Genetics

Genetic Control of Ethirimol Resistance in a Natural Population of *Erysiphe graminis* f. sp. *hordei*

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ABSTRACT

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Resistance in field isolates of *Erysiphe graminis* f. sp. *hordei* to the fungicide ethirimol is controlled, not by one major gene, but by a complex heritable system in which the genetic factors involved are primarily additive. Particularly favorable gene combinations established in resistant genotypes may be broken through recombination, leading to some breakdown of resistance. No evidence was obtained for linkage between resistance to ethirimol and three virulence genes in the pathogen. The implications of the use of ethirimol in disease control strategies are discussed.

Additional key words: quantitative inheritance.

Ethirimol (2-ethylamino-4-hydroxy-5-*n*-butyl-6-methylpyrimidine) has been used throughout Britain since 1970 as a seed treatment to control barley powdery mildew (which is caused by *Erysiphe graminis* D.C. f. sp. *hordei*). Following reports of resistance in 1971 (17), surveys were conducted that established the extent to which the mildew population varied in its response to ethirimol (8,15,17). Many levels of resistance were found, but significant differences between single-pustule isolates suggested that genetic factors were involved. These isolates were stable and prolonged culture on treated plants did not alter their responses to ethirimol (8). However, within each isolate the range of response

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was wide and, although the bioassays used by different workers were not alike, shallow dose/response lines always were produced (7,15,16).

Fungal resistance to chemicals frequently is controlled by a few readily identified genes (6), although often this resistance is induced in the laboratory rather than obtained from field populations. Occasionally more complex heritable systems are involved (2,11), similar to those that control antibiotic production (12), growth (14), and pathogenicity (3). How many loci control resistance and how these interact may well influence the resistance response in field situations.

Therefore, I examined the genetics of resistance to ethirimol to try to explain why, despite widespread resistance within field populations, disease control is still possible.

MATERIALS AND METHODS

Mildew cultures. Cultures were obtained as single-pustule isolates from independent field populations collected 1973–1977 (Table 1), when use of ethirimol in England was widespread (9). None of these cultures was obtained from populations selected by cultivars with corresponding resistance genes, but DH 4, DH 14, and DH 19 were all isolated from the ethirimol-treated crops. Cultures were maintained on detached primary barley leaves (cultivar Proctor) as described elsewhere (7), and every 10 days transferred to fresh leaf material by using a small paintbrush. Resistant cultures readily infected plants grown from seed treated with commercial rates of the fungicide. Assuming a gene-for-gene relationship between the powdery mildew pathogen and barley (18), the reaction of each culture on several differential barley cultivars was used to identify pathogen virulence genes used in the genetic work (Table 1), although a wider set of differentials was used periodically as a check on contamination.

Crosses. The techniques were based on earlier reports of successful crosses using *E. graminis* (see 18 for references). Two compatible cultures were inoculated onto 4-wk-old Proctor plants growing in a suitably adapted isolated propagator (10). Cleistothecia formed 3–4 wk later and were removed when the leaves had senesced. After at least 1 mo at 4 C, cleistothecia were surface sterilized in 1% sodium (w/v) sodium hypochlorite, rinsed in sterile distilled water, and spread over moist filter paper. These papers were attached inside the lid of a clear polystyrene box (Stewarts Plastics, Croydon, U.K.) that contained detached leaves lying on water agar (OXOID No. 3, 5 g/L). When kept moist, cleistothecia began to dehisce after 5 days at 15 C in continuous light. The lid was then transferred to cover fresh leaf material every 5 days. Ascospores germinated to form colonies that were cloned to provide sufficient material for bioassay. Analysis of virulence genes (13) in the progeny showed that recombinants occurred in the expected frequencies, and that selfing was unlikely. Resistance to ethirimol apparently did not affect the viability of ascospores. Progeny sizes were limited by the numbers produced from cleistothecia, and by what could be handled in each bioassay.

