Parasitic Fitness and Intrastrain Diversity of Benomyl-Sensitive and Benomyl-Resistant Subpopulations of *Venturia inaequalis*

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ABSTRACT

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Benomyl-sensitive and -resistant strains of *Venturia inaequalis* were isolated from two commercial apple orchards. In a greenhouse study, four fitness parameters (incubation period, colonization, sporulation, and infection efficiency) were measured on potted McIntosh apple trees infected with the isolates. The resistant and sensitive isolates from one orchard (Schultz) were not significantly different for three of the four fitness parameters. However, the resistant isolates from a second orchard (Barnard) were less fit than the sensitive isolates for all but one fitness parameter. Similarly, the relative fitness parameters, derived from a multiplicative model, were not statistically different for the two isolate types in the Schultz orchard but were different for those in the Barnard orchard. We hypothesize that removal of benomyl from the spray program in the Barnard orchard may allow reversion of the population to sensitivity, whereas this would not occur for the Schultz orchard. Unlike the means, the diversities (Simpson's Diversity Index) of the two subpopulation samples from each orchard were less definitive and dependent on interpretation of the cluster analysis performed.

For any given plant pathogenic fungus, there exist many different parasitic and saprophytic parameters that contribute to its overall biological fitness. Although selection by a fungicide for resistance may be the major process, selection for other characteristics must also occur. The theory of genetic homeostasis states that many characters of a population in a given environment are held in a complex balance, and consequently an entire population shift will not occur because of fungicide selection alone (3). Keiding (10) and later Georghiou (7) proposed that this multidimensional selection process consists of two phases. First, there is selection for individuals with resistance regardless of their other traits. The members of this subpopulation may or may not be

more fit than the sensitive subpopulation for any given fitness parameter. The second phase involves a progressive organization of the genetic background of these individuals for greater overall fitness. Once highly fit resistant strains occur, the selection process proceeds rapidly.

The genetic variation necessary to produce the resistant strains originates by gene mutations and recombinations of these mutations among individuals of the parent, sensitive population. Consequently, with respect to various fitness parameters, the resistant subpopulation can be considered a derivative of the sensitive subpopulation. We hypothesize that the differences between the means and diversities of the two subpopulations for any given fitness parameter are a function of the duration of the selection pressure for that parameter. Resistant subpopulations that have undergone relatively long periods of selection would have means and diversities identical to their parent population, provided both were subjected to the same environmental conditions.

Data to test the above hypothesis are minimal. Sampling of the initial resistant subpopulation at its low frequencies, and, hence, detection of any change in fitness is difficult with present techniques (18). However, populations that are predominantly resistant should have sensitive and resistant subpopulations with approximately equal means and diversities. Our objectives were to compare the means and diversities of benomyl-sensitive and -resistant *Venturia inaequalis* (Cke.) Wint. isolates for four parasitic fitness parameters in vivo: incubation period, colonization, sporulation, and infection efficiency. Because the isolates were obtained from orchards in which the resistant subpopulation was dominant, we hypothesized that the means and diversities of the frequency distributions of each subpopulation should be similar.

MATERIALS AND METHODS

Orchard history. Two commercial Pennsylvania apple orchards (*Malus domestica* Borkh.) were used in the study. The Barnard orchard, which consisted of standard-size trees of the cultivar Delicious, was located in southeastern Pennsylvania near Kennett Square. In 1978 and 1979, benomyl was applied to this orchard for the first six sprays and then combined with mancozeb for the remaining six to eight sprays. From 1980 through 1982, the early season sprays consisted of benomyl in combination with thiram; the mid- to late-season sprays continued to be the benomyl-mancozeb combination. In 1983 benomyl was removed from the spray program and replaced with dodine or captan.

The Schultz orchard, which was situated in northwestern Pennsylvania near North East, consisted of semidwarf trees of the cultivar McIntosh. In 1982 and 1983, benomyl was used alone in this orchard for the first spray application. The following four applications consisted of benomyl in combination with glyodin plus either dodine or triforine. The remaining five to seven sprays were either mancozeb, dodine, or captan. Although records before 1982 were not available, a similar type of program was thought to have been used.

