Density Dependent Sporulation of *Erysiphe graminis* f. sp. *tritici*

D. I. Rouse, D. R. MacKenzie, and R. R. Nelson

Department of Plant Pathology, University of Wisconsin, Madison 53706; Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge 70803; and Department of Plant Pathology, Pennsylvania State University, University Park 16802, respectively.

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ABSTRACT

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An exponential decrease in cumulative number of spores produced per colony as the number of colonies per primary leaf increased was observed for two isolates of *Erysiphe graminis* f. sp. *tritici* on plants of wheat cultivars Blueboy, Redcoat, and Knox. This relationship was observed beginning 8–14 days after inoculation depending on cultivar and isolate. The magnitude of the effect of colony numbers per leaf on spores per colony was measured by the slope of the regression of the logarithm of cummulative spores per colony versus colonies per leaf. The slope increased until 20 days after inoculation when spore production ceased. With the exception of isolate 112 on Knox, maximum spore production per primary leaf occurred when there were ~25 colonies per leaf. Additional colonies per leaf resulted in decreased total spore production. Thus, a mechanism of density dependent regulation of growth of *E. graminis* f. sp. *tritici* is reduction in spore production per colony as the number of colonies per leaf increase. Since this relationship was different for different isolate-cultivar combinations, spore production per colony as a function of number of colonies per leaf should be considered as a possible component of rate reducing resistance.

Additional key words: components of resistance, epidemiology, powdery mildew, slow mildewing.

Population biologists, including epidemiologists, are interested in factors regulating population growth rates and population size. Mechanisms of population regulation resulting from reduction of an individual's reproductive success due to increasing density of the population are referred to as being density dependent. Density dependent population growth may occur by two general mechanisms: adults may produce fewer progeny as population density increases, and progeny may have a reduced chance of reaching adulthood as the population becomes larger. For foliar diseases caused by fungal plant pathogens, the adult individual can be defined as a single pustule, colony, or lesion that produces spores as progeny.

When Vanderplank (14, page 20) formally introduced the logistic model and several extensions of it to plant pathologists, he identified the limitation in amount of healthy susceptible plant tissue as a means by which survival of progeny (spores) could be reduced as density of lesions increased. This is due to the decreasing chance a spore will land on uninfected plant tissue as the number of lesions increase. A spore that lands on diseased tissue and thus causes no increase in disease constitutes a multiple infection. Multiple infections do not directly reduce the number of offspring (spores) produced from already existing lesions. Rather, multiple infections reduce the chances that colonies developing from those spores will reach adult or reproductive age (will become sporulating lesions). Another mechanism of density dependent population regulation could be direct reduction in number of offspring produced by existing adults due to limitation of nutrient resources as the population increases. For example, the number of spores produced might decrease as increasing numbers of lesions competed for limited nutrient from the host. Alternatively, spore production per lesion might be reduced as number of lesions increased due to a host resistance response that was dependent on lesion density.

There are only a few reports in the literature relating fungal spore production per lesion to number of lesions present on a leaf. Yarwood (16) demonstrated that sporulation per pustule decreased with increased pustule density on primary bean leaves. His results were consistent with results obtained for bean trifoliolate leaves by Imhoff et al (3). Leonard (4) reported decreased sporulation per pustule of *Puccinia graminis* f. sp. *avenae* as the number of pustules per leaf increased. Leonard's (4) data suggest a negative exponential relationship between sporulation per pustule and number of pustules per leaf. Two preliminary studies have been conducted relating spore production of *Erysiphe graminis* f. sp. *tritici* to colony density (8; and R. Schein, *personal communication*).

The present study was undertaken to determine if sporulation of *E. graminis* f. sp. *tritici* was affected by the density of powdery mildew colonies on individual leaves. The specific objective was to quantify the relationship between number of conidia produced per colony versus number of colonies of powdery mildew on individual primary leaves over time.

