Density-Dependent Fitness Interactions in the Bean Rust Fungus

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ABSTRACT

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Several paired comparisons of urediniospore production per uredinium at ranges of densities of five isolates of *Uromyces appendiculatus* on two susceptible bean cultivars were made. They revealed that isolates with very large (to 1.4 mm diameter) uredinia were more strongly influenced by crowding than were isolates with large (to 1 mm diameter) uredinia. Negative regression slopes of log of spore production per uredium over log of density (uredinia per unit area) of two isolates with very large uredinia were significantly steeper than were slopes of three isolates with large uredinia in all five comparisons that were made of the two classes of isolates. Slopes of log of reproduction on log of density were also significantly steeper in a more favorable growth chamber environment than in a less favorable greenhouse environment for a single isolate of each of the uredinial size classes. Hence, uredinial size and reproductive potential at low densities (about 1 uredinium/cm²) cannot be used alone to describe selective advantage of a very large uredinium; at high densities such a reproductive advantage is obviated. The results suggest an upper limit on spore reproductive rate per unit area of host tissue for the bean rust fungus.

Additional keywords: population genetics, prudent parasitism.

Fitness of organisms, including plant pathogens, is not a static parameter. Fitness can be defined as relative or absolute reproduction, often measured as the number of viable offspring. This number is influenced by the environment, and is not likely to be uniform over time any more than the total environment is uniform over time. An ever present, dynamic component of the environment for most foliar pathogens is competition among infection units (lesions, pustules, colonies, or uredinia) for the nutrients that, as parasites, pathogenic microorganisms obtain from the host tissue. Many studies have shown that absolute fitness is density dependent for rusts (7,8,16,19) and powdery mildews (12). These studies all demonstrate that as uredinia or colonies become more crowded they produce fewer urediniospores or conidia $\times$ infection unit^{-1} $\times$ time^{-1}.

In population ecology, density dependent fitness has been demonstrated in several different organisms (3,11,14). More important, in some cases genotypes differ in the degree to which they respond to changes in density (2,5,9,15). In *Drosophila melanogaster* two genotypes have even been shown to reverse rank order of fitness in two different densities (2). Examples of density dependent fitness interactions among variants of plant pathogens have not been demonstrated, to our knowledge.

The objective of these studies was to measure and compare the effects of density on fitness of several isolates of the bean rust fungus, *Uromyces appendiculatus* (Pers.) Unger, that have infection types ranging from large to very large on two cultivars of bean (*Phaseolus vulgaris* L.) for use in mathematical descriptions of evolutionary process in rust fungi.

MATERIALS AND METHODS

Two sets of experiments were conducted involving two cultivars of bean and five isolates of the bean rust fungus. In all experiments, bean seeds were germinated in vermiculite and bean seedlings transplanted singly to 11.7-cm-diameter clay pots containing steamed, sandy loam soil. Inoculation of seedlings, each with a single isolate of the fungus, was done when primary leaves were less than half expanded using an atomizer and suspensions of urediniospores in soltrol 170 oil (Phillips Petroleum Co.). Oil was allowed to evaporate from leaves for about 1 hr, and plants were placed in 100% RH in chambers for about 18 hr. Plants were transferred to benches in a 24 C greenhouse under natural light. Concentrations of urediniospores were varied from 0.5 to 10 mg/ml of oil to obtain a range of densities of uredinia. In addition, different amounts of inoculum suspension were applied to different plants. Uredinia were counted 9 days after inoculation. In some trials, only some of the inoculated plants were selected in order to obtain an even distribution of densities and to avoid plants with obviously uneven uredinal coverage.