Isolation and inoculation. During the summer of 1983, a preliminary survey showed that 74 of 120 lesions (62%) sampled from the Barnard orchard were found to be benomyl resistant. The same percentage of resistant lesions (62/100) was observed in the Schultz orchard. In fall of that year, apples with sporulating lesions were collected from each orchard, one fruit per tree. The trees sampled were randomly scattered throughout the orchards. A single spore was isolated from only one sensitive or resistant lesion on each apple. Lesions were resistant if their conidia germinated normally on water agar amended with benomyl at 5 mg a.i./L; sensitive conidia produced nonseptate, malformed germ tubes. Isolates were confirmed as being resistant or sensitive by their growth or lack of growth, respectively, on benomyl-amended potato-dextrose agar slants. Conidia for inoculation were produced on malt-extract broth wick cultures grown at 18 C (17). Sensitive and resistant isolates from the Barnard orchard were designated BS and BR, respectively, followed by an identification number; Schultz orchard isolates were designated SS and SR, also followed by a number. A culture of each isolate has been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

One-year-old McIntosh trees budded onto Malling 7A rootstock were grown in the greenhouse. Each tree was planted in a 64-oz juice can using a steam-sterilized mix of 2 parts soil plus 1 part peat moss plus 1 part perlite. A dormant oil was applied for insect control. The trees were watered periodically to maintain proper soil moisture and fertilized on a weekly basis. Only one shoot was allowed to develop on each tree.

On 1 May 1984, five trees or shoots were inoculated with conidia from each isolate. Conidial suspensions of 50,000 spores per milliliter were atomized at 25 psi onto the youngest four leaves of each shoot. Leaves were sprayed until a uniform deposition of minute droplets appeared; runoff of the suspension did not occur. The youngest, most fully open leaf was tagged to indicate the topmost leaf inoculated. The droplets were then allowed to dry before the plants were placed in a wetting chamber. Incubation in the chamber was for 36 hr of wetting at 18–20 C, the equivalent of a heavy Mills infection period (12).

Fitness parameter estimation. Beginning 6 days after initiation of the wetting period, the total number of visible lesions on each shoot was recorded. These observations were continued for approximately 14 days or until new lesions were no longer produced. From these data, the median incubation period, defined as the number of days from inoculation to the day when 50% of the lesions were visible, was calculated.

After cessation of lesion production, the lesion and leaf areas were measured. The outline of each leaf blade was traced on a clear acetate using red ink felt-tipped markers. The outlines of lesions were similarly traced but also 'colored in' using black felt-tipped markers. When the acetate was pulled through a leaf area recorder (Model LI-3000, Li-Cor, Inc., Lincoln, NE) only the black regions were measured, thus providing an estimate of lesion area. A paper copy of each acetate tracing was also made. When the paper cutouts of each leaf were put through the recorder, an estimate of leaf area was calculated. The average fungal colony size was calculated by dividing the lesion area by the total number of lesions.

Infection efficiency can be defined as the ratio between the number of colonies formed to the number of spores applied on a per unit area basis. Given that the inoculum concentrations for each isolate were identical and assuming that all leaves received the same amount of suspension per unit area, then the leaf area was a measure of the number of spores applied. Thus, infection efficiency was approximated by calculating the ratio between the number of lesions formed and the area of the leaf.

The final fitness parameter examined was sporulation. With an atomized water spray, conidia were gently washed off lesions into a funnel that emptied into a test tube. The conidia from all lesions on a tree were combined into a single sample. The concentration of spores in the sample was then determined using a hemacytometer; a minimum of 250 conidia per sample were counted. The sporulation for each tree replicate of each isolate was estimated as the number of conidia produced per unit area of lesion.

After all assessments were taken, the trees were pruned and allowed to develop another single shoot. The above procedures were then repeated, with inoculation commencing on 6 July. Pesticides other than dormant oil were not needed during the first replication, although one spray of dicofol (30WP, 4 g/L) and pirimicarb (50WP, 1.3 g/L) was applied 1 wk before the second inoculation to control mites and aphids.

Statistical analysis. The data for each orchard and dependent variable (fitness parameter) were subjected to an analysis of variance in which the replicate, benomyl-sensitivity, and interactive effects were examined. The means for each isolate, calculated over the five trees, were used in the analyses. A second set of ANOVAs, similar to those above, were also performed. However, in these cases, the raw data sets were used so that the error mean squares included the within isolate variation. The

isolate means were then compared using the LSD values calculated from these error mean squares.