MATERIALS AND METHODS

Isolates 85 and 112 of *E. graminis* f. sp. *tritici* used in this study were collected from commercial wheat fields in central Pennsylvania and maintained under lamp chimneys on seedlings of the cultivar Chancellor (CI 12333). Infected plants used as the source of inoculum for experiments were shaken briefly ~12 hr prior to their use to remove old conidia. Seedlings of Knox (CI 12798), Blueboy (CI 14031), and Redcoat (CI 13170) were grown in 10 × 20-cm flats in a soil mix containing soil, sand, and peat (2:1:1, v/v); 20 to 30 seeds of each of two cultivars were planted in two rows, one cultivar per row, the length of a flat and about 2 cm from the edge. All experiments were conducted in a single growth chamber at 18 ± 2 C and a photoperiod of 16 hr at ~14,000 lux.

Primary leaves of 8-day-old plants were inoculated in a settling tower by shaking individual infected leaves over the tower. Leaves to be inoculated were previously weighted at the tips of the leaf blades so they would lay horizontally, abaxial side upward, across a wire screen. Primary leaves of each of the cultivars were selected on the basis of similar size. Measurements of primary leaves of each of these cultivars grown under conditions similar to those described above were made with a leaf area meter (LiCor Inc., Lincoln, NE 68504). Estimates of mean leaf areas for cultivars Blueboy, Redcoat, and Knox were 342, 352, and 346 mm^2, respectively. The

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pooled standard error for the three cultivars was 11 mm^2. Differences in the inoculum dose per leaf and thus differences in numbers of colonies per leaf were established by covering all but two leaves with a paper shield and shaking a single infected leaf over the settling tower. After the conidia had settled, the shield was moved to expose two more leaves in addition to those initially exposed and another infected leaf was shaken over the settling tower. This process was repeated until the 16–20 primary leaves of each cultivar in a flat had been uncovered and inoculated. Six days after inoculation, plants in the flats were thinned to four plants of each cultivar (two cultivars per flat) with care being taken to leave plants with different numbers of colonies distributed randomly over the entire leaf surface. Six flats were randomized in a 90 × 20-cm area 20 cm from the walls of the growth chamber. A total of eight primary leaves of each cultivar-isolate combination were sampled with each leaf containing a different number of colonies of *E. graminis* f. sp. *tritici*.

Starting on the seventh day after inoculation, all conidia produced from colonies on each inoculated leaf were collected with a liquid impinger attached to a vacuum pump. The liquid impinger consisted of two 2-mm (inside diameter) glass tubes extending through a rubber stopper attached to a 25 × 200-mm test tube containing 5 ml of 0.3% Tween-80 solution. One glass tube was curved and slightly tapered at one end to facilitate spore collection. This tube extended inside the test tube to within 5 mm of the surface of the solution. The second glass tube was attached to a hose leading to the vacuum pump. After collecting a sample a small quantity of 0.3% Tween-80 solution was drawn through the sampler to remove conidia adhering to the wall of the glass tube. The spore suspensions were adjusted to a volume of 50 ml with a 1.0% NaCl solution and counted with a model B Coulter Counter (Coulter Electronics Industrial Division, 2601 North Mannheim Rd., Franklin Park, IL 60131). Conidia were collected between 0800 and 1000 hours 7, 8, 10, 11, 13, 14, 16, 17, 18, 19, and 20 days after inoculation. Conidia produced on days 9, 12, and 15 (when no samples were collected) were assumed to be collected in samples taken the following day. This assumption seemed reasonable since if appreciable numbers of conidia had been dislodged from the leaf surface, powdery mildew would have been expected to occur subsequently on healthy susceptible wheat plants placed in various parts of the chamber for monitoring the presence of airborne conidia. Also, examination of the data by plotting cumulative numbers of spores versus days after inoculation gave no indication that large numbers of conidia had been lost due to not sampling on days 9, 12, or 15.