The first set of experiments was a preliminary one, using the bean cultivar Topcrop on which the two isolates of the bean rust fungus, KW7-1 and W73-2, developed a large (about 1.1 mm diameter at 12 days after inoculation at 25 C) and very large (about 1.4 mm diameter) uredinium, respectively, at low densities, measured with a 15X calibrated eye lupe. The two single uredinial isolates have been described (6). For each of several runs plants were placed, with isolates alternating, in 16 isolation chambers about $8 \times 8 \times 20$ cm with open fronts. Each chamber had a sheet aluminum trough fitted above the pot but below the leaves of the plant. Every 2–3 days, urediniospores produced on the one to two primary leaves of each plant were collected into glass vials set below the trough by tapping first the leaves and then the trough. Urediniospores were stored in a dessicator as they accumulated until production decreased because of senescence of leaves. They were weighed to 10^{-4} g. Weights were expressed as grams per uredinium. Densities were expressed as uredinia per leaf.

The second series of experiments involved the cultivar Bush Blue Lake and isolate W73-2 plus three additional isolates of the bean rust fungus. Two of the four isolates, U2-1 from Minnesota and W73-2 from Wisconsin produce very large, fully compatible uredinia on Bush Blue Lake that are about the same size as those of W73-2 on Topcrop. The other two isolates, S1-1 and S1-5, were both obtained from a single collection on the cultivar Seafarer grown near Saginaw, MI. These two isolates are not identical for virulence on a set of differential bean lines and have been used in genetic studies of virulence (4). They both produce large uredinia that are slightly but distinctly smaller than those of KW7-1 on Topcrop, hence the comparisons in these experiments were between isolates that differed slightly more than did the two isolates in the preliminary experiments. For each isolate 25 plants were inoculated with a range of urediniospore densities, of which about 20 plants were selected for measurement. In these experiments, urediniospore production was obtained for each leaf by shaking over a large funnel and sweeping the urediniospores into a preweighed plastic vial. Collections were made three times at

3-day intervals, beginning 11 days after inoculation. Urediniospores were stored in a desiccator and weighed 3 days after the last harvest and again a month later on a balance with an accuracy of about ± 0.05 mg. At the end of the experiment the length and width of each infected leaf were measured. Leaf area was estimated using the empirical equation $Y = -8.82 + 1.01X$ ($r = 0.98$ based on measurement of lengths, widths, and electronically digitized areas of 11 fully expanded leaves) in which X is leaf length × leaf width and Y is leaf area. Density was expressed as uredinia/cm^2.

Two of the isolates used in the second set of experiments were also used in identical runs in the growth chamber at 22 C, 15-hr photoperiod at 410 μE m^{-2} sec^{-1} light intensity in order to obtain urediniospore yields over the same range of densities in a different environment.

Densities were logarithmically transformed because their untransformed distributions were not symmetrical about the mean, and, hence, not normal. Urediniospore yields also were logarithmically transformed to reflect the expectation of a linear relationship ln (urediniospores) log of leaf area available to each uredinium. This expectation was supported by the obvious nonlinear relationship when the dependent variable was not transformed.

Simple linear regression analysis of transformed data and t-tests of regression slopes were used to detect isolate × density interactions.

RESULTS

Figure 1 illustrates the scatter plots and regressions of logarithmically transformed urediniospore yields on log of uredinial densities for the two virulent bean rust isolates KW7-1 and W73-2 on the bean cultivar Topcrop. The regression slope of W73-2 was significantly ($P < 0.01$) less (more negative) than that of KW7-1, indicating that W73-2 is more rapidly and adversely affected by crowding than is KW7-1. The ratio of urediniospore production by the two isolates was 4.55 at the Y-intercept and becomes equal at a density of 138 uredinia/leaf. Especially for the scatter plot of KW7-1, the residuals were not homoscedastic, and it was apparent that the points were not linearly arranged; rather they are asymptotic with the X axis at higher densities.

