Contrasting Effects of Asexual Reproduction and Random Mating on Changes in Virulence Frequency in a Field Collection of *Uromyces appendiculatus*

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Published as paper 20,847 of the contribution series of the Minnesota Agricultural Experiment Station based on research conducted under Project 22-73 supported by USDA Competitive Grant 58-5759-G-1.

Accepted for publication 17 February 1994.

ABSTRACT

Groth, J. V., and Ozmon, E. A. 1994. Contrasting effects of asexual reproduction and random mating on changes in virulence frequency in a field collection of *Uromyces appendiculatus*. Phytopathology 84:566-569.

A single local field collection of urediniospores of *Uromyces appendiculatus* was used to initiate a series of sexual (including the entire life cycle of the pathogen) and asexual (urediniospores only) generations on Pinto U.I. 111, a susceptible and presumably nonselective cultivar of bean (*Phaseolus vulgaris*). In parallel studies of five complete or 10 uredinial generations, virulence frequency was monitored on 15 differential bean lines to which the original collection was polymorphic. These 15 lines were placed in four groups whose members shared the same effective virulence gene. In every case, frequencies of virulence quickly diverged for sexual vs. asexual cycles. For three of the groups, which contained the putative effective genes *Ur C, Up 1,* and *Ur epi,* asexual cycling resulted in more change in gene frequency than did sexual cycling. The fourth group, with the line containing *Ur N,* changed only during the first cycle but changed positively for the asexual and negatively for the sexual cycle.

Additional keywords: fitness, polymorphism, selection, sexual reproduction.

Virulence frequency dynamics in plant-pathogenic fungi are poorly understood (6). Often, large changes in virulence in a pathogen can be linked to increases or decreases in the frequency of a corresponding resistance gene in the host (13). Change does not always occur, however; and knowledge is limited of the forces that allow change to begin or that influence the rate and magnitude of virulence frequency change (3,5).

Plant-pathogenic fungi can reproduce sexually or asexually, and those with a more complex life cycle may undergo regular,

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seasonal alternation of a single sexual cycle with several asexual cycles. This mode of reproduction is common in nature and is thought to be genetically advantageous to many organisms because it permits recombination followed by several cycles of intense selection on the recombinant progeny (12,14). Highly fit genotypes are not only conserved in asexual cycles, but they can become predominant quickly. Among microorganisms, all major groups of fungi, algae, and even some parasitic insects, such as aphids, exhibit alternation of sexual and asexual cycles. Yet apparently this is not the best strategy of reproduction for every species. In various ways, many fungi have departed from regular alternation. Members of the Deuteromycotina are quite successful in spite of their total lack of true sexual reproduction. All degrees of reduction in the frequency of sexual reproduction can be found among species within most groups of fungi. At the other extreme, most smut fungi appear to be obligately sexually reproducing, if one assumes that haploid sporidial budding is minimal in nature.

With nearly all plant-pathogenic fungi, disease epidemics are sustained by asexual, rather than sexual, reproduction. Because of this, and perhaps because methods for working with the pathogens asexually are simpler and more available, nearly all empirical data on virulence frequency change is limited to asexual generations (6).

Alexander et al (1) documented that virulence changes that are specific to certain host genotypes occurred readily in a bulk field collection of *Uromyces appendiculatus* (Pers.:Pers.) Unger that was cycled through five uredinial generations on Slimgreen, a partially and, as far as is known, nonspecifically resistant bean (*Phaseolus vulgaris* L.) cultivar. Virulence frequency usually decreased, but virulence to one bean line, US#3, increased. The objective of this study was to compare changes in virulence in similar serial cycles of asexual generations with cycles that include the entire sexual and asexual macrocycle of the bean rust fungus.

MATERIALS AND METHODS

Origin of the experimental population. Field collection P21 of bean rust was made in August 1982 in Renville County, Minnesota, from a commercial field of Pinto U.I. 111, a cultivar with no observable resistance to rust collected from dry beans in Minnesota (8). Urediniospores of this collection were stored at −50 C after drying in a desiccator for 48 h.

Asexual generations. A suspension of urediniospores in Soltrol 170 light oil (Phillips Petroleum Company) was sprayed on partially expanded unifoliate leaves of 50–60 Pinto 111 plants with an atomizer inoculator. After the leaves had dried for 1 h, the plants were misted with water and placed in 100% relative humidity chambers for approximately 24 h, after which they were placed in a greenhouse at 20–25 C. Trifoliate leaves were removed to maintain the unifoliate leaves. Plants with 50–200 uniformly distributed uredinia per leaf were retained in each cycle. The urediniospores were harvested every 2–3 days during a period of approximately 14–21 days after inoculation by gently tapping the plant stems over clean aluminum foil. The spores were placed in #00 gelatin capsules and desiccated for 2–3 days over anhydrous calcium sulfate at 10 C. All collections made during a given period were bulked, sealed in plastic, and stored at −50 C. Two asexual generations were produced during the time period of one complete sexual generation.

