Components of Rate-Reducing Resistance in Seedlings of Four Wheat Cultivars and Parasitic Fitness in Six Isolates of *Erysiphe graminis* f. sp. *tritici*

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

Graduate student, Evan Pugh professor, associate professor, and laboratory technician, respectively, Department of Plant Pathology, The Pennsylvania State University, University Park 16802.
Current address of senior author: Department of Plant Pathology, University of Wisconsin, Madison 53706.

ABSTRACT

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Sporulation capacity and infection efficiency of six isolates of *Erysiphe graminis* f. sp. *tritici* collected in central Pennsylvania were measured on winter wheat cultivars Blueboy, Redcoat, and Knox. Only sporulation capacity was measured on a fourth cultivar, Vermillion. Sporulation capacity was measured as the cumulative number of conidia sampled from individual colonies on primary leaves at 24-hr intervals and was estimated by the parameter X_m in the logistic equation by using a nonlinear regression routine. Differential interactions were found between isolates and cultivars where the ranking of isolates changed by cultivar. Infection efficiency was determined by counting the number of conidia out of groups of ten that had formed elongating secondary hyphae 36–48 hr after inoculation. Two-way analysis of variance identified significant isolate by cultivar interactions for infection efficiency. The change in ranking and the significant statistical interaction in the analysis of variance of isolates by cultivars with respect to sporulation capacity and infection efficiency demonstrates genetic variability for these traits in wheat and *E. graminis* and indicates the possibility for erosion of slow mildewing resistance.

Additional key words: sporulation capacity, infection efficiency, powdery mildew of wheat, *Triticum aestivum*, horizontal resistance.

Several lines of wheat have been identified as possessing slow-mildewing resistance (eg, 13). This type of resistance could result from the single or combined effects of: increasing the latent period (the time from penetration of a hypha from a germinated spore to the production of spores from the resultant colony), decreasing the sporulation capacity (the number of spores produced during the life time of a colony, measured as the rate of cumulative sporulation over time) and the infection efficiency (the proportion of spores successfully completing the infection process). Shaner (13,15) identified the components of slow-mildewing on adult plants of wheat cultivar Knox and found that sporulation capacity and infection efficiency on Knox were reduced compared with a sister selection Vermillion while latent period was not affected by either cultivar. Redcoat, a soft red winter wheat cultivar grown widely in Pennsylvania, appears to be slow-mildewing in the field. However, the components of its resistance have not been characterized.

The longevity of slow-mildewing may depend in part on whether there is a differential interaction between genes conditioning the components of slow-mildewing in the host and genes conditioning the components of parasitic fitness in the pathogen. The interaction term in a two-way analysis of variance provides one method of statistically testing interactions between host and pathogen. A statistically significant change in the ranking of isolates on cultivars provides a second method. Studies of several diseases have demonstrated differential interactions with respect to the components of nonhypersensitive, partial, or field resistance and parasitic fitness (5–8,11,17).

In this study, comparisons of relative sporulation capacity, rate of cumulative sporulation, infection efficiency, and latent period were made among several isolates of *Erysiphe graminis* DC f. sp. *tritici* em Marchal (Egt) and four winter wheat cultivars Knox, Vermillion, Redcoat, and Blueboy. In a preliminary study, statistically significant interactions were found for sporulation capacity, rate of cumulative sporulation, and latent period between several isolates of Egt and wheat cultivars Chancellor, Blueboy, and Baart (12).

MATERIALS AND METHODS

Isolates 10, 19, 66, 85, 112, and 136 of Egt 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). When rated on a scale of 0–4 similar to that proposed by Finkner et al (3) these isolates produced essentially identical reaction types on the cultivars Knox, Vermillion, Redcoat, and Blueboy with the exception of isolate 66 on Redcoat which produced reaction type 1. None of these cultivars contains any known resistance genes. The isolates were tested on a set of near-isogenic wheat lines containing one each of the known genes for resistance to powdery mildew. The six isolates represented several races.

Infection efficiency. Eight-day-old seedlings of each of the cultivars Knox (CI 12798), Vermillion (CI 13080), Redcoat (CI 13170), and Blueboy (CI 14031) planted in 5-cm diameter pots in a mixture of soil, peat, and sand (2:1:1, v/v) were inoculated separately with Egt isolates 85 and 112 in a settling tower by shaking infected leaves over the tower. Plants used as the inoculum source were shaken approximately 12 hr prior to their use to remove old conidia. By selecting leaves with the same number of mildew colonies and by using the settling tower, approximately uniform levels of conidia were deposited on inoculated leaves. Inoculated plants of each cultivar were placed in a growth chamber at 18 C for 36–48 hr with a 16-hr photoperiod at a light intensity of approximately 14,000 lux. Primary leaves of inoculated plants were subsequently removed and taped to glass slides with the upper leaf surface firmly against the glass to minimize loss of ungerminated conidia during subsequent staining with 0.1% lactophenol cotton blue. Several leaves were checked before and after staining to confirm that ungerminated conidia were not washed off the upper leaf surface.