A preliminary experiment was conducted with cultivars Blueboy and Redcoat and isolates 85 and 112 of *E. graminis* f. sp. *tritici* by using the procedures described above. Since these experimental procedures were adequate, the same procedures were used to obtain the experimental results presented below, including those for cultivar Knox as well as for Redcoat and Blueboy. Results of the two experiments were similar; therefore, only results of the second experiment that included Knox are reported below.

All data were expressed in terms of cumulative number of conidia produced per colony. Least-squares linear regression was used to analyze the relationship between cumulative number of spores per colony and number of lesions per leaf.

RESULTS

A negative exponential trend was evident in the relationship between number of colonies and cumulative number of conidia produced per colony. This is illustrated in Fig. 1 for isolate 85 of *E. graminis* on cultivar Knox. Linear, semilog, and log-log regression models were compared to determine the best means of expressing the relationship between spore production per colony and numbers of colonies per leaf for all isolate-cultivar combinations. All regressions were compared on the basis of level of significance of the regression (F-test), coefficients of determination (R^2), standard deviation of the slope, and analysis of the plot of residuals versus predicted values. It was determined that overall, the semilog model was most appropriate for describing the relationship between cumulative number of conidia produced per colony and number of colonies per leaf. Nevertheless, for isolate 85 on Blueboy the log-log model was marginally better and for isolate 112 on Knox the linear model was somewhat better. In both of these cases, the coefficient of determination was slightly lower (5–10%) for the semilog model (Table 1). For both isolate 85 on Blueboy and isolate 112 on Knox, however, residual plots for the semilog model were random, indicating an acceptable fit of the data. Also F-values were significant ($P \leq 0.05$) for the same days as the alternative model (the log-log model for isolate 85 on Blueboy and the linear model for isolate 112 on Knox). The relationship between number of colonies and $\log_e$ number of conidia produced per colony was not apparent 7 days after inoculation, but became statistically significant 8–14 days after inoculation depending on cultivar and isolate (Table 1).

Figure 2 presents the results of fitting the semilog regression model to the cumulative number of spores produced per colony versus the