Sexual generations. Inoculation and collection of urediniospores were made as for asexual generations. Immediately after the last spore collection, the plants were moved to a greenhouse at 15–20 C. Most of the uredinia had converted to telia by 6–8 wk after inoculation. After all leaves were collected (at senescence), they were washed overnight in running tap water to soften them and to remove contaminant phylloplane organisms. Teliospores were scraped off into distilled water, strained through cheesecloth to remove small leaf pieces, and collected by vacuum suction on glass microfiber filters. They were air dried overnight and stored at 10 C (7).

Dry teliospores (250 mg) from pads stored on filters were suspended in about 1 ml of distilled water and ground gently with a mortar and pestle to separate individual spores. Approximately 10 drops of the suspension (up to 50 mg of teliospores, dry weight) were placed on each of five relatively thick 2% water agar plates and spread out with the pestle to 0.5 cm from the plate edge. The plates were left open until no surface water remained. They were then closed and placed in a 100% relative humidity chamber under north window light until the teliospores germinated. Plates were examined at approximately 9:00 A.M. When at least 1–2 metabasidia per 100× microscope field were seen, the plates were inverted on top of clear plastic cylinders over young Pinto 111 plants, creating a high humidity atmosphere. Two plants were used per pot and 15–30 pots per generation. The mature basidiospores were shot off the metabasidia onto the leaves. The plates were left over the plants for 1–3 days, depending on the rate of basidiospore production. If basidiospores were still being produced, the plates were transferred to new plants or, if not, to the 100% relative humidity chamber to initiate another round of germination.

After 1–2 wk, five to 300 pycnia appeared on the upper surface of each leaf (the average number per leaf was approximately 100). The number of pycnia per generation was 3,550–10,000; the average was 6,000. When they exuded nectar, pycnia were mass fertilized by rubbing the leaves with an alcohol-rinsed finger that had been dipped in distilled water, spreading the spermatia over all the pycnia on the leaf, and transferring pycniospores among leaves, thus approximating random mating in a large population (9). This was done daily until no more nectar appeared. Fertilized pycnia produced aecia in about 7 days on the lower sides of the leaves. When the aecia began to break, the aeciospores were collected by vacuum aspiration directly into #00 gelatin capsules, desiccated 2–3 days at 10 C, sealed in plastic, and stored at −50 C.

A suspension of aeciospores in Soltrol was used to inoculate 50–60 plants as previously described and produce the first (F_1) generation of urediniospores. These were collected, and the plants were kept at 16–18 C for teliospore production.

Virulence rating. Virulence frequency was determined on 15 differential lines or cultivars of snap and dry beans that had produced mixed infection types (necrotic flecks and normal uredinia) when inoculated with the original collection. This indicated that the collection was polymorphic for virulence on the bean line or cultivar. Partially expanded unifoliate leaves were inoculated with four samples per generation, two from sexual cycles (aeciospores and urediniospores) and two from asexual cycles (both urediniospores). Approximately 2 wk after inoculation, infection types on plants were scored according to the scale of Groth and Shrum (8), and the percentage of virulent types observed was calculated. Infection types 0–3 (chlorotic fleck, necrotic fleck, necrotic fleck with a small amount of sporulation, and minute uredinia, respectively) were considered avirulent reactions, and types 4–9 (nonnecrotic uredinia of increasing size) were considered virulent reactions. Leaves with a total count of 200 or fewer uniformly distributed infections were chosen for scoring to avoid unreliable counts caused by overcrowding. Data from plants or leaves with low counts (fewer than 50) were pooled. Minimum total counts per sample were 1,000 from at least six plants.

RESULTS

The 15 bean lines on which virulence changes in collection P21 were monitored were placed in four groups on the basis of similarity of virulence dynamics in both sexual and asexual lineages. Previous work had established that each group contained at least one bean line with an effective resistance gene that was unique for that group (4). Lines within each group are thought to share the same effective gene on the basis of similarity of frequencies of virulence in the present study. In some cases, this was confirmed by genetic work (2) or by serial transfers of bulk samples of P21 between differential lines that resulted in loss of polymorphism, strongly suggesting gene identity (E. A. Ozmon, *unpublished data*). Figure 1A shows the changes in virulence frequency on the nine lines containing effective gene *Up2* (4) also known as *Ur C* (2). Among the nine lines, changes in frequency

