Population Biology of the *Rhizoctonia solani* Complex

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The *Rhizoctonia solani* complex represents an economically important group of soilborne basidiomycete pathogens that occur on many plant species throughout the world (41). Identification and study of *Rhizoctonia* species has always presented a challenge because of their ill-defined taxonomy and our poor understanding of their natural history. Although identification of isolates as *R. solani* is largely dependent on vegetative characters, members of this group are known to be associated with a *Thanatephorus* teleomorph, or sexual stage. Several recent reviews discuss the taxonomy of different groups in *Rhizoctonia* (34,41,44). Although previous research has focused primarily on taxonomic characterization and identification of *R. solani*, very little information about their population biology and genetics is available. In this paper, we review some recent progress in understanding the population biology and genetics of fungi in the *R. solani* complex and discuss possible research avenues for investigating *R. solani* genetic diversity in natural populations.

Anastomosis Grouping

The study of population biology and genetics in *Rhizoctonia* has been confounded by the growing realization that *R. solani* is actually a large species complex composed of many genetically distinct groups with very diverse life histories (34,41). Current classification within the *R. solani* complex is based largely on grouping of isolates into anastomosis groups (AG). At least 12 AG have been described within the *R. solani* complex (Table 1). Many AG, including AG1 through 4, 6, 8, and 9, have been subdivided further into subgroups that differ for one or more biochemical, genetic, or pathogenic characteristics (16,23,24,26,28). These studies all suggest that genetically diverse AG and their subgroups represent independent evolutionary groupings within *R. solani* (44).

Anastomosis grouping still represents the single most important advance in our understanding of the genetic diversity within *Rhizoctonia*. Adams (1) recently stated that "outsiders frequently complain about preoccupation with the characterization and identification of *R. solani* AG and subgroups…but these studies have matured our concept of the evolutionary unit in *Rhizoctonia*." Although the mechanisms and biological relevance of anastomosis reactions are still not completely understood, they are believed to represent somatic interactions between genetically distinct or similar fungal individuals. Recent studies have contributed greatly to our understanding of somatic interactions in many fungi, including *Rhizoctonia*. The paradigm underlying all of these studies is the concept of fungal individualism established by Rayner et al. (37–39), which shows that genetically distinct individuals can be distinguished by somatic incompatibility interactions. In *R. solani*, somatic incompatibility (or compatibility) is observed most directly at the microscopic level between paired isolates (40). Pairing of isolates belonging to the same AG results in hyphal fusion (anastomosis), leading to either acceptance (self-pairings) or rejection (somatic incompatibility). Pairings between AG do not result in hyphal fusion, suggesting greater genetic differences between isolates (i.e., different species, etc.).

The criteria used to delimit AG in *R. solani* are summarized in Table 2 (28). Four classes of reactions can be distinguished according to the degree of interaction (and presumed genetic relatedness) between hyphae from interacting isolates. Interactions between AG (C0 and C1) generally result in little or no hyphal fusion between isolates. Within an AG, two types of interactions (C2 and C3) are most relevant for the study of population biology. The C2 reaction, also referred to as the killing reaction, represents a somatic incompatibility response between genetically distinct individuals. The C3 reaction (perfect fusion) between two isolates is indicative of genetic identity or near-identity. For population studies, C2 reactions provide a means of defining individuals within a population, whereas C3 reactions are expected to occur between clones or very closely related individuals. The ability to distinguish between C2 and C3 reactions provides a means of identifying what constitutes a population in different *R. solani* AG but only rarely has been used to characterize *R. solani* populations (28,35).

Interpretation of anastomosis reactions is not always straightforward. The four hyphal interaction phenotypes (C0 to C3) represent a continuum, and reproducibility of AG interactions can be affected by factors such as laboratory environment, nutritional conditions, and genetic instability (14,17,51). In general, adherence to standardized protocols for AG testing appears to provide more reproducible results with certain *Rhizoctonia* spp. (40). The modified 4′,6-diamidino-2-phenylindole staining procedure recently described by Kulik and Dery (20) also may be a useful tool for studying *Rhizoctonia* population biology and genetics by substantially reducing the time required to determine microscopic hyphal interaction phenotypes. The development of less laborious methods of determining somatic interactions and genetic relatedness of *Rhizoctonia* isolates is needed.