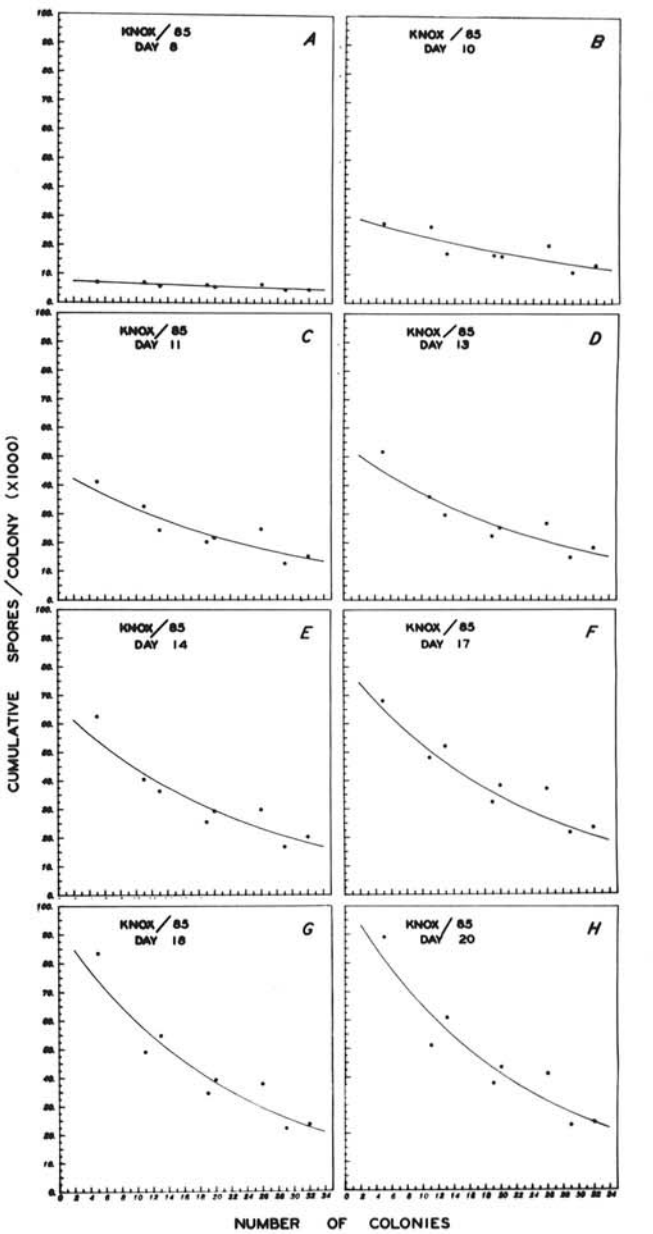

Fig. 1. Plot of cumulative spores per colony versus number of colonies per primary leaf for isolate 85 of *Erysiphe graminis* f. sp. *tritici* on cultivar Knox 8, 10, 11, 13, 14, 17, 18, and 20 days after inoculation. The solid line represents a least squares fit of the model: cumulative spores per colony = $k \cdot \exp(b_1$ [number of colonies per leaf]).

number of colonies per leaf for each cultivar-isolate combination. As days after inoculation increased, the effect on sporulation of number of colonies per leaf became more pronounced (Fig. 2). By 20 days after inoculation significant sporulation in all of the isolate-cultivar combinations had ceased. The slope of the semilog regression equations became progressively more negative over time for all cultivar/isolate combinations (Table 1). Cumulative numbers of spores produced and rate of sporulation (cumulative number of spores produced per day) were affected by cultivar and isolate regardless of number of colonies present on leaves. For example, isolate 85 produced fewer spores than isolate 112 on each of the cultivars. This effect was more pronounced when there were only a few colonies per leaf. The combination of isolate 112 on Knox was unique in that regression coefficients (b_1) for cumulative sporulation per colony versus number of colonies per leaf were much larger than for any of the other isolate-cultivar combinations (Table 1).

Multiplication of number of colonies times number of spores produced per colony as estimated from the semilog regression model provided an estimate of total spore production per leaf. For all of the cultivar/isolate combinations except isolate 112 on Knox, maximum spore production per leaf occurred with ~25 colonies per leaf (Fig. 3). Maximum spore production per leaf ranged from 3.4×10^6 for isolate 112 on Knox to 0.4×10^6 for isolate 85 on Redcoat.

DISCUSSION

Rates of sporulation and sporulation capacities (total number of spores a colony can produce during its infectious period sensu Vanderplank [14, pages 97–99]) for leaves with small numbers of colonies (≤5) were similar to those reported in a previous study using the same isolates of *E. graminis* and the same wheat cultivars (11). These sporulation capacities are higher than those reported by Pady et al (7) but they obtained data for only 8 days from adult wheat plants under unspecified conditions. The environmental conditions for conidial production were close to optimal in this study (15). Ward and Manners (15) reported sporulation rates of 4,000–5,000 conidia per day 9 days after inoculation for single colonies per leaf of *E. graminis* under optimal conditions. Comparison of their results with spore production on leaves with less than five colonies in the present study indicated similar sporulation rates.

There are several potential explanations for the observed relation between sporulation per colony and number of colonies of *E. graminis* f. sp. *tritici* on a leaf. One of these is that colony size might be inversely related to number of colonies on a leaf. The six isolate-cultivar combinations used in this study each produced a reaction type rating of 4 based on the standard 0–4 scale of Mains and Dietz (5). This scale is based on size of colonies (abundance of mycelium). Colony size of these isolates on primary leaves of Redcoat, Blueboy, and Chancellor had been previously estimated with a digitizer (Numonics Corp., Hancock St., North Wales, PA 19454). Colony size was found to vary between 5 and 8 mm^2 on each cultivar. Individual leaves of each cultivar from which colony size measurements were taken contained from 4 to 50 colonies. The correlation between number of colonies and average size of colonies on a leaf was low ($r = 0.10$). Although exact measurements were not made during the experiment reported herein, there was no observable trend of decreasing colony size with increasing numbers of colonies per leaf.