Infection efficiency was determined as the mean number of conidia in 150 groups of 10 conidia that had formed elongating secondary hyphae on the upper leaf surface 36–48 hr after inoculation. The groups of 10 conidia were selected randomly from inoculated leaves until 150 groups had been counted. Ten groups of these 10 conidia counts were averaged to obtain 15 replications

from the original 150 groups of 10 conidia. These 10 groups were often but not always naturally associated by having been selected from the same leaf. Approximately 20 leaves were examined for each cultivar-isolate combination. The data were collected in this way to make possible the estimation of the discrete distribution of infection efficiency that was subsequently used in another paper (13). The data were analyzed using a two-way analysis of variance and paired comparisons of the paired means for each cultivar-isolate combination (10, p. 474). The experiment was repeated twice and the same relationships were observed between cultivar-isolate combinations in each experiment.

Sporulation. Primary leaves of 8-day-old seedlings of Knox, Vermillion, Redcoat, and Blueboy were weighted at the tips of the leaf blades so they would lie horizontally across a wire screen. The upper leaf surface was inoculated with one of the six Egt isolates using the procedure of shaking infected leaves over them as described above. Inoculated plants were placed in a growth chamber at 18 C with a photoperiod of 16 hr and a light intensity at canopy height of approximately 14,000 lux. Between 10 and 15 plants were inoculated per replicate for each cultivar-isolate combination so that plants with similar small numbers of distinct colonies could be selected for subsequent sampling. A single
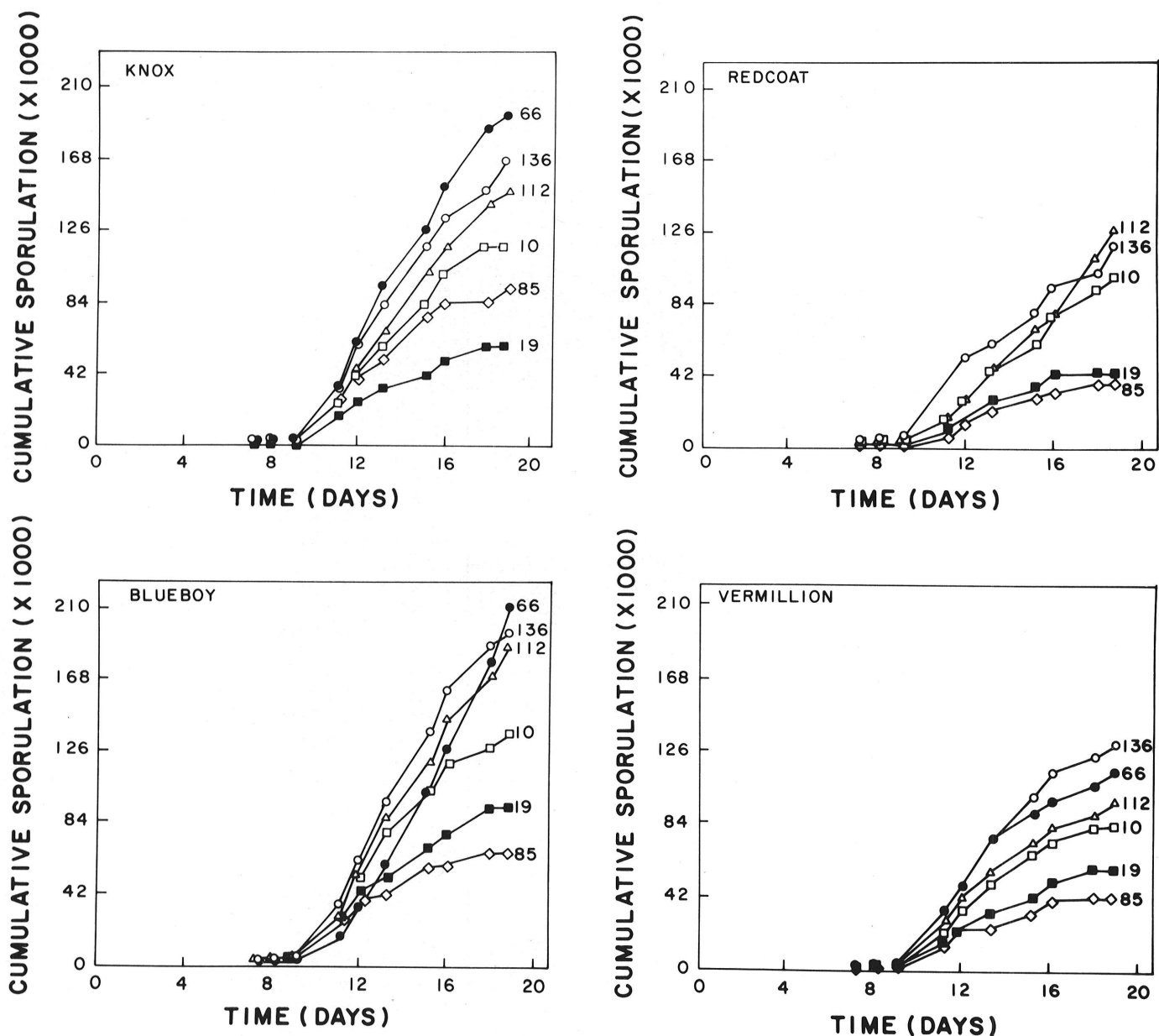
TABLE 1. Infection efficiency of two isolates of *Erysiphe graminis* f. sp. *tritici* on primary leaves of the winter wheat cultivars Knox, Redcoat, and Blueboy

