Symposium on Sclerotinia

Histology and Physiology of Pathogenesis in Plant Diseases Caused by *Sclerotinia* Species

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Diseases caused in economically important plants by *Sclerotinia sclerotiorum* (Lib.) deBy. and closely related species occur worldwide, cause considerable damage, typically have been unpredictable and difficult to control culturally or chemically, and host resistance to disease has been inadequate. For these reasons, it is imperative that as much basic knowledge as possible be gained about the taxonomy, biology, physiology, ecology, and host-parasite interactions of these important and devastating plant pathogens to enable us to discover and develop new ways to control them. This paper is limited to a resumé of the available information on the host-parasite interaction–the process of pathogenesis–in diseases caused by *S. sclerotiorum* and related species, including *S. minor* Jagger and *S. trifoliorum* Erikss.

The present knowledge about the process of pathogenesis and the host-parasite interaction in the Sclerotinia diseases is not complete, and details about specific areas of physiology are especially limited. This state of the science is surprising. Anton deBary, a pioneer in mycology and the study of fungal physiology, chose *S. sclerotiorum* to be one of his myriad subjects of study. His classic works (8,9) included studies on the germination of ascospores, the penetration of the host "membranes", the involvement of an "unshaped, dissolved ferment" (or enzyme) in disease development, and the presence and possible role of oxalic acid, detected in the form of calcium oxalate, in host tissue. In

addition, he demonstrated that an unidentified thermolabile material which could destroy plant tissue in the absence of the pathogen could be extracted from diseased carrot. These discoveries were remarkable for a scientist involved in the dispute over the cell theory and the cause-effect relationship of fungi and plant disease.

With this distinguished legacy, our knowledge of the physiology of Sclerotinia diseases should be far more advanced than it is today. However, with the knowledge currently available, I will attempt to construct a generalized series of events that describe the mechanisms of pathogenesis in plants diseased by *Sclerotinia* spp. fungi.

HISTOPATHOLOGY

Considerable information exists on the histopathology and histochemistry of Sclerotinia diseases and, in general, a clear understanding of the host-parasite interaction at the tissue level is available. That information is prerequisite to understanding the host-pathogen relationships at the cellular level, and may provide the basis for formulation of disease control measures.

Penetration of the host. The ability of *Sclerotinia* spp. to invade and the mode of penetration of host tissues depends upon the type of inoculum, the nutrient status of the fungus, the properties of the host, and the effects of the surrounding environment. The latter will be covered in the epidemiology paper of this symposium. Two types of inoculum, germinated ascospores and mycelium from sclerotia or ascospores, can initiate infection. In some Sclerotinia-caused diseases, ascospores are considered to be the primary source of inoculum. Germinated ascospores can produce simple, single appressoria capable of entering the host if nutrients are present (1,32).

DeBary (8,9) learned that ascospores require external nutrients for penetration of the host. He showed that Sclerotinia hyphae attacked only after being "properly nourished and developed." Penetration occurred when hyphae were placed in a drop of nutrient solution on the host. In water alone, ascospores germinated but the hyphae were unable to form appressoria on the host surface and penetrate. More recent studies by Purdy (32) and Abawi et al (1) confirmed these early observations on *S. sclerotiorum*. Ascospores of *S. trifoliorum* from clover infected leaves of lettuce, broccoli, Brussels sprouts, ladino clover, red clover, broad bean, and snap bean (32). In contrast, ascospores from lettuce and tomato isolates germinated but did not infect the host leaves unless they were partially senescent. Ascospores of all isolates required nutrients for infection to occur. Additionally, infection was usually directly through the cuticle, although germinating ascospores produced a diffusible substance that entered the stomatal opening and disorganized the cellular contents. There are reports of entry of germtubes via open stomata by mycelium of *S. trifoliorum* on a specific clover cultivar (31) and *S. sclerotiorum* on potato leaves (17). Penetration of most hosts, however, is not via stomata but directly through the cuticle (1,5,24,31,32). Stomatal penetration requires further study.

Mycelial infection, rather than infection directly from germinated ascospores, appears to be the primary means of host penetration (2,32). In addition, a source of organic matter for inoculum nutrition usually is a prerequisite for penetration, whether the original source of inoculum is germinated ascospores that invade senescent bean blossoms before infecting bean leaves (1) or germinated sclerotia (32). Adams and Tate (2) described an exception to this, in which direct infection of lettuce plants by *S. minor* occurred in the absence of available organic matter. This exception may be due to the fact that sclerotia of *S. minor* can germinate by producing a mass or "plug" of mycelium that apparently has sufficient nutrient reserves to allow direct penetration. Sclerotia of *S. sclerotiorum* isolates have not been observed to germinate in this manner, but only by production of hyphal strands that require external organic matter before infection can occur (32).

Appressoria are formed unless penetration occurs directly via stomates as with some hosts (17,31). Usually appressoria are complex, multicelled, dome-shaped structures variously referred to as appressorial masses (32), large appressoria (5), cushion-shaped appressoria (1) or infection cushions (24,31). Formation of these organized structures requires contact stimulus (1,9,32). After contact with the host, the hyphal strands branch dichotomously, form finger-shaped structures, and eventually develop into dome-shaped infection cushions (1,5,24,32). Three distinct types of hyphae were observed in cross-sections of infection cushions (24): densely safranin-staining, thin-diameter hyphae on top of the cushion similar to that on the host surface; inflated, granular, lightly safranin-staining hyphae in the center; and dichotomously branched penetration hyphae similar in texture to those near the center of the cushion.

