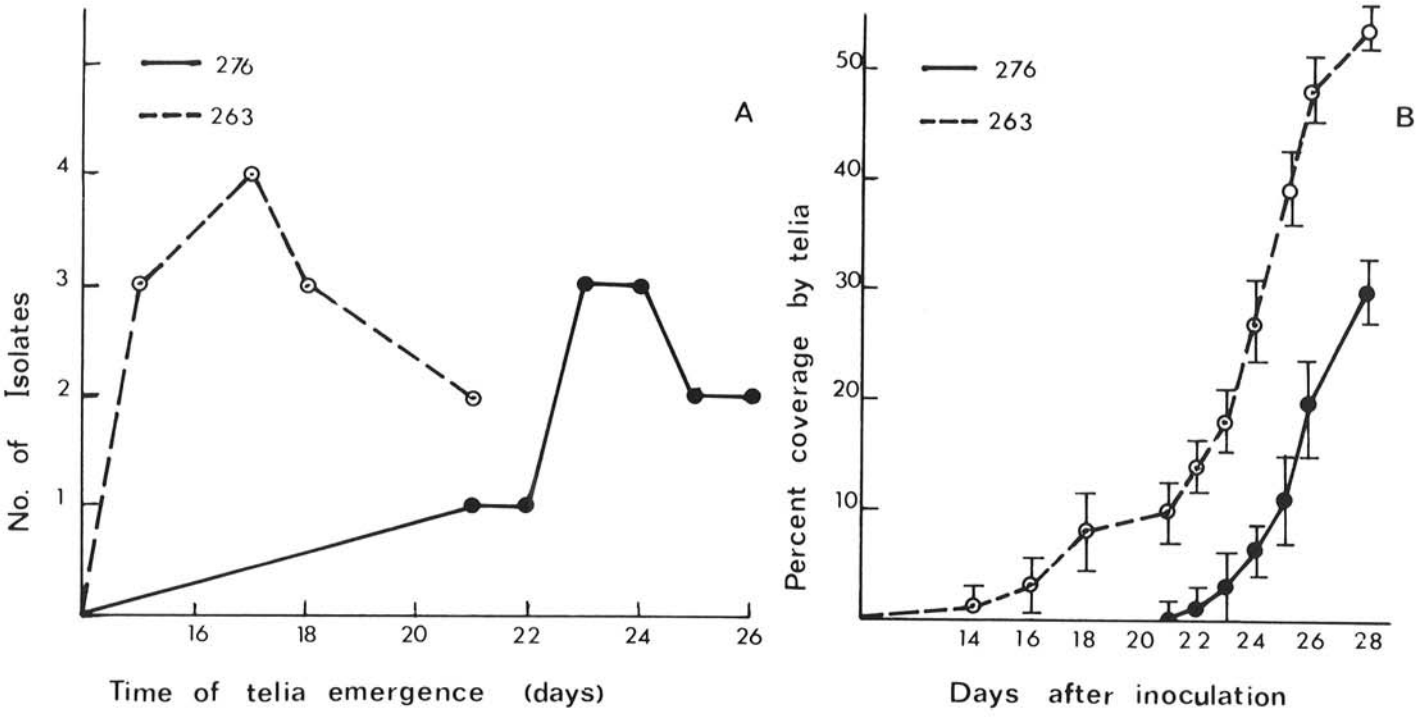

Fig. 3. Number of urediospores per liter of air trapped into Rotorod sampler over field plots, over a 20-day period. Each data point is an average of five plots in two experiments. Vertical bars indicate ± SE of the mean.

populations on races 276 and 263. Moreover, the studies reported herein were made on the oat cultivar Markton, which is universally susceptible to crown rust, thus eliminating the possibility of selective influence of the host genotype on parasitic strains.

This research has dealt with the effect of some elements of parasitic fitness on the development of races 276 and 263. A similar approach was adapted by Katsuya and Green (5), Brown (2), Nelson (9), and Lesovoi (6).

Our results reveal that race 276 is superior to 263 in urediospore competitiveness in mixture (Table 1) and in urediospore viability over a period of hostless conditions (Table 2). The number of uredia and the number of spores per uredium produced by race 276 were higher than those produced by race 263 (Table 3). In field tests, infectivity and duration of urediospore yields for isolates of race 276 showed a higher rate of pathogenic development than those of race 263 (Figs. 1 and 2). In addition, the longevity of urediospore production of race 276 exceeded that of race 263, which began to produce telia earlier (Fig. 3). The cumulative effect of these factors over a number of generations may influence the capability of the pathogen to survive and propagate in *A. sterilis* populations.