Bioassays. For in vitro bioassays (7), young conidia were germinated for 24 hr on cellophane membranes overlaying nutrient agar incorporating different concentrations of ethirimol. Responses were assessed by measuring germ-tube length at each dose, and ED_{50} values were calculated from the logistic curve of these lengths against the logarithm of the dose. Progeny were arranged in a completely randomized design during inoculum production, and all progeny from a particular cross were assayed together, on two separate occasions, each constituting one replication. The same ethirimol-sensitive standard also was assayed on each occasion, and where differences in sensitivity were significant, progeny ED_{50} values were adjusted accordingly. In the analysis of variance, each ED_{50} was weighted according to its particular standard error, although this seldom differed significantly between progeny. ED_{50} values are expressed as logarithms ($\log_{10} \mu g \cdot ml^{-1}$) that have been multiplied by 10^3 to avoid use of bar numbers. Only in Table 1 are values given in micrograms per milliliter.

RESULTS

Selection for altered ethirimol sensitivity in asexually reproducing clones. Eight clones were established from an ethirimol-sensitive culture (DH 16). Bioassays revealed that these did not differ greatly from each other and that the mean ED_{50} value of all clones was similar to that of DH 16. Nevertheless, six second-generation clones were selected from the most resistant clone and six more were selected from the most sensitive one. These two groups did not differ when bioassayed (Fig. 1), and the mean ED_{50} value of all clones in each group remained no different from the parent. Repeated similar attempts to select for both increased resistance to ethirimol and greater sensitivity failed. Repeated cloning from single pustules resulted in some loss of pathogenicity, however, and a few daughter clones were difficult to maintain after three to four generations of selection.

Crosses. The frequency distribution of progeny in each of the four crosses was continuous and approximately normal (Fig. 2), with no indication of segregation of major genes affecting sensitivity to ethirimol. Separate analyses of variance of progeny ED_{50} values (4) for each cross provided estimates of environmental (σ_e^2) and genotypic (σ_g^2) variation, as well as heritability (h^2) (Table 2). Variation in sensitivity to ethirimol was significant in crosses that involved only genotypes of intermediate sensitivity (eg, crosses 2 and 3) with heritability around 60%. Some progeny exceeded the

TABLE 1. Characteristics of *Erysiphe graminis* f. sp. *hordei* cultures genetically analyzed for resistance to the fungicide, ethirimol

Culture	Year isolated	Ethirimol sensitivity ED_{50} (μg/ml)	Confidence limits ($P = 0.01$)	Virulence genes[a]
DH 29	1977	0.017	0.008–0.036	*v*g *v*as *v*v
DH 31	1977	0.030	0.015–0.061	*V*g *V*as *v*v
DH 16	1975	0.098	0.040–0.240	*V*g *v*as *v*v
JS 16	1973	0.130	0.070–0.263	*V*g *V*as *v*v
JS 21	1975	0.510	0.170–1.52	*v*g *v*as *v*v
DH 4	1975	1.05	0.510–3.27	*v*g *V*as *v*v
DH 19	1976	9.55	4.66–21.3	*v*g *v*as *v*v
DH 14	1976	17.8	8.87–35.6	*v*g *v*as *V*v

[a] The differential barley cultivars used were: Zephyr (*M*lg); Hassan (*M*las); Maris Mink (*M*lg, *M*las); Vada (*M*lv), and Abacus (*M*lg, *M*lv). *V* = avirulent, *v* = virulent.