Although separate analyses for each parameter aid in comparing isolates, a single fitness parameter (fp) incorporating each dependent variable would be more indicative of the reproductive success of an isolate in an epidemic. A measure of this overall fitness parameter was calculated using a multiplicative model:

$$\text{fp}(i) = 1/\text{mip}(i) * \text{col}(i) * \text{spor}(i)\ \text{ie}(i),$$

where mip(i), col(i), spor(i), and ie(i) were the median incubation period, colony size, amount of sporulation, and infection efficiency, respectively, for isolate i. The dimensions for the terms in the model were:

$$1/\text{day} = 1/\text{day} * \text{cm}^2/\text{lesion} * \text{spores}/\text{cm}^2 * \text{lesions}/\text{spore},$$

which is the same dimension that is derived for reproductive rates. Division of fp(i) by fp(max), the maximum fp value obtained for any given observation (tree), produced a dimensionless relative fitness parameter (rfp), which can be viewed as a measure of the relative reproductive success. The sensitive and resistant isolate rfp means were compared within each orchard using a standard F test; calculations were performed on the isolate averages. In addition, individual isolate means were compared using the LSD procedure. In this case, however, the raw data were used in the calculations to account for within isolate variation.

Another approach for examining the combined effect of the fitness parameters was to perform a multivariate statistical procedure such as principal components analysis. This analysis produced a set of equations, each a linear combination of the four fitness parameters, from which new variables or scores were computed. Plots of standardized scores from each principal component equation were examined to determine if resistant and sensitive isolates tended to group together. Only those isolates that were examined in both replicates were included in the analyses.

To estimate the diversity within each subpopulation, isolates had to be placed into groups according to their similarity with respect to each of the fitness parameters. This grouping was accomplished by performing a cluster analysis on each subpopulation sample within each orchard. The analysis was based on the Euclidean distance measure, and amalgamation of isolates into clusters was performed using a single linkage algorithm. The proportion of a group or cluster within a sample was then calculated by dividing the number of isolates in that group by the total number of isolates in the sample. These proportions were used in calculating Simpson's Diversity Index (6):

$$C = 1 - \sum_{i=1}^{n} p_i^2$$

where p = proportion of group i in the subpopulation sample, n = number of groups, and C is the probability that two isolates chosen at random in the subpopulation will belong to different groups.

All ANOVAs and the principal components analyses were performed using the GLM and PRINCOMP procedures, respectively, or PRODAS (2). The PM2 routine of BMDP (1) was used for the cluster analysis.

RESULTS

Separate analyses. The experimental replicates, as indicated by the low P-values obtained in the ANOVAs, were significantly different in seven of the eight cases (Table 1). This was attributed primarily to differences in greenhouse environmental conditions.

In the first replication, the average daily temperature (C) and relative humidity (%) were 20 ± 1.0 and 65 ± 11, respectively; values of 22 ± 1.5 and 80 ± 6.7, respectively, were obtained during the second replication. Although the relative humidity was more favorable during the second replication, the warmer temperatures, often reaching highs near 27 C, may have had an important influence in slowing fungal growth. Most isolates had extended latent periods, smaller colonies, lower disease severities, and produced fewer spores under the warmer conditions.

The sensitivity effect, which examines the difference between the sensitive and resistant isolates, was significant for three of the four fitness parameters in the Barnard orchard: incubation period, colony size, and sporulation (Table 1). The sensitive and resistant Schultz orchard isolates, however, were only significantly different for the infection efficiency parameter. The replicate by sensitivity interaction was insignificant regardless of the orchard or fitness parameter examined. Thus, the differences between the two types of isolates were consistent across both replications.

The resistant Barnard orchard isolates had, on the average, a longer incubation period, smaller colonies, less sporulation, and fewer lesions per unit area of leaf than the sensitive isolates (Table 2). As previously indicated by the outcome of the F tests, the resistant and sensitive isolate means for the Schultz orchard were very similar for all but the infection efficiency parameter. In both orchards, none of the resistant isolates had statistically smaller incubation periods or statistically greater colony size, sporulation, and infection efficiency than any sensitive isolate. However, some of the sensitive isolates did have statistically greater fitness than any one resistant isolate for each of the parameters.

Combined analyses. The results of the rfp analyses were in agreement with the results from the separate fitness parameter analyses. In the Barnard orchard sample, the sensitive and resistant isolates had rfp means of 0.24 and 0.08, respectively. The F test in the ANOVA indicated that these two values were significantly different at the $P = 0.02$ level. The sensitive and resistant Schultz orchard samples had rfp means of 0.11 and 0.04, respectively, and were not significantly different at the 0.05 level ($P = 0.08$). Regardless of the F test outcomes, the sensitive isolates in both orchard samples tended to have higher fitness (Table 3). The BS8A and SS45 isolates were most fit, and their 'success' could be attributed to their high degree of sporulation and infection efficiency (Table 2). Note, however, that their rfp does not have the value of one, as might be expected. This occurred because the rfp was calculated using the raw data.

