Sclerotinia Species: Summary and Comments on Needed Research

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My assignment for this symposium is summarization and commentary on various points of information on the biology and epidemiology of *Sclerotinia* spp. discussed by the other symposium speakers. I will emphasize, however, areas of investigation in which additional information apparently is needed for the development of procedures for more consistent and effective control of the diseases caused by these fungi.

These complex and interesting fungi would merit considerable scientific research effort even if they were not important plant pathogens. Plant pathologists have an additional primary incentive to reduce or prevent the considerable economic and food production losses that they cause each year. To accomplish this goal we need more in-depth and thorough information on all facets of the biology of these fungi, and with special emphasis on the biological and environmental factors that affect their capacity to cause plant disease under natural conditions. Although a large amount of material has been published on *Sclerotinia* spp., each of the symposium speakers has pointed out informational gaps and areas in which we can speak in general terms, but lack detailed and specific data. These gaps doubtless limit our ability to predict the occurrence of epidemics and to devise effective control procedures.

What still needs to be determined if we are to control the diseases more effectively? In attempting to answer this question, I have experienced considerable difficulty determining what portion of the published information has general applicability, what part may apply only to special situations, and what is controversial and uncertain. Among the several possible reasons for this, I suggest that we have expended too much effort on studies in artificially controlled conditions and on in vitro studies, and not enough additional time attempting to determine whether our in vitro findings apply in a variable natural environment. Furthermore, we have not determined adequately the range of variability of these fungi nor the mechanisms controlling this variability; too often we have assumed that observations of a single or a few isolates in artificial conditions is applicable to the whole of the variable population wherever it occurs. This has resulted in some successful control measures but many failures, and also has produced a body of literature with many conflicts and disagreements that are difficult if not impossible to interpret. For example, if a report states that a 4-yr rotation failed to control white mold of bean, but does not include information on whether apothecia were produced within the field, what conclusion can be drawn? Nothing with any degree of certainty because the inoculum might have been blown in from adjacent fields or hedgerows. Similarly, if a report suggests that an epidemic caused by *S. sclerotiorum* in lettuce is attributable to mycelial germination of sclerotia instead of ascospores, but provides no information on numbers of sclerotia in the soil or whether apothecia were produced, a similar uncertainty remains. In the following sections, I will indicate some other areas of uncertainty in which more in-depth and definitive work seems to be needed.

Taxonomy. I have chosen taxonomy as the first topic for discussion because it seems to be a prime source of uncertainty and controversy, both at present and historically.

Most plant pathologists, I suspect, are relieved to hear that we can continue to use the generic name, *Sclerotinia* instead of *Whetzelinia*. However, this is a relatively unimportant issue compared with the criteria used for designation of specific names for the isolates used in our research. Most published work on *Sclerotinia* spp. pertains to the biology and pathology of three species, *S. sclerotiorum, S. trifoliorum,* and *S. minor* that Kohn (second paper in symposium) has discussed. Thus, I will limit my discussion to them.

When these names are used I fervently hope that the respective names will indicate possession of certain unique biological and pathogenic characteristics in addition to the few morphological ones that were recommended by Kohn for their delimitation. Some of the information that should be known and associated with the names is indicated in the following questions (doubtless you can think of others of equal importance): For all three species, what is the range of variability and how is this controlled genetically? How similar genetically and biologically are these species? What would tests for similarities and differences in DNA homology, ribosomal protein patterns, serology, and occurrence of hyphal anastomosis or formation of barrier lines between isolates reveal? Also (and of more importance to plant pathologists) do the names indicate occupance of a definite ecological niche, or possession of unique pathogenic capabilities such as greater or less virulence on some hosts than on others. For example, does the name, *S. trifoliorum*, indicate that isolates of it are specialized pathogens of forage legumes, produce apothecia in the fall of the year instead of the spring, and that its ascospores can initiate infection without an exogenous food source? These attributes have been reported as unique characteristics of isolates designated as *S. trifoliorum*, and to indicate differences between *S. trifoliorum* and *S. sclerotiorum*. In many instances, however, authors of these reports have not stated the basis for identification of the isolates. Thus, readers either must accept on faith that the isolates were identified correctly or, if they question this, will have some uncertainty and confusion about the supposed differences between the two species. In many instances, the only available clue of what was used for identification was host of origin; in most instances isolates of *S. trifoliorum* were derived from forage legumes whereas isolates of *S. sclerotiorum* were from lettuce or some other vegetable crop. Thus, I have suspected that host of origin has exerted a major influence on identification of the isolates. If so, the reported differences between species may reflect variability of individual isolates rather than characteristics of two different species.

In this context, several other questions arise from Kohn's delimitation of *S. trifoliorum* to include only isolates with dimorphic ascospores and sclerotia with tomentum hyphae on the surface cells of sclerotia. How many of the reports of unique characteristics for *S. trifoliorum* would pertain to isolates that conform with this more restricted delimination of the species? What is the biological significance of ascospore dimorphism and what variabiity is evident in single-spore cultures derived from the different sized spores? What is the range of variability and the ecological niche of this species? In other words, what biological information is correlated with the morpohological characteristics recognized by Kohn and how consistent is the correlation?