Another important factor influencing the outcome of AG pairings is the genetic state of interacting isolates, which may be either homo- or heterokaryotic or both (1). For pairing studies involving homokaryotic strains especially, sexual interactions also are possible, and these may complicate interpretation of anastomosis interactions. The dynamics of asexual and sexual interactions are poorly understood in most fungi (3,39), especially in *R. solani*, because

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heterokaryons are not easily distinguished from homokaryons (1, 10). The occurrence of homokaryons in nature also increases the possibility of interactions between homo- and heterokaryotic isolates. The role of all these hyphal interactions in the interpretation of anastomosis tests is not well understood and deserves further investigation in a population biology context.

Looking Beyond the AG

The separation of *R. solani* into distinct AG and subgroups provides a long-sought foundation for examining population biology and genetics. Depending on one's definition, several hierarchical organism groupings below the AG level might be considered as species, populations, individuals, and clones. The population level (and lower) is still the least well understood in *R. solani,* and the population genetic structure also remains virtually unknown within any *R. solani* AG.

Mating Compatibility Systems

Some of the first questions addressed by *Rhizoctonia* workers focused on determining the type of mating compatibility system associated with specific AG. Mating systems govern patterns of sexual reproduction in natural populations and have a direct effect on population structure. In other basidiomycetes, heterothallic mating systems promote higher levels of outcrossing, whereas homothallism can lead to a higher proportion of inbreeding in populations through self-mating. Homothallism by itself, however, does not necessarily prevent outcrossing from occurring in homothallic fungal species, which has been observed with other fungal genera (3).

Unfortunately, analysis of mating compatibility in *R. solani* is not as straightforward as in other groups of basidiomycetes that have regular monokaryotic and dikaryotic life histories. In all groups of *R. solani*, hyphae of both homokaryons and heterokaryons are multinucleate and lack clamp connections and, thus, lack diagnostic morphological characteristics for detecting mating reactions. Instead, researchers studying *R. solani* have traditionally relied on the formation of aerial tuft hyphae within the interaction zone between paired homokaryons to assess heterokaryon formation (36, 46). In our laboratory, studies with DNA markers to confirm crosses have found that tuft formation may not always be associated with heterokaryon formation (10). Assessment of mating behavior is further complicated by the fact that most field isolates of *R. solani* that sporulate in nature do not readily sporulate in vitro (4,34).

Several AG in *R. solani* that have been investigated possess either homothallic (self-fertile) or bipolar, heterothallic mating systems (Table 1). At least two AG (AG1-IC and AG4) have heterothallic, bipolar mating systems controlled by a single genetic factor with multiple alleles (1). Initial studies suggest that the mating system of AG8 is also bipolar (48). Several AG with heterothallic mating systems exhibit homothallic behavior as well, through a process known as homokaryotic fruiting (2). Homothallic mating behavior has been reported for several AG, including AG1 through 4. Homothallism can have predictable consequences for population structure, because progeny that arise through homothallic self-mating are genetically identical to each other and to their parent. Both heterothallic and homothallic AG also may undergo recombination through the process of heterokaryon-homokaryon (di-mon) mating originally described by Buller (8). For di-mon mating to occur, basidiospores must germinate on soil or plant tissue and produce a viable homokaryotic mycelium capable of interacting with resident heterokaryotic mycelium. However, previous studies have shown that *Thanatephorus* basidiospores are difficult to germinate and that single basidiospore isolates usually are less virulent and have more limited saprophytic capabilities than their parental heterokaryon (9,49). Although di-mon mating in *R. solani* is easily demonstrated in laboratory crosses (10,17), little is known about its prevalence in populations.

The extent of homothallic versus heterothallic reproduction in most AG and subgroups is unknown. In most *R. solani* AG and subgroups, the asexual stage often is viewed as the predominant stage in their life history, although the sexual stage of many *R. solani* AG (e.g., AG1 through 5) is frequently observed in agricultural fields. Hymenia of *Thanatephorus* spp. consisting of basidia, basidiospores, and sterigmata often form near the base of a plant within the canopy at or just above the soil surface. More critical studies are needed to assess the prevalence of homothallic and other forms of asexual reproduction in natural populations. In general, the relative importance of sexual and asexual reproduc-

TABLE 1. List of anastomosis groups (AG) in the *Rhizoctonia solani* complex, with information about mating systems, genetic markers, and population structure