Principal components 1 and 2 in the Barnard orchard explained 92% of the total variation (Table 4). The eigenvector for principal component 1 was dominated by high positive weights for the sporulation and infection efficiency variables and a relatively large negative weight for incubation period (Table 5). An isolate that sporulates abundantly, efficiently infects the suscept, and has a

TABLE 1. Analysis of variance[a] to determine the importance of benomyl sensitivity of *Venturia inaequalis* and experimental replication for four fitness parameters in two commercial orchards[b]

	Degrees of freedom		Median incubation period		Colonization		Sporulation		Infection efficiency	
Source	Barnard	Schultz	Barnard	Schultz	Barnard	Schultz	Barnard	Schultz	Barnard	Schultz
Model	3	3	<0.01	0.02	<0.01	0.44	<0.01	0.04	0.01	<0.01
Rep	1	1	<0.01	<0.01	<0.01	0.30	<0.01	0.01	<0.01	<0.01
Sens	1	1	<0.01	0.48	0.03	0.47	0.06	0.33	0.30	0.02
Rep × sens	1	1	0.31	0.48	0.69	0.34	0.64	0.44	0.85	0.07
Error	20	21								

[a] P - values for the F test of the effect given that all others have been entered into the model (Type III).

[b] The Barnard orchard data consisted of six sensitive and seven resistant isolates; the Schultz orchard data comprised seven sensitive and seven resistant isolates.

short incubation period will produce a high Prin1 score. In contrast, the principal component 2 eigenvector represented the less fit isolate. Prin2 is dominated by a very large negative weight for colonization and a high positive weight for incubation period. Isolates with long incubation periods and small colony sizes will produce the highest scores.

Principal component 1 for the Schultz orchard, which described 63% of the total variation (Table 4), had an eigenvector somewhat similar to that generated for the Barnard orchard (Table 5). However, because of the moderately large negative weight for colonization, a Schultz isolate would also need to have small colonies to produce a large Prin1 score. Principal component 2 explained an additional 22% of the total variation (Table 4) and had a large negative weight for incubation period with a major positive weight for colonization (Table 5). Prin2 can be interpreted as representing isolates with short incubation periods and large colonies.

A plot of Prin2 against Prin1 for the Barnard orchard emphasized the importance of the latter component in separating sensitive and resistant isolates (Fig. 1A). Very little separation occurred along the Prin2 axis, whereas a distinct separation between isolate types occurred along the horizontal Prin1 axis. Resistant isolates tended to have negative Prin1 scores, whereas sensitive isolates were positive for this component; resistant isolates were less fit in the parameters investigated than sensitive isolates. This outcome was in agreement with other results (Tables 1–3).

The graph of Prin2 against Prin1 for the Schultz orchard was similar to that generated for the Barnard orchard (Fig. 1B). However, the separation of isolate types occurred in a different fashion. Resistant isolates must have long incubation periods and/or large colonies coupled with a low degree of sporulation and infection efficiency in order to produce a negative Prin1 score. The relatively large sporulation and infection efficiency capacities of the sensitive isolates (Table 2) were most likely the cause of their positive Prin1 scores. The only major exception to this situation was isolate SS7, which had a low degree of sporulation and infection efficiency but a large average colony size (Table 2). This isolate appeared in the upper left corner of the graph (Fig. 1B).

Intrastrain diversity. The cluster analysis, using a hierarchical classification procedure, placed the isolates into groups that had similar fitness parameter attributes (Table 6). The technique began by joining the two most similar isolates, as determined by the Euclidean distance measure, into a single cluster. In the next and subsequent steps, either the next closest isolate was added to the cluster or two other isolates were joined to form a second cluster. This procedure continued until all isolates belonged to one cluster.