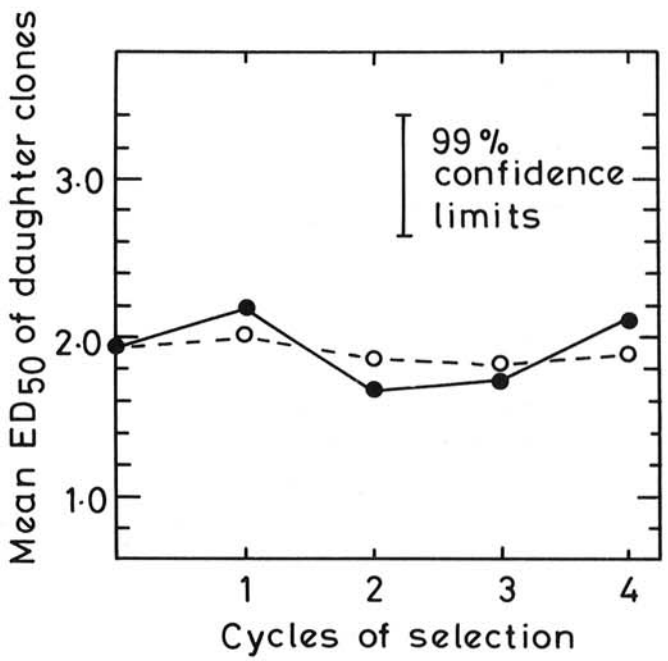

Fig. 1. Attempted asexual selection of culture DH 16 of *Erysiphe graminis* f. sp. *hordei* for greater resistance (●——●) or increased sensitivity (O----O) to the fungicide, ethirimol.

sensitivity of either parent, but parental and progeny means were similar, indicating that gene action was probably additive.

Fewer progeny were available from the cross between two ethirimol-sensitive genotypes (cross 1). These progeny were somewhat less sensitive to ethirimol than their parents, but significant genetic effects were not detected, indicating some similarity between the two parents. In the cross between resistant genotypes, some progeny were significantly more sensitive to ethirimol than either parent, but none was more resistant. Consequently, the progeny mean was lower than that of the parents, although an estimate of the contribution of gene interaction (i) was not significantly different from zero.

Interaction of pathogen virulence and resistance to ethirimol. Ethirimol-resistant forms were found more frequently in mildew populations from barley cultivars possessing the Sultan resistance gene (*M*las), than in populations from those without this gene (19). Possible linkage between some virulence genes in the pathogen and resistance to ethirimol could be examined in the four crosses analyzed here. No evidence of an association between resistance to ethirimol and any of these genes was observed (Table 3). Progeny virulent on cultivar Sultan (*vas*) were somewhat more resistant than avirulent progeny, but these differences were not statistically significant.

DISCUSSION

E. graminis f. sp. *hordei* varies considerably in its response to the fungicide ethirimol. Undoubtedly, some of this variation is environmental, whereas variation caused by cytoplasmic factors seems unimportant, because attempts to alter sensitivity to ethirimol by selection of asexual progeny were unsuccessful. From analysis of crosses 2 and 3 it is clear that a genetic component for resistance to ethirimol exists. As all assays were standardized by reference to the same ethirimol-sensitive culture, the correlation between parental and progeny means (Table 2, correlation coefficient = 0.992, $P<0.01$) provides additional evidence for the involvement of some genetic component. However, the continuous nature of the data suggests that resistance is not simply controlled by one gene, but is under more complex genetic control. More