Anastomosis group	Mating system	Genetic markers employed[a]	Population structure
AG1	Primarily heterothallic[b]	Isozymes, rDNA, and RAPD RFLP	Outcrossing
AG2	Presumed homothallic	Isozymes, rDNA, and RAPD RFLP	Clonal/outcrossing
AG3	Presumed homothallic	Isozymes, DNA fingerprinting, rDNA, and scn DNA RFLP	Clonal/outcrossing
AG4	Primarily heterothallic[b]	rDNA and RAPD RFLP	Outcrossing
AG5	?	rDNA RFLP	?
AG6	?	rDNA RFLP	?
AG7	?	rDNA RFLP	?
AG8	Primarily heterothallic[b]	Isozymes, DNA fingerprinting, rDNA, and RAPD RFLP	Clonal/outcrossing
AG9	?	Isozymes and rDNA RFLP	?
AG10	?	rDNA RFLP	?
AG11 or AGBI	?	?	?

[a] RAPD = randomly amplified polymorphic DNA; rDNA = ribosomal DNA; RFLP = restriction fragment length polymorphism; scn DNA = single-copy nuclear DNA; and ? = data not available.
[b] Several AG with heterothallic mating systems may exhibit homothallic behavior as well through a process known as homokaryotic fruiting.

TABLE 2. Classification of hyphal anastomosis reactions using the criteria of MacNish et al. (28) to delimit anastomosis groups (AG) in *Rhizoctonia solani*

Reaction	Phenotype	Nature of genetic relationship between isolates
C0: No interaction	Hyphae grow past each other, no recognition	Isolates have no genetic relationship and belong to different AG
C1: Hyphal contact only	No evidence of wall or membrane contact, reaction may or may not be accompanied by cell death	Isolates have a distant genetic relationship and belong to either the same or different AG
C2: Killing reaction	Wall fusion (anastomosis) evident, with cell death of anastomosing and adjacent cells, somatic incompatibility response often macroscopically visible	Isolates represent genetically distinct individuals that belong to the same AG
C3: Perfect fusion	Wall and membrane fusion evident, point of anastomosis not clearly visible, cell death absent	Isolates are genetically identical or closely related, individuals belong to the same AG and may represent clones

tion and their relative contribution to genetic diversity and structure in natural populations has not been addressed in many fungi (3). Is the observed genetic diversity in different *R. solani* AG and subgroups associated with asexual or sexual reproduction or a combination of both?

Adams' recent review (1) provides some interesting insights into the significance of asexual and sexual reproductive strategies in *R. solani*. For some *R. solani* AG, wind-dispersed basidiospores may serve as the primary inocula for inciting foliar diseases of various cultivated plant species under favorable environmental conditions (32). In general, however, basidiospores are not the primary inocula responsible for infections, and most *Rhizoctonia* diseases result from initial infection by propagules (sclerotia or mycelia, often in association with plant debris) that are able to survive for many years in soil. Disease resulting from infection via sclerotia or mycelia often appear as clusters (aggregates) of infected plants within a field, whereas disease resulting from basidiospore infection often appears as a random, uniform pattern of infected plants throughout the field (32). Although basidiospores may not be of epidemiological significance for most *Rhizoctonia* root and seedling diseases during a growing season, they may have important ecological and population consequences. Innate mechanisms of basidiospore dispersal (i.e., repetitive spore germination and forcible discharge at night), as well as the immigration and vegetative spread of sclerotia and mycelium associated with agricultural practices, are well documented and would contribute significantly to the temporal and spatial distribution of *R. solani* genotypes in natural populations. Meiotic reproduction may provide a mechanism for generating genetic diversity in a population even if the primary mode of reproduction is asexual (30).

Molecular Markers in *Rhizoctonia*—The Need for Population Genetics

Future advances in understanding *Rhizoctonia* population biology almost certainly will rely heavily on the application of genetic markers to characterize the genotypic structure of populations. A variety of molecular markers have been applied to the study of *Rhizoctonia* spp. at different taxonomic levels (Table 3). The majority of these studies have been aimed primarily at establishing genetic differences between AG and subgroups (34,44). Awareness of the differences between groups should now help researchers focus attention on the considerable amount of genetic variation that exists within AG.