Because cluster analysis lacks an inferential basis, the amalgamation process did not converge on any particular grouping. However, the amalgamation distance, a measure of the separation between clusters, was used to choose the step having the 'best' grouping. If the difference between the distances of any two steps was considerably less than the maximum difference (between the first and last steps), then that grouping was termed feasible. In the sensitive isolate sample from the Barnard orchard, BS12 was added to a cluster of isolates (BS21 and BS25A) in the second step (Table 6). Because BS12 was almost the same distance from the cluster that the two isolates in the cluster were from each other (1.9 vs. 1.8, respectively), then the grouping performed in the second step was deemed reasonable. The difference between the two steps was only 10% of the maximum. In the third step, however, isolate BS8A was much more distant from the cluster formed in step 2 than isolate BS12 was from the cluster formed in step 1. The difference between steps 2 and 3, 0.4 or 40% of the maximum, was considered too great to allow for amalgamation. Consequently, the clustering presented in step 2, which had a diversity index of 0.56, was chosen as the best grouping.

As indicated by the relatively large amalgamation distances, most resistant isolates in the Barnard orchard were different in their fitness attributes (Table 6). Only isolates BR23 and BR15 in step 1 were considered similar enough to warrant grouping; the corresponding value for C for this step was 0.78. The resistant isolates from the Schultz orchard also had relatively large amalgamation distances. In this case, no isolates would be grouped and C would be 0.80. However, relative to the maximum difference obtained, the grouping presented in step 3, with $C = 0.32$, could also be considered a reasonable stopping point. In contrast, the sensitive Schultz orchard isolates had relatively short amalgamation distances. Assuming that a difference of 23% of the maximum was insignificant, then all but isolate SS7 could be grouped together (step 4). A diversity of 0.28 was associated with this level of clustering.

TABLE 2. Comparison[a] of benomyl-sensitive and -resistant isolates of *Venturia inaequalis* for four fitness parameters in two commercial orchards

Isolate[d]		Median incubation period[b] (days)		Colonization (cm^2/lesion)		Sporulation conidia/mm^2 lesion)		Infection efficiency[c] (lesion/cm^2 leaf)	
Barnard	Schultz	Barnard	Schultz	Barnard	Schultz	Barnard	Schultz	Barnard	Schultz
BS8A	SS7	10.5	10.7	0.36	0.61	7,364	2,670	1.10	0.29
BS12	SS35	10.6	11.3	0.32	0.32	4,067	5,096	0.57	0.88
BS21	SS36[e]	10.3	11.7	0.32	0.25	5,357	4,310	0.85	0.30
BS25A	SS45	10.2	10.3	0.38	0.28	5,529	8,495	0.50	1.11
BS28A	SS55	10.0	10.8	0.60	0.32	3,365	4,560	0.21	0.57
BS35[e]	SS67	10.8	11.1	0.56	0.24	2,658	6,122	0.26	0.81
	SS71		10.4		0.27		6,380		0.95
BR5	SR1[e]	11.6	10.0	0.30	0.41	4,080	7,578	0.49	0.92
BR15	SR13	11.4	10.3	0.36	0.42	2,501	6,223	0.44	0.48
BR23	SR21	11.0	11.1	0.34	0.31	2,445	2,969	0.36	0.34
BR30[e]	SR32[e]	12.9	11.0	0.31	0.36	1,792	4,713	0.14	0.14
BR31	SR33	10.8	10.5	0.37	0.37	2,883	5,106	0.48	0.38
BR38	SR43	11.4	11.4	0.30	0.29	5,611	3,662	0.59	0.14
BR39	SR54	11.8	12.0	0.25	0.48	2,260	3,989	0.33	0.19
LSD ($P = 0.05$)		0.9	1.1	0.13	0.18	2,305	3,313	0.36	0.59
Resistant mean[f]		11.6	10.9	0.32	0.33	3,082	4,891	0.40	0.37
Sensitive mean[f]		10.4	10.9	0.42	0.38	4,723	5,376	0.58	0.70

[a] Each mean is an average of two replicates, five observations per replicate.
[b] The number of days from inoculation to the day when 50% of the lesions were visible.
[c] Assuming the conidia for each isolate were deposited onto the leaf surfaces at approximately the same average density, then the number of lesions per unit area of leaf could be used as an estimation of relative infection efficiency.
[d] Sensitive isolates: BS or SS; resistant isolates: BR or SR.
[e] Examined in only one of the two experimental replications.
[f] The F test for the sens factor in Table 1 provided a direct comparison of sensitive vs. resistant isolate means.