TABLE 1. Regression coefficients and associated statistics[y] for regression of $\log_e$ (cumulative number of spores produced per colony of *Erysiphe graminis* f. sp. *tritici*) versus number of colonies per primary leaf for each of three cultivars of wheat and two isolates of the fungus

Cultivar	Day after inoculation	Isolate 85					Isolate 112				
		b_0	b_1	F	R^2	S_{b1}^2	b_0	b_1	F	R^2	S_{b1}^2
Knox	7	1.38	−0.009	3.43	25.7	0.005	1.59	0.018	5.01	36.4	0.008
	8	2.05	−0.014	11.81*[z]	60.7	0.004	2.23	0.012	10.90*	58.6	0.004
	10	3.44	−0.028	12.35*	61.9	0.008	3.99	−0.004	3.76	28.3	0.004
	11	3.82	−0.036	21.79*	74.8	0.007	4.45	−0.012	14.23*	65.4	0.003
	13	3.99	−0.038	27.20*	78.9	0.007	4.63	−0.014	14.54*	65.9	0.003
	14	4.20	−0.040	33.29*	82.2	0.007	4.78	−0.015	12.86*	62.9	0.004
	16	4.40	−0.043	37.76*	84.0	0.007	4.95	−0.017	12.35*	61.9	0.004
	17	4.47	−0.043	42.80*	85.7	0.006	5.03	−0.017	11.04*	58.9	0.005
	18	4.53	−0.044	47.53*	86.9	0.006	5.08	−0.018	10.17*	56.7	0.005
	19	4.56	−0.045	45.52*	86.4	0.006	5.15	−0.019	10.45*	57.5	0.006
	20	4.63	−0.045	43.88*	86.0	0.007	5.18	−0.019	10.53*	57.6	0.006
Redcoat	7	0.04	−0.010	0.60	6.0	0.013	0.35	0.0134	1.98	12.3	0.0095
	8	0.62	−0.013	0.59	6.2	0.017	1.16	0.0029	0.277	−11.5	0.0056
	10	1.91	−0.011	0.70	4.5	0.013	2.50	−0.0009	0.127	−14.3	0.0026
	11	2.33	−0.012	2.88	21.2	0.007	3.16	−0.0104	10.08*	56.5	0.0033
	13	2.56	−0.013	5.04	36.6	0.006	3.51	−0.0158	18.73*	72.0	0.0036
	14	2.77	−0.016	7.51*	48.2	0.006	3.90	−0.0227	27.52*	79.1	0.0043
	16	3.25	−0.026	34.44*	82.7	0.004	4.43	−0.0315	32.91*	82.0	0.0055
	17	3.46	−0.031	34.13*	82.6	0.005	4.60	−0.0344	35.23*	83.0	0.0058
	18	3.59	−0.034	33.42*	82.2	0.006	4.77	−0.0374	35.80*	83.3	0.0062
	19	3.74	−0.039	27.13*	78.9	0.007	4.88	−0.0396	35.92*	83.3	0.0066
	20	3.80	−0.041	27.21*	78.9	0.008	4.93	−0.0405	36.60*	83.6	0.007
Blueboy	7	0.50	0.0104	0.87	−2.0	0.0112	1.50	0.0103	6.05	41.9	0.00418
	8	1.14	0.0064	0.38	−9.6	0.0103	1.95	0.0039	0.62	−5.7	0.0049
	10	2.91	−0.0182	3.71	27.9	0.0094	3.99	−0.0196	6.91	45.8	0.0075
	11	3.36	−0.0258	9.86*	55.9	0.008	4.41	−0.0246	11.58*	60.2	0.0072
	13	3.55	−0.0286	11.80*	60.7	0.0083	4.63	−0.0276	17.74*	70.5	0.00656
	14	3.71	−0.0308	12.27*	61.7	0.0088	4.86	−0.0321	20.92*	74.0	0.00702
	16	3.91	−0.0343	17.12*	69.7	0.0083	5.08	−0.0357	27.58*	79.2	0.0068
	17	4.01	−0.0359	18.25*	71.1	0.0084	5.21	−0.0379	29.62*	80.3	0.00697
	18	4.09	−0.0375	21.07*	74.1	0.0082	5.29	−0.0394	31.00*	81.1	0.0071
	19	4.14	−0.0384	21.81*	74.8	0.0083	5.37	−0.0409	33.48*	82.3	0.0071
	20	4.16	−0.0386	21.87*	74.9	0.0083	5.39	−0.0413	33.51*	82.3	0.00714