Figure 2 presents scatter plots and regressions for the four rust isolates on the bean cultivar Bush Blue Lake. Improvements in methodology were reflected in improved regressions and fidelity to the assumptions of good regression analysis. In all four comparisons of pairs of differentially virulent isolates, slope differences were highly significant at $P < 0.01$ except W73-2 vs.
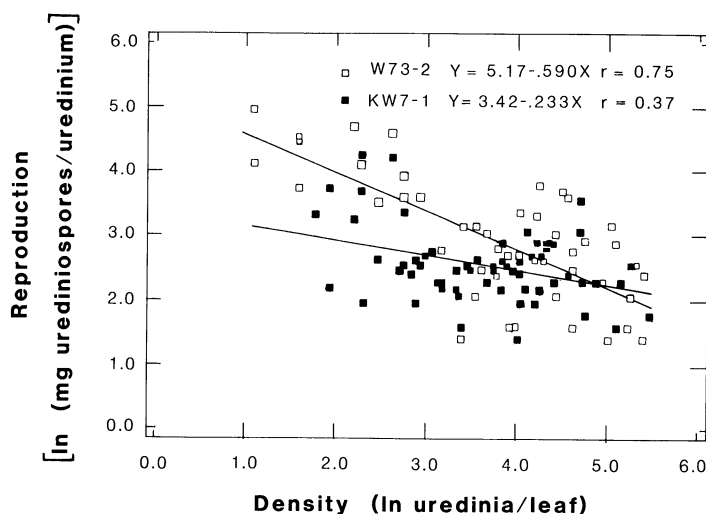
S1-1, which was significant at $P < 0.05$. Again the isolate having the largest uredinium at low densities had the steeper slope (U2-1 vs S1-1, t = 4.60; U2-1 vs S1-5, t = 4.83; W73-2 vs S1-1, t = 2.65; and W23-2 vs S1-5, t = 2.89). The regression fits were also improved compared with Figure 1 in all instances, with no apparent asymptotic tendencies in the scatter plots.

Figure 3 presents regressions of urediniospore production over density in two different environments for isolates U2-1 and S1-1 of the fungus, representing the two groups of isolates based on uredinial size. The growth chamber was the more favorable environment for both isolates as determined by sporulation per uredinium at low densities. Highly significant steeper slopes were found in both isolates for the growth chamber environment as compared to the greenhouse environment (U2-1, t = 11.23; S1-1, t = 3.86).

Within the two categories of virulence that the five isolates of this study exhibit, comparisons were made at the same range of densities as in Figure 2 only for the four isolates that were on Bush Blue Lake. On that cultivar, it was found that S1-1 produced slightly more urediniospores/uredinium at all densities than did S1-5, but slope comparisons showed no difference (t = 0.44) in response to crowding. These two isolates have been studied genetically (4) and, although they came from the same Michigan field collection, they are genetically distinct. The other two isolates, W73-2 and U2-1, were not significantly different with respect to fitness at low densities, although slope comparisons showed that W73-2 (b = −0.59, r = 0.89) is slightly but significantly (t = 2.31, $P = 0.05$) less affected by crowding than is U2-1 (b = −0.76, r = 0.94).

DISCUSSION

The five isolates of the bean rust fungus used in this study all can be considered virulent on the bean cultivars that they were associated with. They fall into at least two categories of virulence, however, that are visibly distinct. The nature of the interaction of urediniospore production and crowding is best explained by considering that uredinia derive nutrients from a zone of leaf tissue (13) whose area is directly proportional to size and sporulation rate of the uredinium. Hence larger uredinia would begin to interfere with one another at lower densities than would smaller uredinia. A logarithmic transformation of urediniospore production should be more linearly related to crowding than should untransformed urediniospore numbers as the zones to support each uredinium become smaller with crowding in a logarithmic fashion.