DISCUSSION

In general, the sensitive and resistant isolate samples from the Schultz orchard had similar means and rfp's for the characteristics examined. Although these were only four of many possible phenotypic traits that contribute to overall fitness, these similarities suggest that the two subpopulations have stabilized with the resistant strains being dominant. In a greenhouse study, McGee and Zuck (11) observed no major change in the original 1:1 ratio of resistant to sensitive *V. inaequalis* isolates after passing them through several sporulation cycles on McIntosh seedlings. It is speculated that these isolates, like those from the Schultz orchard, have undergone enough selection so that their two subpopulations would be of equivalent parasitic fitness. In terms of Keiding's hypothesis (10), strains that have passed through the second phase of selection are fit in all the various traits in addition to resistance; the 'age' of selection is related to fitness and, hence, persistence. Assuming this to be the case in the Schultz orchard, then removal of benomyl from the spray program would not result in a reversion back toward sensitivity.

The results from a variety of field surveys support the concept of persistence. After removal of benomyl, resistant strains of *V. inaequalis* were still present in orchards after 2 yr in South Australia (16) and New York (8) and after 3 yr in Victoria, Australia (15). High levels of benzimidazole resistance in *V. pirina* Aderh. on pear were reported for 4 yr after removal of these fungicides (14). Dodine resistant isolates of *V. inaequalis* were recovered after 10 yr in the absence of dodine selection pressure (8). In a region in Greece where conditions favorable for disease required six to eight sprays of triphenyltin hydroxide per year, benomyl-resistant strains of *Cercospora beticola* Sacc. on sugar beet remained at 90% of the population from 1973 to 1975 (5). Hypothetically, a selection against the resistant and for the sensitive is necessary for reversion to sensitivity and to counterbalance the selection pressure imposed by the at-risk fungicide. Such a situation occurs by using two fungicides that have negatively correlated cross resistance between the two subpopulations (4). These examples of persistence and the similarity of fitness parameters for the Schultz orchard subpopulations indicate that 'unnecessary' genes may not reduce fitness.

The resistant isolates obtained from the Barnard orchard, unlike those from the Schultz orchard, were less fit than the sensitive isolates. On the average, they had longer incubation periods, produced smaller colonies with less sporulation, and had a lower infection efficiency. Given the spray program, this result contradicts what might be expected. In the 5 yr before 1983, benomyl was used either alone or in combination with thiram during the first half of the growing season. The thiram, however, was added for rust control and is only moderately effective against apple scab. Thus, the early-season spray program was essentially equivalent to the application of benomyl alone. This 5-yr period should have been of sufficient duration to allow the resistant subpopulation to increase in fitness relative to the sensitive. Sexual reproduction between the two subpopulations, along with any new mutations, should have produced an equivalence in fitness.

One possible explanation was that the addition of a gene for resistance actually bestowed decreased fitness in the parameters investigated. The resistant strains are only more fit in the presence of the fungicide and its removal should result in a reversion of the population to sensitivity. Resistant strains of *V. nashicola* Tanaka & Yamamoto on pear persisted for 5 yr after removal of selection pressure by benzimidazoles (9). However, the highly resistant strains were slowly replaced by intermediate and weakly resistant strains as well as by sensitive ones. It was speculated that strains having the allele controlling high resistance were at a disadvantage in the absence of fungicide selection (9). Although this interpretation explains the results of the Barnard orchard, it does not agree with observations from the Schultz orchard. Nevertheless, the most fit strains in each orchard were of the sensitive type.

The sensitive and resistant Barnard isolates may have different fitness for an entirely different reason. The Barnard strains evolved

TABLE 3. Comparison of relative fitness parameters[a] (rfp) of benomyl-sensitive and -resistant isolates of *Venturia inaequalis* obtained from two commercial orchards

Barnard orchard		Schultz orchard	
Isolate[b]	rfp	Isolate[b]	rfp
BS8A	0.518	SS45	0.244
BS25A	0.281	SS7	0.132
BS21	0.270	SR1	0.130
BS12	0.231	SS71	0.122
BR38	0.156	SR13	0.078
BR31	0.125	SS35	0.078
BR5	0.109	SS55	0.072
BR15	0.053	SS36	0.059
BS28A	0.049	SS67	0.049
BR23	0.042	SR32	0.041
BR30	0.040	SR33	0.036
BR39	0.036	SR21	0.029
BS35	0.033	SR54	0.019
		SR43	0.009
LSD = 0.185		LSD = 0.166	

[a] Proportion that an isolate is of the maximum fitness; see text for details concerning derivation and assumptions.
[b] Sensitive isolates: BS or SS; resistant isolates: BR or SR.