What are the characteristics of a "good" genetic marker for examining populations? Some of the molecular approaches with potential for addressing genetic diversity at the population level in *R. solani* include isozymes (23,25,42), restriction fragment length polymorphism (RFLP) (11,15), DNA fingerprinting (5,29,45), and randomly amplified polymorphic DNA (RAPD) (12,50). One criterion to consider is the utility of a particular marker for inferring an unambiguous genotype for each individual (strain) in a given population. Single-locus genetic markers such as isozymes and DNA-based RFLP are probably most useful for analyzing population structure because they provide information on a locus-by-locus basis that can be analyzed by standard population genetic methods. To identify genetically distinct strains (clones) in a population, methods that survey many loci at once (such as RAPD or DNA fingerprinting) might provide a higher level of discrimination than single-locus markers. Because each method has its own advantages and limitations, in many instances it may be desirable to use a combination of different methods. Issues that need to be considered concerning any molecular method include practical considerations regarding methodological and logistical aspects (sample preparation, reproducibility, cost, and safety), as well as analytical considerations regarding the genetic basis of a marker (e.g., sensitivity to genetic differences and dominance).

TABLE 3. Genetic markers employed previously to study genetic variation in different *Rhizoctonia solani* anastomosis groups (AG) and their utility at different taxonomic levels

	Utility of genetic marker			
Genetic marker[a]	Above species level	Species/AG level	Population level	Reference
Cellular fatty acids	X	X		16
DNA fingerprinting			X	5, 29, 45
DNA/DNA hybridization	X	X		21
Electrophoretic karyotyping		X	X	18
Isozymes/zymograms		X	X	23, 26, 28, 42
RAPD RFLP		X	X	10, 12, 43, 47, 50
rDNA RFLP	X	X		11, 15, 24–26, 33
scn DNA RFLP		X	X	15

[a] RAPD = randomly amplified polymorphic DNA; rDNA = ribosomal DNA; RFLP = restriction fragment length polymorphism; and scn DNA = single-copy nuclear DNA.

A variety of cytoplasmic genetic elements have been described in *R. solani*, including double-stranded RNA (dsRNA) and DNA plasmids (reviewed in reference 44), which may potentially be useful genetic markers. Although some studies have suggested a role for these cytoplasmic elements in pathogenicity, more extensive surveys of both plasmid and dsRNA elements have failed to find any clear associations (6,7). Although several studies also have demonstrated that dsRNA and plasmid diversity are clearly linked with differences among AG, more work is needed to understand the patterns of cytoplasmic element diversity within an AG. For example, are plasmids or dsRNAs transmitted via hyphal anastomosis when C2 or C3 interactions occur or can they be transmitted via basidiospores? No studies, to our knowledge, have attempted to address the distribution and transmission of cytoplasmic elements as genetic markers in *R. solani* populations. Such studies clearly are needed before we are able to understand more about the occurrence and transmission of cytoplasmic elements in natural populations.

Mitochondrial DNA (mtDNA) is another untapped source of cytoplasmically inherited markers for investigating *Rhizoctonia* population biology. In other groups of fungi, patterns of mtDNA diversity have revealed a great deal about patterns of clonality and genetic structure in agricultural and wild fungal populations (13, 19,31). As with dsRNA and plasmids, very little is known about mtDNA diversity in different *R. solani* AG. Future studies need to address basic questions about the transmission and inheritance of mtDNA, as well as the utility of mtDNA as a marker for population genetic studies. For example, what is the relationship between mtDNA haplotype and *R. solani* AG/subgroup? Are certain mtDNA haplotypes associated with specific dsRNA and plasmids? How comparable is the genetic diversity of different *R. solani* AG/subgroups based on nuclear versus mitochondrial-based DNA markers?

Few of the studies described above have gone much further than to simply demonstrate the potential utility of genetic markers for understanding population biology in *Rhizoctonia*. Future studies need to address many fundamental questions about the life history of *R. solani* in natural populations: (i) what is the genetic nature of individuals and populations; (ii) does recombination occur; (iii) how prevalent is clonal reproduction; and (iv) how are populations established and maintained? In all cases, both variation and heritability of genetic markers need to be established. In addition, markers must be stable enough that different laboratories are able to repeat the results of different studies (22,43).

Population Biology

In contrast to many papers presented in this symposium, the study of *Rhizoctonia* population biology and genetics is just beginning to emerge. Several recent studies on AG8 demonstrate how molecular markers might be integrated with hyphal anastomosis behavior to examine population biology in *R. solani* AG8. Most isolates belonging to AG8 are serious pathogens on cereal crops, especially in Australia, where they are associated with bare

patch disease (27). Methods that have been used to characterize AG8 isolates include hyphal anastomosis, pectic isozymes (zymograms), RAPD, and DNA fingerprinting. Using hyphal anastomosis criteria and pectic zymograms, MacNish et al. (28) studied distribution patterns among AG8 isolates in several fields in West Australia. Hyphal anastomosis tests were performed to distinguish between self (C3) and non-self (C1 and C2) reactions among isolates from the same and different fields. Isolates from the same field showing self-identity (C3) reactions always belonged to the same pectic zymogram group (ZG), as would be expected for genetically identical strains (28). Based on these two criteria, their study also found that certain clones belonging to two ZG (ZG1-1 and 1-2) were distributed over very broad geographic distances, including West Australia, Washington, and Oregon. To date, five ZG have been identified within *R. solani* AG8 (ZG1-1 to 1-5), and isolates from the same disease patch usually belonged to the same ZG.