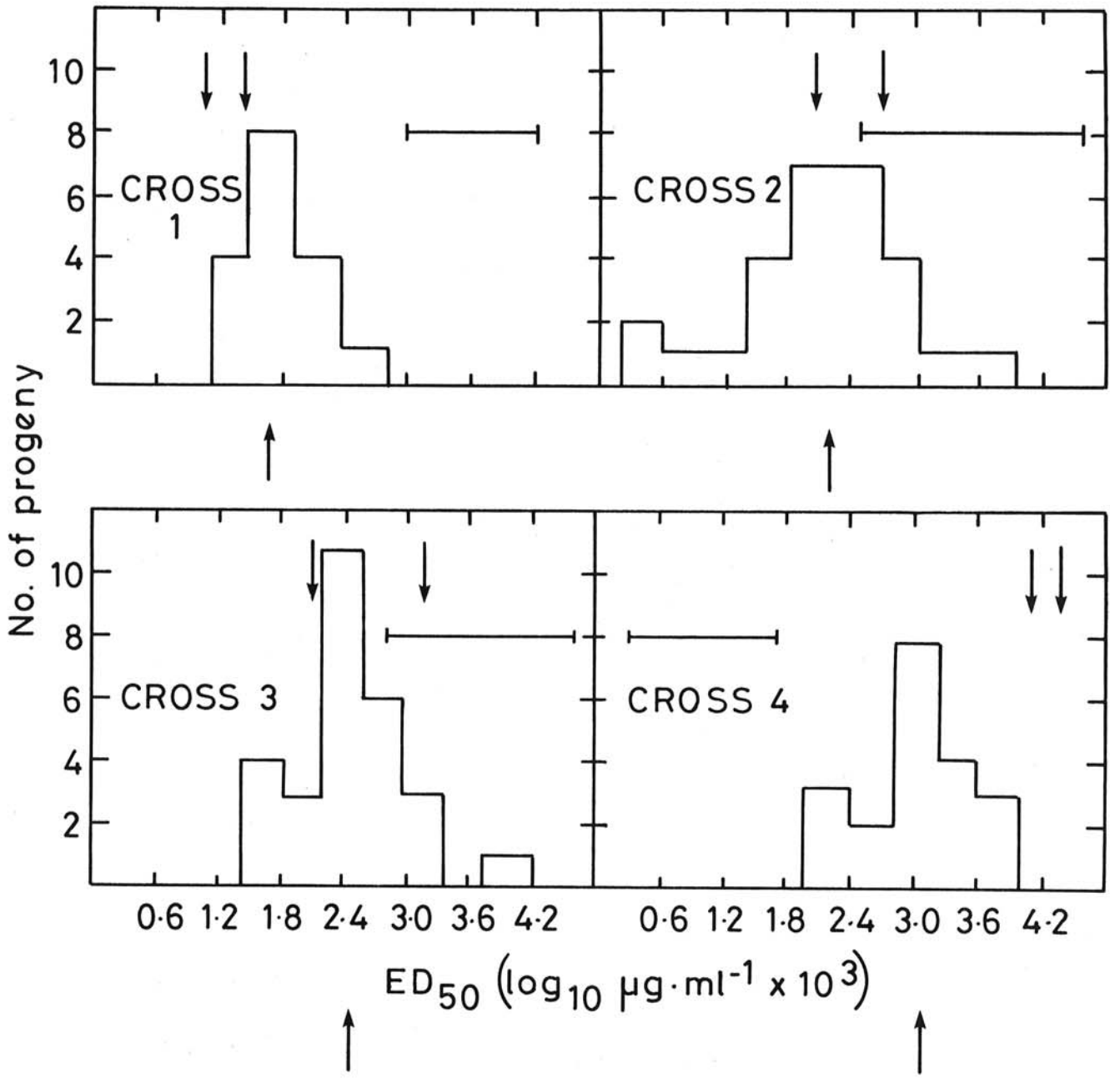

Fig. 2. Sensitivity to ethirimol of single-ascospore progeny from four crosses of *Erysiphe graminis* f. sp. *hordei* isolates. Upward arrows (↑) indicate progeny mean ED_{50} ($\log_{10}$ $\mu g \cdot ml^{-1} \times 10^3$), downward arrows (↓) indicate parental mean ED_{50}, and horizontal bars (|——|) indicate LSD, $P = 0.05$.

TABLE 2. Inheritance of ethirimol resistance in four crosses between cultures of *Erysiphe graminis* f. sp. *hordei*

Cross	No. of progeny analyzed	Mean ED_{50} values ($\log_{10}$ $\mu g \cdot ml^{-1} \times 10^3$) Parental P	Progeny F_1	Components of variance[b] i[a]	σ_g^2	σ_ϵ^2	h^2
DH 29 × DH 31	17	1.31	1.79	+0.48 NS	0.045	0.193	0.23 NS
JS 16 × JS 21	28	2.41	2.23	−0.18 NS	1.70***	2.72	0.62*
JS 16 × DH 4	28	2.62	2.47	−0.15 NS	0.815***	1.36	0.59*
DH 14 × DH 19	20	4.12	3.09	−1.03 NS	0.156	0.361	0.43 NS