One reason that the regression for KW7-1 shown in Figure 1 is heteroscedastic even after logarithmic transformation is that the points were accumulated from several separate sets of plants. The greenhouse environment varied from one set to another, mostly because of seasonal effects in greenhouse light and temperature (daytime highs in late spring sometimes reached 35 C). While density should have been a random variable with respect to time, in fact more of the low density points of KW7-1 were obtained later in the experiment, during spring when conditions were more favorable for reproduction. This was not the case for W73-2. This would contribute to the apparent heteroscedasticy of KW7-1 specifically, as shown by inspection of the scatter plots of the two in Figure 1 as well as to the lower r^2 value of KW7-1. Experiments on Bush Blue Lake avoided this problem as points for each regression were obtained entirely in one run, with compared isolates being run simultaneously.

The interactions shown in Figures 1 and 2 depend on the particular measure of disease increase that was used, namely urediniospores per uredinium. Other measures of reproduction or disease, such as urediniospores per leaf, per unit area, or percentage disease might or might not show the interaction. Because we are interested in the implications of the interaction on pathogen evolution in response to resistance in the host, uredinial fitness is the measure of importance; a uredinium represents one individual or one unit of successful reproduction. As Barrett (1) has pointed out, one of the simplifying assumptions that has been made in describing pathogen fitness as a parameter in genetic

Fig. 1. Scatter plots and regressions of logarithmically transformed urediniospore yields over density for isolates W73-2 and KW7-1 of the bean rust fungus on the bean cultivar Topcrop. Slopes of the two regressions are significantly different ($P = 0.01$).

models of pathogen increase is that all genotypes respond identically to crowding. Departure from this assumption will necessitate greater complexity in mathematical descriptions of pathogen increase, and the manner in which crowding slows reproduction of different genotypes of the pathogen.

Perhaps the most far-reaching implication of these interactions is in how they affect predictions about the durability of resistance. Skylakakis (17) has stated that both the intensity and duration of selection are important in determining the likelihood that a selectively favored virulent or fungicide resistant form of a pathogen will predominate. The interactions shown in this work should reduce both the intensity and duration of selection for the very large uredinial forms of the bean rust pathogen as the epidemic progresses and crowding increases.

For years, ecologists have speculated as to whether a special form of group selection is operating in predator-prey, and by extension host-parasite, systems against maximized parasite fitness on the grounds that the best strategy for the parasite is to optimize fitness by balancing short-term reproduction against host survival. This is sometimes referred to as prudent predation (10,18). The controversy is about whether prudent predation represents fitness altruism (sacrifice of the fitness of the individual for that of the group, population, or species) or can be accounted for through maximized fitness of each individual, or Darwinian selection. In rust pathogens, one might think of a type 4 (largest) uredinium as being more fit than a type 3. In the short term, this is

true. But in the long term, it depends on the shape and duration of the epidemic, which might be severely curtailed due to crowding for the larger infection type. It is possible for the overall fitness of the larger infection type to drop below that of the smaller infection type if two conditions are met: 1) urediniospore production of the larger infection type at late, high levels of reproduction is curtailed for an extended period of time due to crowding, and 2) successful reproduction by progeny urediniospores of the larger type is relatively lower than that of the smaller type because of reduced availability of susceptible host tissue (this assumes that effective reproduction is measured as number of successfully reproducing progeny, and that disease increase is from within-field reproduction). Total reproduction of the larger uredinial type, as measured as the area under the curve of reproduction over time would be rendered lower than that of the smaller type as the regression lines of effective reproduction over density of the two uredinial sizes crossed, with the bulk of reproduction occurring at densities above the point where the lines cross. In this case, what appeared to be a case of prudent parasitism is really an example of Darwinian selection (18).