were similar for both sexual and asexual lineages, so data were combined. Sexual frequencies differed significantly from asexual frequencies from the third cycle until the end of the experiment. On these lines, the sexual lineage remained at about the initial frequency, while the asexual lineage declined steadily to a final frequency of about 1.5%. Four bean lines, Pink, B1349, B1431, and B1527, containing another gene tentatively called *Ur epi* (for the cultivar Epicure [2]), are shown in Figure 1B. In this case, the sexual and asexual generations diverged sharply in the first cycle of mating or uredinial reproduction, respectively. The asexual population reached a plateau at approximately 80% after three cycles, while the sexual population, with very low frequencies of virulence, continued to slowly decline through most of the generations. Figure 1C shows the changes of P21 on the bean line US#3, containing the dominant gene *Up 1* (4). Here the asexual lineage slowly increased through three to six cycles to a plateau of about 96% virulence, while the sexual population remained steadily below 60% until the fifth fertilization, when it increased to about 70%. Finally, the bean line 4C-69, known to have a gene that has tentatively been called *Ur N* (2), gave a distinctly different pattern; the asexual population quickly rose and reached a plateau at approximately 90%, while the sexual population decreased and reached a plateau at approximately 18%. In this case, the difference between sexual and asexual populations can be accounted for in the first cycle.

One deviation from this consistency of change must be noted. In Figure 1D at generation 8, the frequency jumped unexpectedly from about 20 to 90% virulence in the sexual cycle. Similar change (12 to 90%) occurred for all the *Ur epi* lines (not shown), suggesting that the sample of aecia was effectively much smaller than the 600 that had been counted and collected from after the mass self of cycle 8. We surmise that either all 600 aecia were not represented in the sample or that because of differential success of some genotypes, a small number of aeciospores gave rise to the next uredinial generation, resulting in a strong founder effect. Similar large changes in virulence frequency were not seen for *Ur C* or *Up 1* lines. Cycle 8 was repeated with additional teliospores from the same batch of cycle 7, and about 6,600 aecia were produced and sampled. This second time, virulence frequencies were consistent with trends of other generations, as shown in Figure 1B and D.

DISCUSSION

In this study, including sexual recombination in the selection cycle of bean rust had a large impact on the rate and direction of change in virulence frequency. Alexander et al (1) first documented with this same collection of the bean rust fungus that virulence frequencies changed rapidly when no evident directional selection relevant to the virulence markers being monitored was being exerted on the population. Their study was based only on asexual reproduction, so that large numbers of new genotypes were not being generated. The direction of change in virulence frequency for asexual cycles in the present study is the same as that in Alexander et al (1) for two of three bean lines included in both, Early Gallatin and US#3. For the third line, B1349, the direction of change was negative in the study of Alexander et al but positive, at least initially, in the present study. This could have been caused by differences in the greenhouse environment; plants in the earlier study were kept in enclosed plastic chambers, whereas they were not in the present study.

Initial frequencies in the two studies were similar for all but Early Gallatin, on which about a 10% lower frequency was observed in the present study than in that of Alexander et al (1). This is understandable, since an additional increase was necessary to obtain enough urediniospores to initiate the large numbers required to complete the present studies with a single founding population and the direction of change of the population was downward in asexual generations in the earlier study.