Cultivar	Infection efficiency[a]	
	Isolate 85	Isolate 112
Knox	1.59 c[b]	2.75 d
Redcoat	2.77 d	4.53 g
Blueboy	4.12 f	3.13 e

[a] Infection efficiency is calculated as the mean number of conidia (of ten observed) that formed elongating secondary hyphae. Data are means of 15 replications.

[b] Letters indicate significant differences in values for infection efficiency ($\alpha = 0.05$) by Tukey's multiple pairwise comparison test.

Fig. 1. Cumulative sporulation per colony of six isolates of *Erysiphe graminis* f. sp. *tritici* on primary leaves of the winter wheat cultivars Blueboy, Redcoat, Knox, and Vermillion. Data points represent the averages of three individual colonies. Time measured in days after inoculation.

individual colony from primary leaves with between two and five colonies on them was sampled every 24 or 48 hr starting 7 days after inoculation and ending 19 days after inoculation. Conidia from individual colonies were collected with a vacuum pump into test tubes containing a 0.3% Tween-80 solution. 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). The experiment was repeated twice with two colonies (replicates) per sampling per cultivar-isolate combination in the first experiment and three colonies (replicates) per cultivar-isolate combination in the second experiment. Data were analyzed by using a nonlinear regression procedure (1) to fit cumulative sporulation versus time to the logistic growth model.

Latent period. Inoculated leaves for each cultivar-isolate combination were visually observed every 4 hr from the time macroscopic signs first appeared until colonies were clearly sporulating. Vacuum sampling of individual colonies 5 and 6 days after inoculation also was attempted.

RESULTS

Infection efficiency. The lowest infection efficiency for each isolate occurred on the cultivar Knox. Blueboy was more susceptible than Redcoat to isolate 85 but the reverse was true for isolate 112 (Table 1). The two-way analysis of variance confirmed a significant interaction between isolates and cultivars. Paired comparisons were significant for isolates on each cultivar (Table 1).

Sporulation. Plots of cumulative sporulation versus time were sigmoid for each cultivar-isolate combination (Fig. 1). A nonlinear

least squares regression produced a statistically significant fit to the logistic model for all sporulation data. The logistic model used in this study is stated as:

$$S = S_m S_0 / [S_0 + (S_m - S_0) e^{-rt}]$$

in which S = cumulative number of spores at time t, S_0 = initial sporulation, r = rate of cumulative sporulation, and S_m = maximum cumulative sporulation (= sporulation capacity). The confidence intervals generated for S_m (= sporulation capacity) and r were used to compare cultivar-isolate combinations for significance. Several of the cultivar-isolate combinations in Fig. 1 do not appear to have completed their infectious period (ceased sporulating) by the end of the 19th day after inoculation. In specific instances this may have been due to the proximity of daughter colonies that had begun to sporulate at the margin of the initial colony being sampled; however, in the majority of instances sporulation had ceased by day 20 based on visual observations. Thus, differences were not observed in the length of the infectious period due to prolonged sporulation by old colonies. There were several changes in the ranking of isolates with respect to sporulation capacity on different cultivars indicating a differential interaction between isolates and cultivars for sporulation capacity. For example, isolates 112 and 136 differentially interacted with Knox and Redcoat. These interactions were statistically significant but small in magnitude. Isolates 66, 112, and 136 produced more conidia on Blueboy and Knox than did isolates 10, 19, and 85. Similarly, the cultivars differed in overall effectiveness to reduce sporulation; cultivar Redcoat significantly reducing sporulation capacity of all isolates compared to Knox and Blueboy (Fig. 2).
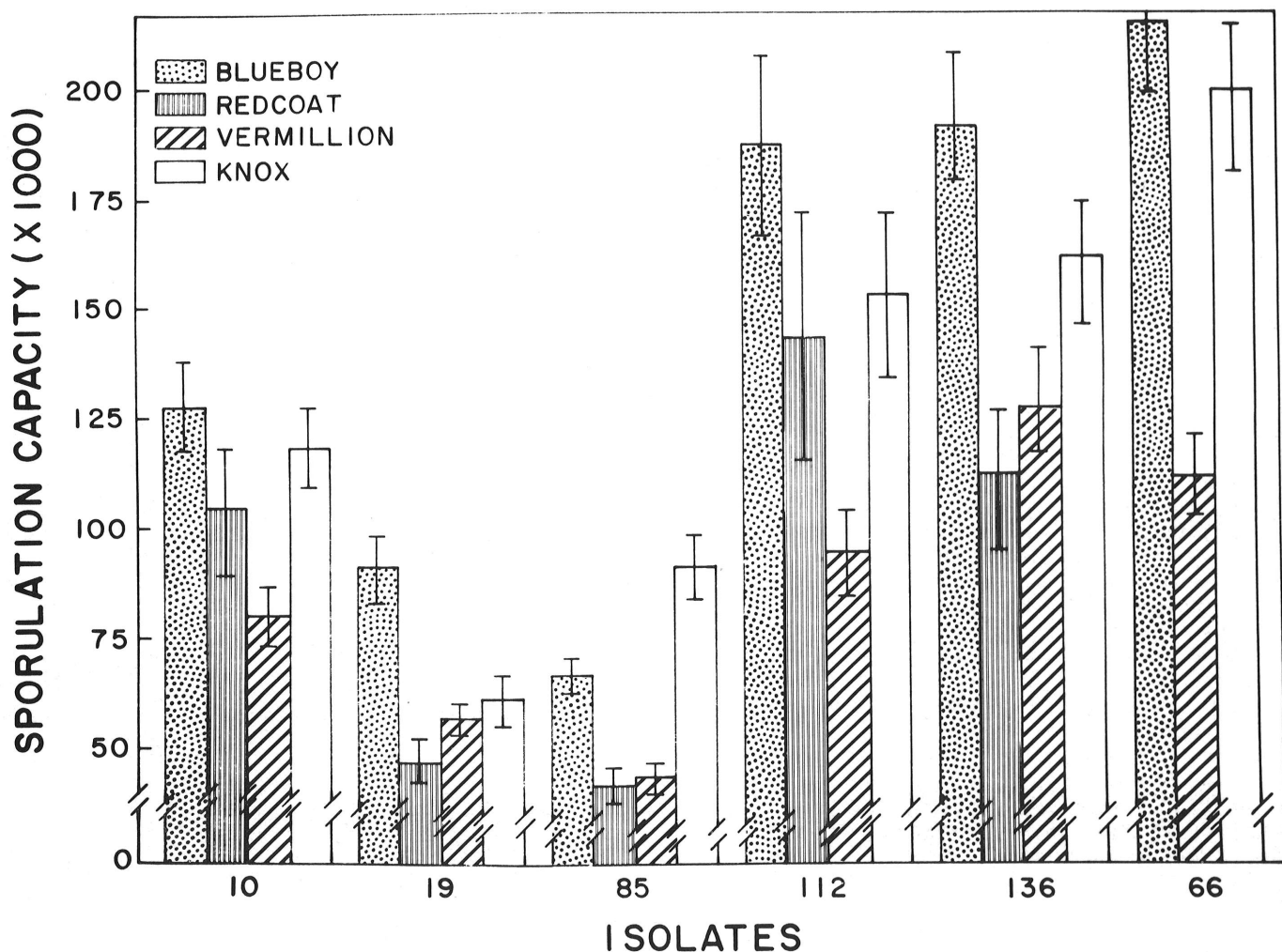

Fig. 2. Sporulation capacity (total number of spores produced per colony) of six isolates of *Erysiphe graminis* f. sp. *tritici* on primary leaves of the winter wheat cultivars, Blueboy, Redcoat, Knox, and Vermillion. The 95% confidence interval is shown for each cultivar-isolate combination.