The interaction shown in this work might operate to minimize the impact on the host of large uredinial reactions by reducing the slope of disease increase at a relatively low level of infection. It is not known, however, whether fewer and larger uredinia will damage the host more or less than will more but smaller uredinia. Therefore the interaction has implications for pathogen evolution
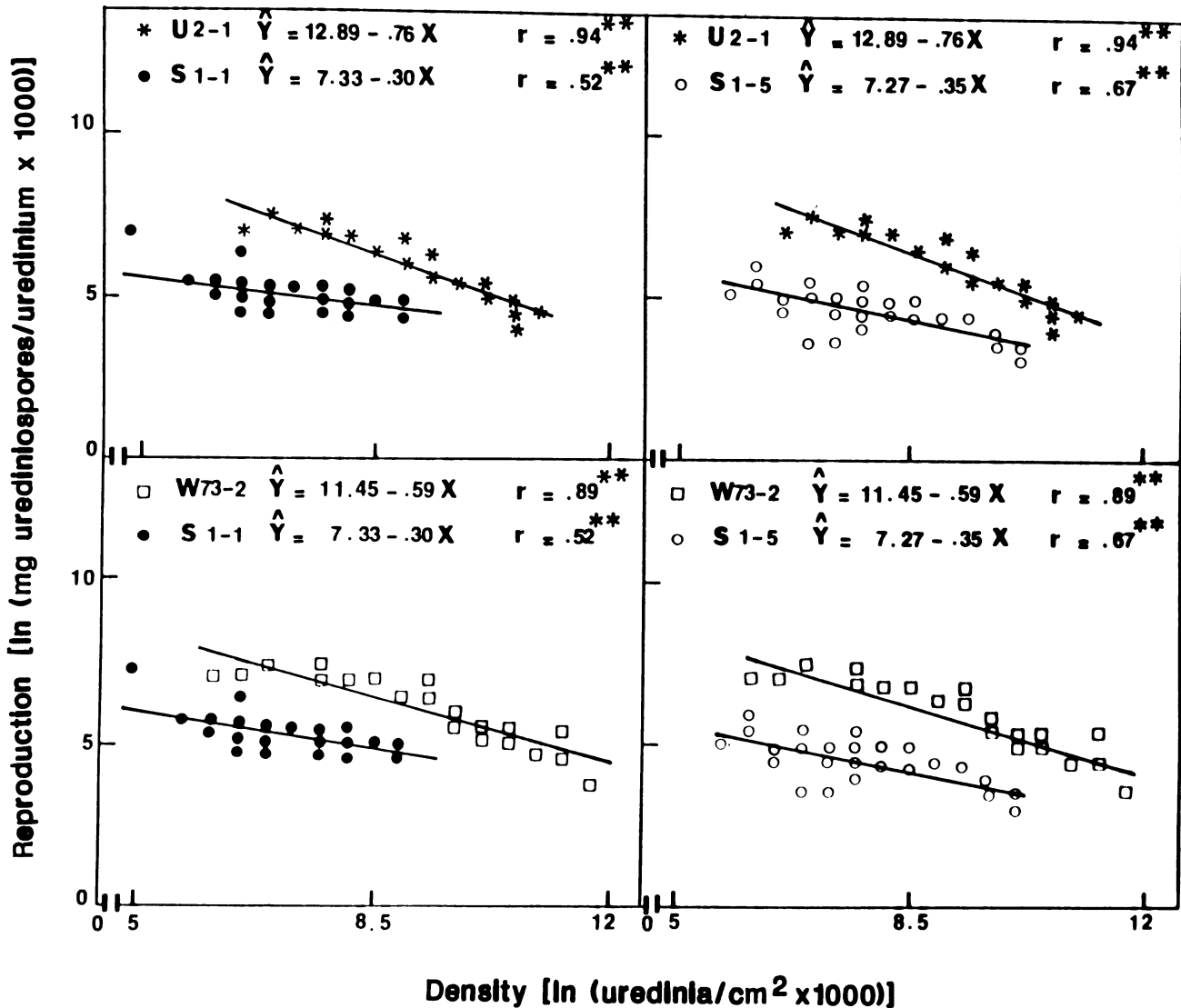