Most of the change in virulence frequency can be ascribed to

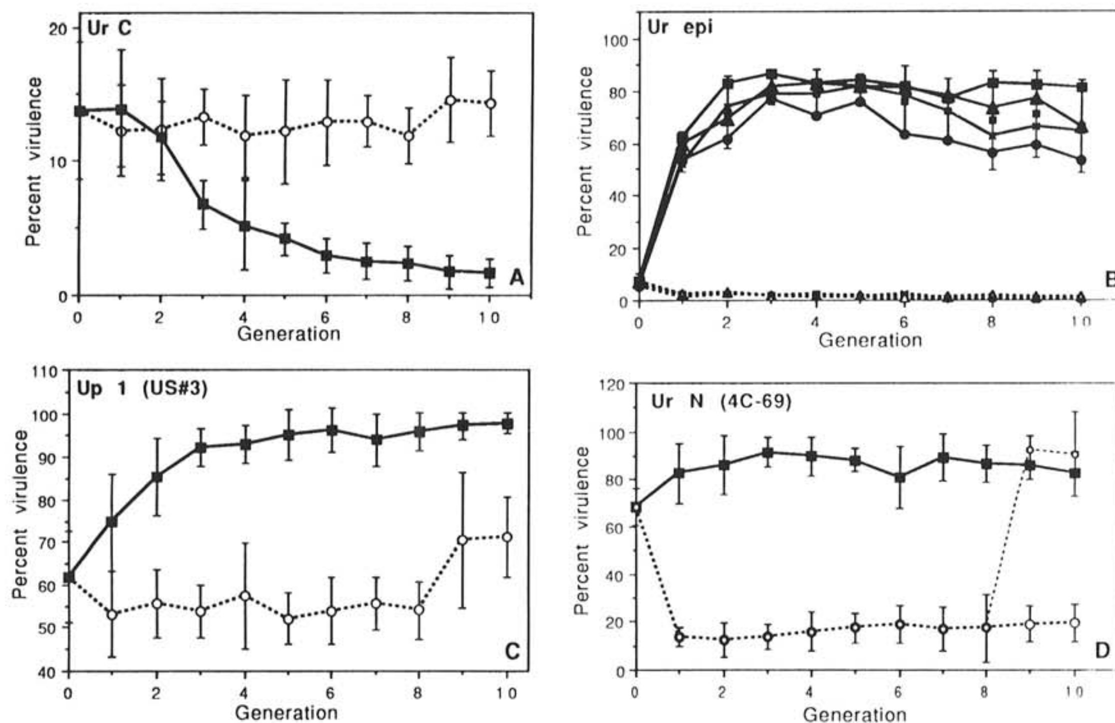

Fig. 1. Changes in virulence frequency in *Uromyces appendiculatus* uredinial field collection P21 on susceptible Pinto 111 beans through five complete generations (sexual-asexual cycles, broken lines) or 10 uredinial generations (asexual cycles, solid lines) on 15 bean lines that are placed in four groups on the basis of possession of effective resistance genes **A,** *Ur C;* **B,** *Ur epi;* **C,** *Up 1;* and **D,** *Ur N.* The *Ur C* gene group (**A**) shows only mean frequencies of the combined data for the nine cultivars or lines Early Gallatin, Bonanza, Executive, Bush Blue Lake, Pencil Pod Black Wax, Improved Tendergreen, Black Eye, 780, and 385. The *Ur epi* (**B**) group is composed of four lines: Pink (●), B1349 (■), B1431 (▲), and B1527 (x), and weighted means of the four are indicated by the smaller, solid, unconnected squares. Single bean lines are shown in **C and D.** Confidence intervals (vertical lines) are based on variation among lines (**A and B**) or among units of count (plants or leaves, **C and D**); *t* distribution ($\alpha = 0.05$).

selection; samples were generally large enough that drift could not account for changes observed, nor would changes due to drift be expected to be as consistent from cycle to cycle as were observed. The instance of apparent drift noted above occurred in the smallest sample of aecia obtained in the entire study.

Most of the divergence between sexual and asexual generations was accounted for by changes in the asexual populations. Except on *Ur N,* the sexual population maintained and ended with virulence frequencies that were not significantly different from the original frequencies. This suggests that in the field, selection has operated at the level of the alleles at individual loci rather than the whole genotype, at least at the beginning of the growing season. This would tend to eliminate alleles with major negative fitness effects. In this study, regular recombination might be expected to homogenize genotypes for fitness, given that fitness is a function of the small effects of many genes. The asexual population is clonal, and the unit of selection is the whole genotype. The number of genotypes would be fixed at the beginning and limited relative to the number being generated during each of the five sexual cycles of the sexual population. This apparently allows selection in the asexual population to change the frequency of virulence during the cycles, with the rate of change tapering off as the frequency approaches fixation (except on *Ur epi*). Whole, invariate genotypes can be expected to have greater mean fitness differences than do genotypes that are regularly recombining their genes. It seems more likely that changes in the frequency of virulence are a result of hitchhiking (11) rather than of direct fitness effects of the virulence. Asexual reproduction causes virulence to be effectively linked to all other genes; fitness of predominant races containing a virulence gene will determine the direction and rate of change of that virulence.

The initial population may or may not have been in Hardy-Weinberg equilibrium for the virulence genes that were monitored. If it was not, change might be expected to occur only at the first sexual cycle in the absence of selection, if the assumptions that the initial population is large and randomly mating are met. This is evident only for *Ur N.* The rapid decrease during only the first sexual cycle that is seen for this gene could result in part from an initial population that is inbred (having a lower frequency of heterozygotes than expected from Hardy-Weinberg equilibrium) if virulence is recessive or from an overdominant gene (having more heterozygotes than expected) for dominant virulence. There is no apparent change in subsequent generations of sexual *Ur N,* which is consistent with the expectation that, ideally, Hardy-Weinberg equilibrium can be reestablished in a single sexual cycle. However, the decrease in frequency in either case is too large to be entirely ascribed to this random mating effect. Selection must have also been operating against virulence to *Ur N* in the sexual population. The only other results that can be cited regarding Hardy-Weinberg equilibria in this fungus employ an isozyme locus. In a previous study, P21 was unfortunately not polymorphic for the allozymes of phosphoglucose isomerase, and of three other collections (not used in this study) that were polymorphic, the only one that was not in Hardy-Weinberg equilibrium had a large excess of heterozygotes (10).

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