[a] The parameter "i" (= P−F_1) provides an estimate of nonadditive gene effects.
[b] σ_g^2 = genotypic variation; σ_ϵ^2 = environmental variation; h^2 (= σ_g^2/σ_p^2, in which $\sigma_p^2 = \sigma_p^2 = \sigma_g^2 + \sigma_\epsilon^2$) is a measure of heritability of ethirimol resistance.
[c] Asterisks indicate statistically significant difference from zero, $P<0.05$ (*) and $P<0.001$ (***). NS indicates not significantly different from zero.

definite conclusions concerning the number of genes affecting resistance to ethirimol cannot be drawn from these data. However, detailed analyses of the genetic determination of other quantitative characters in natural isolates of fungi have indicated that several (more than five) genes frequently are involved (4). Effects of the genetic factors involved appear to be additive; crosses between cultures of intermediate sensitivity released a significant increase in variation, suggesting that the genetic architecture of each parent probably involved different combinations of factors. Additivity seems not to hold at high levels of resistance; none of the progeny from the cross between two ethirimol-resistant cultures were more resistant than their parents, although this simply may be a reflection of the small size of the progeny samples. Instead, resistance appeared to decline somewhat, suggesting that the parents were not genetically alike, but that each contained favorable gene combinations changed through recombination. A similar mechanism was thought responsible for the loss of chloramphenicol resistance in *Escherichia coli* (5), and may well represent an important means whereby organisms normally reproducing clonally, but occasionally crossbreeding, maintain variability (20).

An association between resistance to a fungicide and pathogen virulence would suggest some restriction in gene flow within the natural population. Such a link would influence how fungicides and genetic host plant resistance might be manipulated to control powdery mildew. However, these data provide no clear evidence to support such an association, at least for three virulence genes. Furthermore, bioassays over the past 5 yr have not revealed obvious links between resistance to ethirimol and six different virulence genes (*unpublished*). Also, in fungicide trials at several centers in England and Wales (1) the response to ethirimol, in terms of both disease control and yield of cultivars possessing the *M*las resistance gene, has not differed from that of cultivars without this gene. Although there seems no absolute association between resistance to ethirimol and *v*as, which would occur if this gene determined both virulence and resistance to ethirimol, progeny sizes were small and unlikely to detect even moderate linkage. In addition, some association might exist between resistance to a fungicide and factors that, although in some way controlling disease development on a particular cultivar, are not identified by largely qualitative virulence tests. No satisfactory explanation yet exists, therefore, to account for field observations linking resistance to ethirimol and virulence (19).

In Britain, *E. graminis* reproduces sexually in early summer so that ascospore progeny provide an inoculum source for autumn-sown barley. The results presented here suggest that use of ethirimol on these crops is unlikely to increase resistance dramatically. Characters controlled by complex genetic factors generally respond slowly to selection (6). Larger samples might have revealed progeny more resistant than the most resistant used here, but these would only be produced at a low frequency and, therefore, the rate of evolution of resistance would be slow. No evidence was obtained for an association between resistance to ethirimol and pathogen virulence, so any disease control strategy that integrates the use of fungicides and natural host plant resistance may use barley cultivars, at least those with genes *M*lg, *M*las, or *M*lv, without necessarily encouraging resistance to ethirimol. The ethirimol-resistant cultures used infect ethirimol-treated plants. However, they compete poorly with more sensitive cultures (8), and have remained infrequent in natural populations despite widespread fungicide use. But greater fitness may become linked with resistance to ethirimol through recombination. A strategy for control should, therefore, avoid the use of ethirimol during early summer when sexual reproduction occurs.

TABLE 3. Ethirimol resistance and virulence genes in *Erysiphe graminis* f. sp. *hordei*

Cross	Virulence gene	Mean ED_{50} value[a] ($\log_{10}$ $\mu g \cdot ml^{-1} \times 10^3$) Virulent progeny	Avirulent progeny
1	*v*g	1.73 (12)	1.84 (5)
2	*v*g	2.22 (12)	2.26 (16)
3	*v*g	2.62 (14)	2.43 (14)
3	*v*as	2.65 (16)	2.36 (12)
4	*v*v	3.03 (11)	3.11 (9)

[a] The number of progeny in each class is given in parentheses. Differences between mean ED_{50} values for virulent and avirulent progeny are not statistically significant, $P = 0.05$.