Small-sclerotia isolates from forage legumes have been designated *S. minor* in some reports, but Kohn has concluded that all small-sclerotia isolates from whatever source should be assigned to *S. minor*. Here again, however, I think we need more information on the range of variability and biology of this group.

As Abawi and I described in the section on epidemiology, *S. minor* is quite different epidemiologically from *S. sclerotiorum* even though both can cause lettuce drop. Essentially, *S. sclerotiorum* produces ascospores that usually infect via senescent lower leaves whereas *S. minor* produces larger numbers of sclerotia

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that can germinate myceliogenically and infect directly without an exogenous food base. These "competent" sclerotia of *S. minor* can germinate quickly at high moisture tension (>−10 bars) whereas those of *S. sclerotiorum* require a more prolonged period of lower (<−5 bars) moisture tension to produce apothecia and to cause infection via ascospores. Thus, *S. minor* types often predominate in lettuce drop epidemics. On the other hand, some small-sclerotia-type isolates can produce apothecia in vitro. But, so far as I know, there is only one report of the natural production of apothecia by *S. minor*. Nevertheless, this raises several potentially important epidemiological questions that pertain to the range of variability of *S. minor* and similarities and differences between it and the large-sclerotia types. How commonly do *S. minor*-type isolates produce apothecia in nature? Can isolates that produce apothecia also germinate myceliogenically if preconditioned differently? If so, what factors control the two types of germination? If some large-sclerotia isolates can germinate myceliogenically (as indicated by reports of the production of secondary sclerotia), are these types more similar genetically and in other respects to *S. minor* types than are other isolates that only rarely germinate myceliogenically but usually produce apothecia?

The major point of this discussion on taxonomy of *Sclerotinia* spp. is the urgent need for more in-depth information on the range of variability, interrelationships, and biology of these fungi. Without this, I fear that uncertainty and confusion will continue to thwart our efforts.

Physiology of growth and maturation of sclerotia. As discussed by LeTourneau, there is much information on the utilization of different carbon and nitrogen compounds for growth in vitro, presence of various enzymes, gross analyses of sclerotial components, and analysis of materials in the exudation droplets that occur on the surface of sclerotia during development in vitro. At present, we know little about the control of various biochemical changes during the different stages of growth and morphogenesis. Even less is known about the factors that affect the ability of sclerotia to function; ie, to germinate myceliogenically or to produce apothecia, the two types of sclerotial germination usually required for infection. Saito (referred to by L. Kohn) suggested the term "functional maturation" to indicate, for *S. sclerotiorum*, the series of changes requisite for sclerotia to produce apothecia (carpogenic germination); the same term can be applied to *S. minor* to indicate ability to germinate myceliogenically. A third mode of germination that has been called "hyphal" differs distinctly from either carpogenic or myceliogenic. In many published articles failure to recognize and to report whether sclerotial germination was hyphal or myceliogenic appears to be an important source of confusion and uncertainty. A sclerotium capable only of hyphal germination will not infect or produce much mycelial growth unless an exogenous food source is available because the endogenous food reserves of the sclerotium are not utilized.

Saito showed that conventional indicators of sclerotial maturity such as darkening, disappearance of liquid droplets on the surface, and size did not necessarily correlate with capability for carpogenic germination. Furthermore, subtransfers of the same isolate grown on different synthetic media appeared to produce normal dark sclerotia that were as large as those produced on a bean-leaf broth; however, they differed significantly in the time required to reach functional maturity, or even to function at all in some instances. Other definitive information on factors that influence the attainment of functional maturity by sclerotia is meager. For example, it is well known that newly formed sclerotia require a period of "conditioning" to attain the ability to germinate (cool moist conditions or burial in soil for various times is usually specified). Variation in time required for different isolates to attain functional maturity is affected by various environmental conditions such as constant or alternating temperatures that are conducive to or that accelerate preconditioning, but little is known about what changes occur in sclerotia during preconditioning. Saito demonstrated that apothecial stipe initials were formed during preconditioning, and that β-1,3-glucanase activity increased during production of apothecia, but no other changes were detected.

Similar studies on *S. minor* to determine factors affecting its functional maturity are clearly needed. In preliminary studies of functional maturity, we (D. B. Marcum and R. G. Grogan, *unpublished*) have determined that: the time requirements for isolates and even subtransfers of the same isolate are variable; growth media exerted an influence, but results have been variable; sclerotia must be allowed to age for several weeks or months; and after aging, sclerotia must be allowed to dry. The latter has been consistent; other variables notwithstanding, no sclerotia have germinated that were not allowed to dry before germination was induced by remoistening.

The main theme in this section is that a major effort should be made to determine factors that affect the production of potentially functional sclerotia, and to determine the changes that occur in them during preconditioning. After all, sclerotia that cannot function do not cause disease.