[y] Statistical parameters: b_0 = intercept, b_1 = slope, F = calculated value of the F-statistic, R^2 = coefficient of determination, and S_{b1}^2 = standard error of slope.
[z] Indicates significance at $P \leq 0.05$.

Another possible explanation for reduced sporulation per colony as number of colonies per leaf increase is nutrient limitation. *E. graminis* f. sp. *tritici* obtains all of its nutrients from its host. The host's ability to assimilate and translocate nutrients to haustoria could be assumed to be limited. In that case, reduction in spore production with increasing numbers of haustoria or colonies might be due to competition for nutrient both with other haustoria and with plant cells. This food base must be utilized by the fungus for vegetative growth, reproductive growth, and maintenance respiration. Thus, an explanation for the observed decrease in total spore production per leaf when >25 colonies per leaf are present is that all available nutrient from the leaf is being sequestered and that some portion of the substrate used for reproductive growth when there are <25 colonies present is used for maintenance respiration and vegetative growth of additional vegetative fungal biomass if >25 colonies are present. Nutrient limitation could become more important also as photosynthesis was reduced as a result of colonies directly blocking light or by decreased assimilate availability to the plant. Net CO_2 assimilation has been shown to decrease during sporulation of several powdery mildew fungi (1,2,6).

Another possible explanation of reduced sporulation per colony as number of colonies per leaf increases is that host defense mechanisms are turned on as a result of the increasing density of infections or subsequent colonies. In that case, the observed response might be referred to as an induced resistance response.

The quantification of sporulation is an important step in understanding the epidemiology of polycyclic diseases. Rate of sporulation, duration of sporulation, and total spore production are a few of the aspects of sporulation that influence disease progression. The relationship between spore production per lesion and number of lesions per leaf also represent an important aspect of sporulation influencing disease progression. Maximum spore production per leaf was reached in five of the six isolate-cultivar combinations when ~25 colonies were present. This is approximately one-third the total number of colonies that could be accommodated on the surface of the primary leaves of the wheat cultivars used in this study assuming an average colony size of 5 mm^2 and a leaf area of 350 mm^2. This could be particularly important if lesions tended to be aggregated. Powdery mildew of wheat has been shown to have a negative binomial distribution of colonies per leaf implying that leaves with large numbers of colonies occur in the field more frequently than would be expected at random (10).

Several researchers have emphasized the role sporulation might play as a component of rate-reducing resistance. If the effects of lesion or colony density on sporulation are large enough and are not accounted for when different numbers of lesions or colonies are present on leaves of plants being compared for sporulation, it is possible that erroneous conclusions could be drawn from the data. Also, if sporulation capacity is a function of number of colonies on leaves, then the functional relationship between numbers of spores produced per colony and number of colonies per leaf may be considered a component of rate-reducing resistance. The wheat cultivar Knox has been reported to be slow mildewing (9,12). Slow mildewing of Knox has been attributed to reduced infectability and reduced spore production (13). There is a clear difference between