LITERATURE CITED

1. Anonymous. 1972–1977. Results of Experiments at the Experimental Husbandry Farms. Her Majesty's Stationery Office, London.
2. Ben-Yephet, Y., Y. Henis, and A. Dinoor. 1975. Inheritance of tolerance to carboxin and benomyl in *Ustilago hordei*. Phytopathology 65:563-567.
3. Brasier, C. M., and Gibbs, J. N. 1976. Inheritance of pathogenicity and cultural characters in *Ceratocystis ulmi*: hybridization of aggressive and non-aggressive strains. Ann. Appl. Biol. 83:31-37.
4. Caten, C. E. 1979. Quantitative genetic variation in fungi. Pages 35–39 in: J. N. Thompson, Jr., and J. M. Thoday, eds. Quantitative Genetic Variation. Academic Press, New York.
5. Cavalli, L. I., and Maccacaro, G. A. 1952. Polygenic inheritance of drug resistance in the bacterium *Escherichia coli*. Heredity 6:311-331.
6. Georgopoulos, S. G. 1976. The genetics and biochemistry of resistance to chemicals in plant pathogens. Proc. Am. Phytopathol. Soc. 3:53-66.
7. Hollomon, D. W. 1977. Laboratory evaluation of ethirimol. Pages 505–515 in: N. R. McFarlane, ed. Crop Protection Agents: Their Biological Evaluation. Academic Press, London.
8. Hollomon, D. W. 1978. Competitive ability and ethirimol sensitivity in strains of barley powdery mildew. Ann. Appl. Biol. 90:195-204.
9. Hollomon, D. W. 1981. Resistance of barley powdery mildew to fungicides. Agricultural Development and Advisory Service Quarterly Review 39:226-233.
10. Jenkyn, J. F., Hirst, J. M., and King, G. 1973. An apparatus for the isolated propagation of foliar pathogens and their hosts. Ann. Appl. Biol. 73:9-13.
11. MacKenzie, D. R., Nelson, R. R., and Cole, H. 1971. Quantitative inheritance of fungicide tolerance in a natural population of *Cochliobolus carbonum*. Phytopathology 61:471-475.
12. Merrick, M. J., and Caten, C. E. 1975. The inheritance of penicillin titre in wild-type isolates of *Aspergillus nidulans*. J. Gen. Microbiol. 86:283-293.
13. Moseman, J. G. 1968. Reactions of barley to *Erysiphe graminis* f. sp. *hordei* from North America, England, Ireland and Japan. Plant Dis. Rep. 52:463-467.
14. Papa, K. E. 1970. Inheritance of growth rate in *Neurospora crassa*: crosses between previously selected lines. Can. J. Genet. Cytol. 12:1-9.

15. Shephard, M. C., Bent, K. J., Woolner, M., and Cole, A. M. 1975. Sensitivity to ethirimol of powdery mildew from U. K. barley crops. Proc. 8th Br. Insecticide and Fungicide Conf. 1:59-66.
16. Smith, J. E., Payne, R. W., and Bainbridge, A. 1977. Ethirimol sensitivity in populations of *Erysiphe graminis* from plots of spring barley. Ann. Appl. Biol. 87:345-354.
17. Wolfe, M. S. 1971. Fungicides and the fungus population problem. Proc. 6th Br. Insecticide and Fungicide Conf. 3:724-734.
18. Wolfe, M. S. 1972. The genetics of barley mildew. Rev. Plant Pathol. 51:507-522.
19. Wolfe, M. S., and Dinoor, A. 1973. The problems of fungicide tolerance in the field. Proc. 7th Br. Insecticide and Fungicide Conf. 1:11-19.
20. Wright, S. 1956. Modes of selection. Am. Nat. 9:5-24.