Hyphal anastomosis and pectic zymogram criteria also were used for extensive study of AG8 patch dynamics (28). Isolates between ZG produced a C2 hyphal anastomosis reaction, whereas isolates within a ZG produced either a C2 or C3 reaction. These results suggest a greater degree of genetic diversity within a ZG than was detected previously by pectic zymograms. MacNish (27) recently proposed the term "vegetatively compatible population" for a group of isolates that belonged to the same ZG and produced a C3 hyphal anastomosis reaction with each other.

In another study, Yang et al. (47) showed that isolates of AG8 from two locations in West Australia belonging to two pectic ZG were distinguished by RAPD fingerprints. Within each ZG, isolates were characterized by identical RAPD patterns, suggesting these AG8 isolates were clonal or very closely related. Together, these studies suggest that there is limited genetic variability and that extensive clonal reproduction is present within populations of *R. solani* AG8 in West Australia.

More recently, DNA fingerprinting probes that recognize highly repetitive DNA sequences have been developed to study *R. solani* AG8 (29) and AG3 (5) from South Australia. Matthew et al. (29) identified 15 DNA fingerprints for 16 AG8 isolates they examined. Two of the isolates shared a common DNA fingerprint pattern but were recovered 300 km apart, suggesting they might be clones. Another case of possible clonality for multiple isolates was presented by Balali et al. (5) for AG3 isolates from potato and soil in commercial potato fields in South Australia. Of 136 isolates examined, only 38 DNA fingerprints patterns were identified, and 1 DNA fingerprint was associated with ~30% of the isolates. The repeated recovery of similar genotypes from various geographic regions strongly suggests a clonal population structure for AG3 and also possibly for AG8. In these and the other examples mentioned above, evidence concerning genetic structure would be strengthened by larger sample sizes (both within and among populations), as well as by more information about the genotypic data collected. Interpretation of DNA fingerprints in many fungi is complicated by the fact that individual bands are dominant and may not effectively distinguish homo- versus heterozygotes. In the absence of other evidence, DNA fingerprinting utilized in conjunction with somatic compatibility and pectic zymogram patterns is beginning to provide an initial picture of the genetic structure for some *R. solani* AG.

Conclusions

Future progress in understanding *R. solani* population biology will require a combination of the approaches discussed above, including detailed analysis of anastomosis behavior and mating reactions among isolates. Both types of studies (anastomosis and mating) would benefit greatly from judicious application of appropriate molecular genetic markers. The following characteristics would be especially informative for such markers: (i) single locus-specific (nuclear) markers for unambiguous assignment of genotypes to *R. solani* isolates—both isozyme and DNA-based (RFLP) markers are ideally suited for this purpose, because they are codominant and their inheritance can be easily demonstrated through crossing studies. (ii) DNA fingerprinting probes: multilocus genetic markers showing high levels of variation also are needed to assess genetic identity among isolates suspected of being clonal in origin. Methods such as DNA fingerprinting and RAPD profiling could be used in conjunction with hyphal anastomosis testing to assess hypotheses concerning clonal structure in populations. (iii) mtDNA markers (mtDNA RFLP) also could provide additional information about genetic relatedness, evolutionary origin, patterns of cytoplasmic inheritance, gene flow, and subdivision among populations.

Although a clearer understanding of the biology of *R. solani* is slowly emerging, many basic questions about the nature of populations and individuals need to be addressed. In contrast to most research programs aimed at a single species, *Rhizoctonia* researchers are still learning how to integrate population studies with existing knowledge about the complex biological nature of AG. Often, questions about species, populations, and individuals all must be addressed simultaneously. Future progress in this area will require coordinated and cooperative efforts between individual research groups investigating population biology within different *R. solani* AG using common approaches. In this manner, combined results from different laboratories would provide a better and more complete view of *Rhizoctonia* population biology.

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