Fig. 2. Scatter plots and regressions of logarithmically transformed urediniospore yields over density for isolates S1-1, S1-5, W73-2, and U2-1 of the bean rust fungus on the bean cultivar Bush Blue Lake. In each paired comparison regression slopes are significantly different.
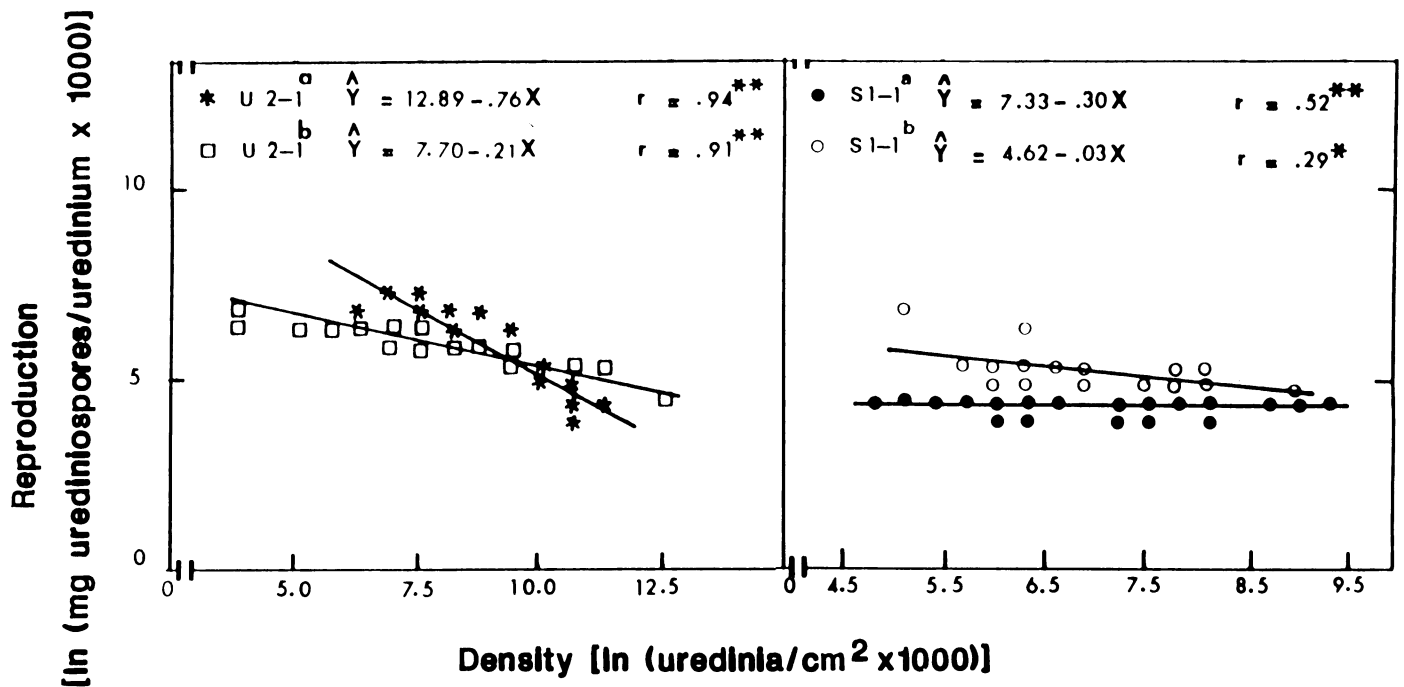
Fig. 3. Scatter plots and regressions of logarithmically transformed urediniospore yields over density for isolates U2-1 and S1-1 of the bean rust fungus on the bean cultivar Bush Blue Lake in a growth chamber vs. a greenhouse environment. Slopes of regressions in the growth chamber environment are significantly steeper for both isolates at $P = 0.01$.

but not necessarily for disease losses. In terms of pathogen reproduction on a per unit area of host tissue basis, the data suggest that on a given host line, there is an upper limit to reproduction by the bean rust pathogen that is independent of uredinial size, at least in the range of sizes used in these studies.

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