Early detection and evaluation of the rate of parasitic fitness in virulent races could be an important element in designing breeding programs for disease resistance. Efforts to breed cultivars resistant to virulent races with low competitiveness are of lower priority than breeding cultivars resistant to virulent races with high parasitic fitness. The latter constitute a serious threat to cultivation of the crop involved. For example, the notorious race 15B of *P. graminis tritici* has become destructive only with the appearance of strains of high parasitic fitness (9) and does not present a serious problem in Canada, where its competitiveness is low. Lesovoi (6) has stressed that wheat leaf rust, race 77, has become dominant over other races with similar virulence, due to superior competitive ability. He also maintains that breeding should pay special attention to that race. Further research is needed to identify other factors that influence the competitive ability of strains of pathogens. As more knowledge is amassed, it may become possible to explain changes in the incidence of physiologic races of cereal rusts in the field more readily.

LITERATURE CITED

1. Brodny, U. 1980. Studies on the nature of mechanisms determining the composition of physiological race populations of *Puccinia coronata* Cda. var. *Avenae* Fraser & Ledingham on *Avena sterilis* L. in Israel. Ph.D. thesis, Tel Aviv University (in Hebrew, with English summary).
2. Brown, J. F. 1974. Factors affecting the relative ability of pathogens to survive in populations. Aust. Plant Pathol. Soc. Newsletter 3:44-45.
3. Edmonds, R. L. 1972. Collection efficiency of Rotorod samplers for sampling fungus spores in the atmosphere. Plant Dis. Rep. 56:704-708.
4. James, W. C. 1971. An illustrated series of assessment keys for plant diseases, their preparation and usage. Can. Plant Dis. Surv. 51:39-65.
5. Katsuya, K., and Green, G. T. 1967. Reproductive potentials of races 15B and 56 of wheat stem rust. Can. J. Bot. 45:1077-1091.
6. Lesovoi, M. P. 1979. Factors determining race dominance in the populations of brown rust pathogen on wheat. Mikologiia i fitopatologiia 13:131-136 (Russian).
7. Martens, J. W. 1973. Competitive ability of oat stem rust races in mixtures. Can. J. Bot. 51:2233-2236.
8. Mikhailova, L. A., and Metreveli, T. G. 1982. Competitiveness of the strains of wheat brown rust causal agent. Mikologiia i fitopatologiia 16:439-444 (Russian).
9. Nelson, R. R. 1972. The evolution of parasitic fitness. Pages 23-46 in: Plant Disease. An Advanced Treatise. Vol. 4. How Pathogens Induce Disease. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York.
10. Roane, C. W., Stakman, E. C., Loegering, W. Q., Stewart, D. M., and Watson, W. M. 1960. Survival of physiologic races of *Puccinia graminis* var. *tritici* on wheat near barberry bushes. Phytopathology 50:40-44.
11. Schein, R. D. 1964. Design performance and use of quantitative inoculator. Phytopathology 54:509-513.
12. Schein, R. D., and Rotem, J. 1965. Temperature and humidity effects on urediospore viability. Mycologia 57:397-403.
13. Sebesta, J. 1986. Plant pathologic and genetic fundamentals of wheat and oats breeding for resistance to rusts, powdery mildew and eyespot. Ph.D. thesis, Czechoslovakia Agricultural Academy, no. 41-03-9 (in Czech, with English summary).
14. Segal, A. 1981. Elements of resistance against *Puccinia coronata* Cda. var. *Avenae* Fraser & Ledingham and their integration in the defense structures of *Avena sterilis* L. populations in natural ecosystems undisturbed by man in Israel. Ph.D. thesis, Tel Aviv University (in Hebrew, with English summary).

15. Simons, M. D., and Michel, L. J. 1964. International register of pathogenic races of *Puccinia coronata* var. *avenae*. Plant Dis. Rep. 48:763-766.
16. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics: A Biometrical Approach. McGraw-Hill, New York.
17. Ted Brown Associates. 1976. Operating instructions for the Rotorod sampler. Technical manual no. 6. Ted Brown Associates, Los Altos Hills, CA.
18. Wahl, I. 1970. Prevalence and geographic distribution of resistance to crown rust in *Avena sterilis*. Phytopathology 60:746-749.
19. Wahl, I., Anikster, Y., Manisterski, J., and Segal, A. 1984. Evolution at the center of origin. Pages 39-77 in: The Cereal Rusts. Vol. I. W. R. Bushnell and A. P. Roelfs, eds. Academic Press, New York.