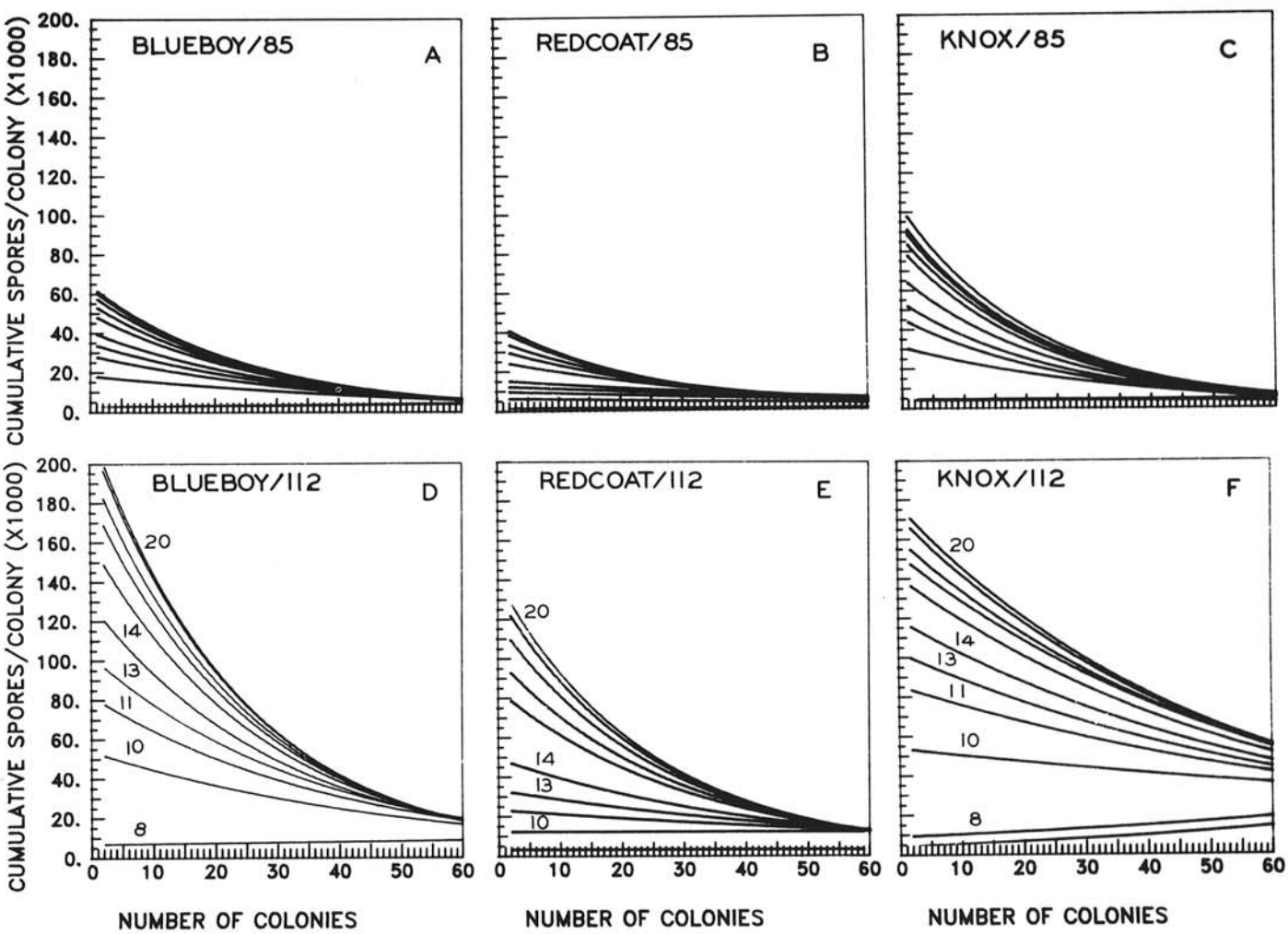

Fig. 2. Fitted semilog regression curves of cumulative spores per colony versus number of colonies per primary leaf 8, 10, 11, 13, 14, 16, 17, 18, 19, and 20 days after inoculation for each of the six combinations of isolates 85 and 112 of *Erysiphe graminis* f. sp. *tritici* on wheat cultivars Blueboy, Redcoat, and Knox.