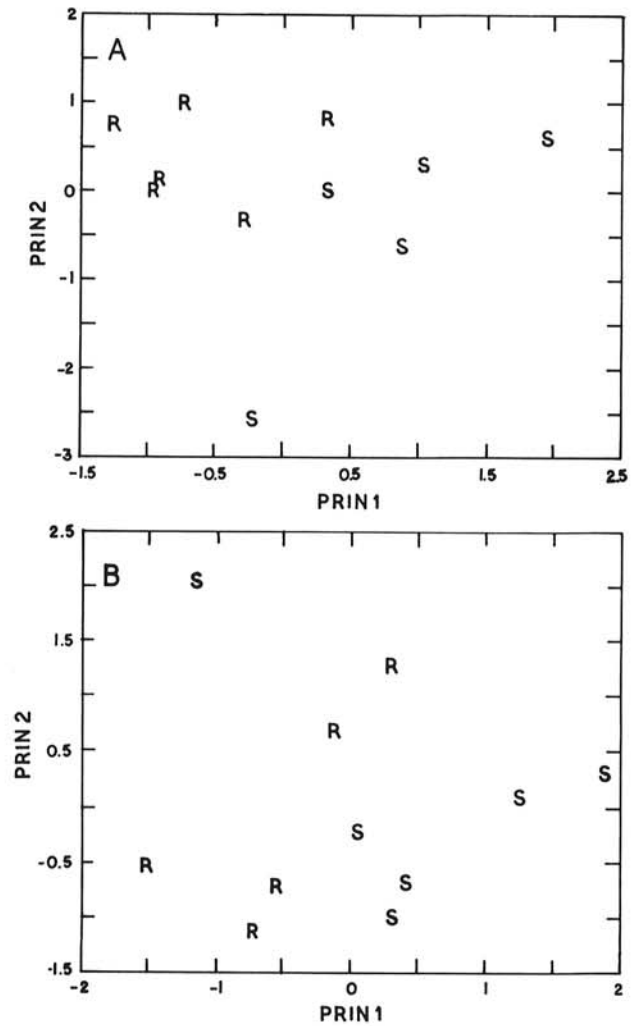

Fig. 1. Plot of principal component 1 (PRIN1) and 2 (PRIN2) scores for the sensitive (S) and resistant (R) isolates from the **A,** Barnard orchard, and **B,** Schultz orchard. Each component is a linear combination of four fitness parameters: incubation period, colonization, sporulation, and infection efficiency. Only those isolates examined in both experimental replications were used in the analysis.

on the moderately susceptible cultivar Delicious but were tested on the highly susceptible cultivar McIntosh. Thus, an interaction between benomyl sensitivity and cultivar resistance could have been the cause for the difference in the fitness parameters. Or, alternatively, if the rate of selection was a function of the cultivar's level of disease resistance, then we would expect the Schultz orchard subpopulations to reach equivalent fitness levels before those in the Barnard orchard. In addition, since the McIntosh trees in the Schultz orchard were semidwarf, spray coverage may have been much more complete than on the large, standard trees in the Barnard orchard. Thus, high disease resistance and a lower degree of spray coverage in the Barnard orchard may have decreased selection pressure.

For any given population, diversity indices are measures of both the species richness and the evenness of the species' abundances (13). The index depends not only on the number of different species but also on the relative abundance of each. We hypothesized that as mutations toward resistance occur among sensitive strains, and as these resistant mutants sexually reproduce with sensitive types, the means and diversities of the two subpopulations should become similar. This process would occur for all the various fitness parameters and, most likely, at different rates. The sensitive and resistant isolates from the Schultz orchard, which had similar means for three of the four fitness parameters investigated, were shown to have diversity indices of 0.28 and 0.32, respectively. Conversely, the sensitive and resistant Barnard isolates were similar only in the infection efficiency parameter and were assigned indices of 0.56 and 0.78, respectively. Although these results agree with expectations, the process by which the indices were derived is tenuous at best; the selection of the 'best' cluster analysis grouping, and thus value of C, was somewhat arbitrary. Furthermore, the diversities could not be statistically compared because this would have required experimentation on isolates obtained from more than one sampling in each orchard.

TABLE 4. Eigenvalues generated from the principal components analysis on the four fitness parameters[a] in each orchard

Principal component	Barnard			Schultz		
	Eigenvalue	Proportion[b]	Cumulative[c]	Eigenvalue	Proportion[b]	Cumulative[c]
Prin1	2.12	0.53	0.53	2.54	0.63	0.63
Prin2	1.55	0.39	0.92	0.87	0.22	0.85
Prin3	0.24	0.06	0.98	0.40	0.10	0.95
Prin4	0.09	0.02	1.00	0.18	0.05	1.00

[a] Incubation period, sporulation, colonization, and infection efficiency.
[b] Proportion of variation explained in model.
[c] Cumulative proportion of variation explained in model.