Pathogenesis. Lumsden has presented a comprehensive account of the various enzymes and metabolic products of *Sclerotinia* spp. that are involved in pathogenesis, but noted that "correlation of various enzymes or toxic substances with virulence has been difficult to demonstrate." However, some positive correlations have been reported.

Even less well understood is the nature of the resistance response of nonhosts and resistant lines of susceptible hosts. Several types of resistant reactions to *Sclerotinia* spp. have been proposed, but none has been studied thoroughly. Comparisons between resistant and susceptible reactions in otherwise similar host tissues should provide needed information both on the pathogenic process and the nature of resistance.

Sclerotial production and survival. Sclerotia, usually formed on aboveground infected tissues, are deposited on the soil surface along with the infected crop debris and are incorporated into the soil at various depths during land preparation for the next crop. Some sclerotia may be produced on incipiently infected tissues after incorporation into the soil, but there is no information on this possibility.

As Adams and Ayers noted, there is surprisingly little information on natural populations of sclerotia in soil, but from limited information, they estimated numbers of sclerotia of *S. sclerotiorum* in infested fields ready for planting to range from near zero to less than 10 per kilogram of soil. In contrast, the numbers of sclerotia of *S. minor* were some 10 to 100 times greater. It seems likely that this difference results from the adaptation of *S. minor* to initiate infection directly from mycelial germination; thus, each sclerotium is an infective propagule whereas the sclerotia of *S. sclerotiorum* that are larger, but less numerous, function by production of apothecia.

For both *S. minor* and *S. sclerotiorum*, however, the numbers of sclerotia at the time of planting seems to be considerably less than expected in view of the large numbers that usually are produced on infected tissues during an epidemic. Thus, the percent of survival, even in the short term, appears to be low. If so, reports of ability of sclerotia to survive in soil for several years must pertain to a relatively small portion of the original population.

The data on time of survival of sclerotia in soil are extremely variable (ranging from a few weeks to 8 yr) and no meaningful interpretation or extrapolation to other soils or situations seems possible. Each case (soil, area, situation, etc) is different and in most reports, insufficient information is provided on environmental and other soil factors such as microflora and fauna, moisture tension and its fluctuations, gaseous and mineral content, physical characteristics, temperature, etc that might have affected the results. Furthermore, the populations of sclerotia are likely to vary due to various factors such as relative maturity, substrate used for production, isolate differences, etc. Another variable, that often hinders interpretation of the data is the methodology utilized to determine survival. For example, if the test for survival involved culturing on a nutrient medium after the recovered sclerotia were washed and surface-sterilized, we know only what portion was capable of hyphal germination in a highly artificial in vitro situation. What we actually need to know for prediction of disease potential is the portion that is capable of myceliogenic or

carpogenic germination under more natural conditions. After all, this is where and how these sclerotia must be able to function to cause disease.

Control. Steadman's conclusion that most diseases caused by *Sclerotinia* spp. "have not been controlled consistently and economically" is disconcerting but undeniably true. Why is this so? As stated previously, I think that we do not understand in sufficient detail how these fungi operate and how various factors interact to enhance or limit their ability to produce disease.

Even though the information on epidemiology of white mold of snap bean is incomplete, the factors affecting development of this disease probably are understood better than those for most other diseases caused by *Sclerotinia* spp. Primarily because of this, white mold of snap bean can be controlled by a single fungicidal spray if applied thoroughly and at the right time. Also, predictions of occurrence of epidemics are possible (at least in some seasons) and methods for screening for disease resistance that simulate exposure to natural infection have been developed, and sources of resistant germplasm have been identified. With additional information on epidemiology, and if sources of resistance that have been identified prove useful, I predict that even more consistent and economical control of snap bean white mold can be achieved. Is this a unique example or can diseases of other crops similarly be brought under control? I believe so, but each disease must be studied in sufficient depth to determine in as complete detail as possible what factors are involved and how they influence disease development. We must develop a much more complete understanding of these fungi and especially of their range of variability and the extent that this influences how they can function to produce disease in various natural situations.

With regard to biological control that probably will and should receive more emphasis in the future, we need to determine what factors affect the survival and functioning of sclerotial antagonists and parasites that have been identified and to search for others yet to be identified. We should determine what is involved in the natural biological control that apparently is operating fairly effectively in some disease situations without our help (suppressive soils) and what can be done to enhance it. I suspect we will find that no single antagonist is responsible for the natural control, but that different combinations are involved, and that the combinations vary in composition from situation to situation. Also physical and chemical characteristics of soils probably exert an influence. If so, should we expect a single antagonist, no matter how effective it appears to be in vitro tests, to be effective when introduced alone into natural soil? I suspect not.

Finally, the reported successes in identifying sources of resistant germplasm for some hosts should encourage development of better methods of screening for additional sources of resistance and continuance of efforts to determine the nature of resistance and to use this new information in screening for resistance.