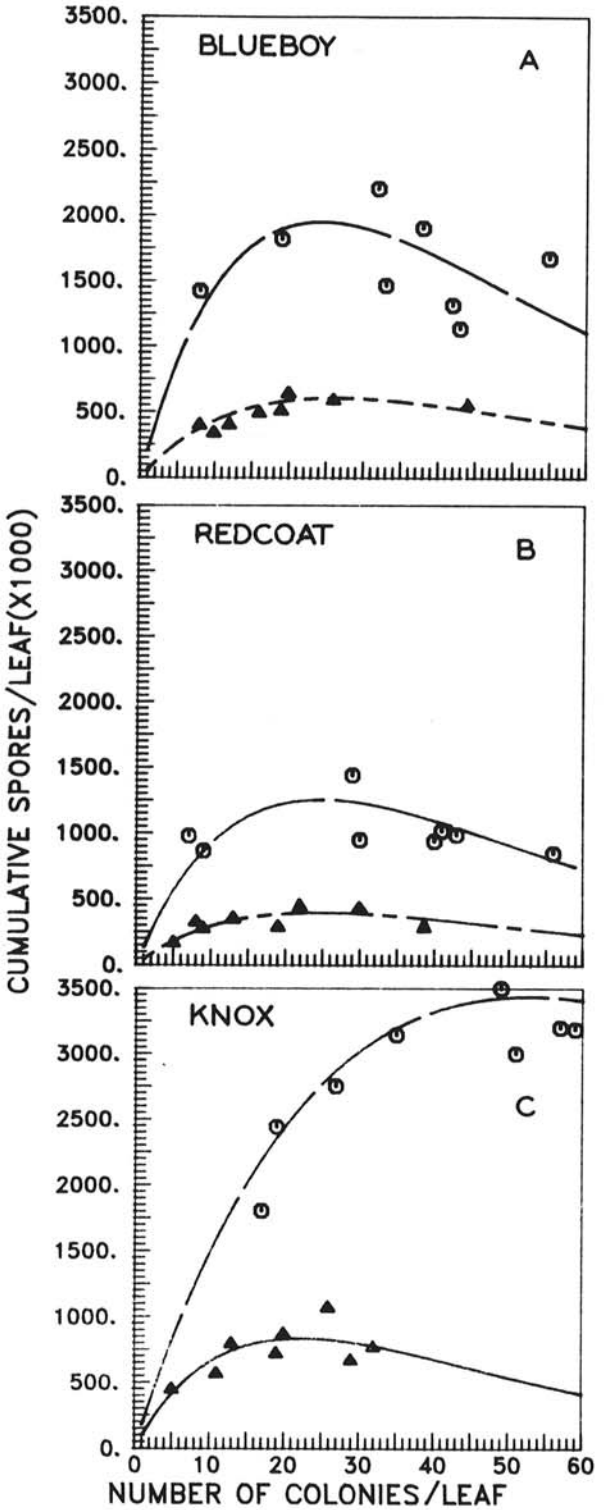

Fig. 3. Relationship between cumulative number of spores of *Erysiphe graminis* f. sp. *tritici* produced per leaf 20 days after inoculation and the number of colonies of isolates 85 (Δ) and 112 (o) on primary leaves of the wheat cultivars Blueboy, Redcoat, and Knox.

the high level of resistance of adult plants of the cultivar Knox and the apparently low level of resistance found on primary leaves of seedlings of this cultivar. In the present study, isolate 112 on Knox had a unique property associated with sporulation capacity compared with the other isolate-cultivar combinations. The regression coefficients for the semilog model of cumulative spore production per colony versus number of colonies per primary leaf for isolate 112 on Knox 20 days after inoculation were much higher than for any of the other isolate-cultivar combinations. These results reflect the much greater spore production of isolate 112 on Knox than on Blueboy or Redcoat at high colony densities. If cultivars were developed in which this phenomenon occurred on adult plants where rate reducing resistance is manifested, resistance might break down under conditions of severe disease pressure.

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