TABLE 5. Eigenvectors for each principal component within each orchard

Variable[a]	Barnard				Schultz			
	Prin1	Prin2	Prin3	Prin4	Prin1	Prin2	Prin3	Prin4
Incubation period	−0.49	0.50	0.67	0.25	−0.43	−0.67	0.61	0.07
Sporulation	0.64	0.18	0.53	−0.53	0.58	0.04	0.37	0.73
Colonization	0.12	−0.76	0.50	0.39	−0.40	0.74	0.54	−0.001
Infection efficiency	0.59	0.38	−0.12	0.70	0.57	−0.02	0.45	−0.68

[a] Incubation period = Number of days from inoculation to when 50% of lesions visible, sporulation = number of conidia/mm^2 of lesion, colonization = cm^2/lesion, and infection efficiency = lesions/cm^2 of leaf.

TABLE 6. Determination of Simpson diversity indices (C) from cluster analysis[a] tree diagrams[b] for the benomyl-sensitive and -resistant subpopulations of *Venturia inaequalis*

Barnard Orchard

Sensitive isolates[c] 8A 21 25A 12 28A	Amalgamation Step	Amalgamation Distance[d]	Prop.[e]	C[f]	Resistant isolates[c] 5 31 23 15 39 38	Amalgamation Step	Amalgamation Distance[d]	Prop.[e]	C[f]
— ——— – ——	1	1.8	···	0.72	— — ——— — —	1	1.6	···	0.78
— ———— ——	2	1.9	0.10	0.56	— ——— — —	2	2.3	0.44	0.67
———— ——	3	2.3	0.40	0.32	———— — —	3	2.3	0.00	0.50
————	4	2.8	0.50	0.00	———— —	4	2.4	0.06	0.28
					————	5	3.2	0.50	0.00

Schultz Orchard

Sensitive isolates[c] 7 45 71 55 67 35	Amalgamation Step	Amalgamation Distance[d]	Prop.[e]	C[f]	Resistant isolates[c] 13 33 43 21 54	Amalgamation Step	Amalgamation Distance[d]	Prop.[e]	C[f]
— — — ——— —	1	1.1	···	0.78	——— — — —	1	2.0	···	0.72
— ——— ——— ——	2	1.6	0.23	0.72	— — ——— —	2	2.2	0.29	0.64
— ——— ————	3	1.7	0.04	0.61	———— —	3	2.3	0.14	0.32
— ————	4	2.2	0.23	0.28	————	4	2.7	0.57	0.00
————	5	3.3	0.50	0.00					

[a] Cluster formation was based on the Euclidean distance measure and amalgamation was performed using the single linkage algorithm; variables analyzed: median incubation period, colonization, sporulation, and infection efficiency.
[b] Each horizontal line in a tree corresponds to a cluster of the indicated isolates.
[c] Replicate averages were used in the analysis; those isolates examined in only one replicate were excluded.
[d] Measure of the separation between two isolates, two clusters, or an isolate and a cluster.
[e] Proportion that the difference in the amalgamation distances between the last two steps is of the largest amalgamation difference; e.g., in step 2 for the Barnard orchard sensitive isolates: Prop. = (1.9 − 1.8)/2.8 − 1.8) = 0.10.
[f] C is the probability that two isolates chosen at random from the subpopulation will have different levels of fitness in some or all of the four parameters examined.

Although the differences between the two orchards could be caused by valid biological phenomena, chance alone may be the cause. The sample size or number of isolates from each orchard may have been inadequate to account for the unknown degree of variability. Furthermore, the objective of an experiment should be to reject the null hypothesis of no differences. However, because the level of resistance in the orchards was high, our goal was to show that there were no differences between subpopulations. If orchards with low frequencies of the resistant population were examined, and if it were feasible to obtain a sample of sufficient size, then we would hypothesize that differences should exist between the subpopulations. Finally, because determination of the fitness parameters required much time and effort, only a single sample was examined. Observations on the subpopulations over time would have supplied the necessary information to properly access the dynamics of the evolutionary process. Regardless of these limitations, the results presented here should provide an initial base for future experimentation.

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