Vermillion also significantly reduced sporulation as compared with Knox and Blueboy with the exception of isolate 19 (Fig. 2). Isolate 66 produced a hypersensitive reaction on Redcoat suggesting the presence of an unidentified race-specific resistance gene in Redcoat which has no known *Pm* genes for resistance to powdery mildew.

The rate of cumulative sporulation differed between some isolates and cultivars although a significant difference in ranking of isolates on cultivars was not observed. Redcoat reduced the rate of cumulative sporulation to a greater overall extent than did the other cultivars for all isolates.

Latent period. The parameter S_0 in the equation used to describe cumulative sporulation represents initial sporulation and could be used in the fitted equation to determine the time when sporulation began and thus the latent period. Estimated time to the calculated S_0 did not differ significantly for cultivars or isolates. This was in agreement with visual estimates of the latent period. Although visual estimation of the occurrence of the first mature conidia in a mildew colony is somewhat subjective in itself, it also was consistent with the failure to obtain countable numbers of conidia from colonies of all cultivar-isolate combinations by vacuuming prior to 6 days, whereas on day 7 countable numbers of conidia were obtained for all cultivar-isolate combinations. Given the precision of our techniques, we conclude that all the cultivar-isolate combinations in this study had latent periods within 24 hr of each other.

DISCUSSION

In the field Redcoat and Knox possess a high level of slow-mildewing resistance compared to Blueboy and Vermillion. However, the major rate-reducing factors in Redcoat and Knox as measured on seedlings differ. Redcoat affects infection efficiency, rate of cumulative sporulation, and sporulation capacity depending on the isolate, while Knox reduces infection efficiency. Shaner (16) showed that sporulation on adult Knox plants is significantly less than on Vermillion. Presumably, the difference between our results (which show Vermillion with reduced sporulation compared with Knox) and those of Shaner (16) is due to differences between seedlings and adult plants. This suggests that care must be taken in developing screening methods to estimate components of slow-mildewing resistance. Our results indicate that the use of screening methods involving seedlings alone could be quite misleading. The different combinations of traits affecting slow-mildewing suggest that improvement in the level of resistance could be made in a breeding program by identifying these components and making the appropriate crosses to combine them.

The interaction of components of slow-mildewing resistance and parasitic fitness indicate that the resistance could at least to some extent erode over time. Available evidence suggests that factors determining the components of resistance that act to reduce the rate of epidemic development often are quantitatively inherited (9). Similarly, it has been suggested that components of parasitic fitness in fungal pathogens are quantitatively inherited (4). A hypothesis based on experiments on a limited number of other host-parasite systems is that the components of slow-mildewing resistance and parasitic fitness of Egt are quantitatively inherited. Thus, while differential interactions have been demonstrated in this study between some of these components, erosion of this type of resistance should be relatively slow compared to that observed for single-gene or vertical resistance because, in a quantitatively inherited genetic system, changes would need to occur in several-to-many loci before a large change would occur in the level of resistance. This is purely conjecture, since the inheritance mechanism for slow-mildewing resistance actually is not known. Statistically significant interactions between elements of rate-reducing resistance and parasitic fitness have been demonstrated in other host-pathogen combinations (2,5–8,11,12,17).

The differential interactions demonstrated in this study show that slow-mildewing resistance cannot be equated with horizontal resistance sensu Vanderplank (18,19). Nevertheless, slow-mildewing resistance reduces the rate of disease progress (15), which is an attribute of horizontal resistance sensu Vanderplank

(18, p. 184). Recently, Vanderplank (20) introduced the term "vertical resistance without host-pathogen specificity" to describe the case in which the ranking order of cultivars for nonspecific resistance changed for different isolates of the pathogen. Vertical resistance without host-pathogen specificity and with its rate-reducing abilities and differential interactions seems to describe the powdery mildew resistance of cultivars Redcoat and Knox. Alternatively, the suggestion by Nelson (9) that horizontal resistance can be defined epidemiologically seems appropriate. In that sense, Knox and Redcoat possess horizontal resistance which is demonstrated by effects on infection efficiency, sporulation capacity, and rate of cumulative sporulation which reduce the rate of disease development.

This study indicates that Egt possesses the inherent genetic ability to adapt to cultivars possessing rate-reducing attributes by selection for greater infection efficiency and sporulation capacity. Future research designed to monitor subtle population shifts toward improved parasitic fitness on specific cultivars is needed to assess the practical importance of this adaptation to overcome rate-reducing resistance.

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