The infection cushions adhere tightly to the host surface and appear to be cemented by a mucilagenous material (5) that stains darker than surrounding material (24,31). This material, and the domed shape of the cushion, apparently allow the cushion to exert considerable force on the cuticle to mechanically gain ingress into the host tissue by way of pore-like infection pegs that form at the tips of appressoria (1,5,24,32). That mechanical entry is the probable means of entry is supported by the following evidence: there is no softening, dissolution, or any modification of the cuticle prior to penetration (5); infection cushions often appear to pinch the surrounding suscept tissue into a slightly convex mound (24); the cuticle is pushed inward at points of penetration (5,24); and the cuticle can remain impervious to the fungus (24) and show no alteration in staining reaction even late in pathogenesis (5,24). Prior and Owen (31) attributed the ability to penetrate clover leaves to the mechanical pressure applied by the infection cushions. However, they mentioned disintegration of the cuticle and epidermal cells, which apparently resulted from enzyme action produced by the infection cushion. Establishment of enzyme involvement in penetration must await further studies. At present, evidence for mechanical entry by *Sclerotinia* spp. into the host seems most prevalent.

Early stages of infection. After penetration of the host cuticle an inflated, granular "vesicle" is formed between the cuticle and the epidermis (5,24,32). These vesicles give rise to "infection hyphae" that develop radially from the infection cushions and invade host tissue exclusively in an intercellular manner (24). The penetration of host tissue by hyphae has been variously described as being intercellular and intracellular (1,32) or indiscriminate, growing in, between and through cells (9). In the advancing infection front, however, this is not the case. The infection process is remarkably well-organized and appears to follow a sequence of events that progressively leads to total invasion and collapse of the host tissue (24).

In bean tissue infected with *S. sclerotiorum* or *S. minor*, large, granular "infection" hyphae grow radially from the vesicles and develop between the cuticle and the epidermal cell layer and intercellularly in the cortex (24). These hyphae are quite different in size, appearance, and safranin staining reaction from those seen in ordinary cultures or on the surface of the host. They resemble, in their inflated, granular appearance, and light safranin-staining reaction, the hyphae in the interior of infection cushions and the vesicles beneath the cuticle.

The subcuticular hyphae orient parallel to one another, branch, and form an organized, fan-shaped, infection front beneath the cuticle (24). The hyphae that move into the cortex develop exclusively intercellularly. After the initial infection period (12–24 hr), the radial hyphal fronts break up into clusters of 18–20 hyphae which become oriented parallel to the bean hypocotyl axis and develop more rapidly upward than downward or transversely. The subcuticular hyphae move more rapidly than those in the cortex and growth is more pronounced on the side of the hypocotyl on which infection occurred. Perhaps the subcuticular region offers less resistance to progress by the hyphae, thus accounting for rapid, aggressive advance up the hypocotyl, which probably is vital to successful disease development. The cortical infection hyphae complete the girdling of the hypocotyl. All hyphae to this point are large and inflated (8.5–34.0 μm, av 19.1 μm) and penetrate tissue exclusively between cells.

The infection hyphae undoubtedly are responsible for breaching the host's defenses and for initial colonization of host tissue. The infection hyphae are associated with the advancing margins of visible lesions on hosts either slightly behind the margin (5,24) or slightly in advance (1). These hyphae probably are responsible for changes in infected host tissue. The changes include histologically detected alterations in pectic materials in cell walls two to three cells in advance of hyphae (24), death of cells in advance (5,15,33), copious accumulation of fluids and water-soaking in advancing margins (15,24,27,33), changes in permeability of cells in advance (15,27,33), and production of enzymes and other substances responsible for pathogenicity (11,23).

Late stages of infection. After colonization of host tissue by Sclerotinia infection hyphae, and 12–24 hr after penetration, small-diameter hyphal branches develop on the infection hyphae about 55 μm behind the advancing hyphal tips (24,31). These "ramifying hyphae" branch profusely. They extensively invade dead or dying host tissue both intercellularly and intracellularly and are capable of readily penetrating cell walls. These hyphae are considerably smaller in diameter than the infection hyphae from which they branched (24,31). They compare in size, and in intensity of staining with safranin, to hyphae in culture or on the host surface (av 8.5 μm in diameter) (24). The broad range of hyphal size from the small-diameter ramifying hyphae to the inflated infection hyphae would account for the extreme variability in diameter, cell length, and shape reported by others (1,31).

Ramifying hyphae readily invade cells and intercellular spaces in the cortex. They also are associated with destruction of the crystalline structure of host cell walls (6, and R. D. Lumsden, *unpublished*). Ramifying hyphae invade the vascular tissue of bean and clover (24,31), although with difficulty. In sunflower *S. sclerotiorum* enters the vessels and interfascicular regions and is considered the cause of vascular plugging and wilting of infected plants (30). Crystals detected in xylem vessels (24) may also contribute to plugging and wilting.

After extensive colonization of tissue, ramifying hyphae emerge from the host tissue, primarily through stomates or breaks in the cuticle (24). Emergence of hyphae from stomates was not observed in potato leaves (17), but protruding hyphal strands were observed on the lower surfaces of bean leaves (1). These tufts form mycelial wefts and, eventually, cottony growth on the surface of mature lesions. Sclerotial initials, consisting of clumps of short, barrel-shaped cells, give rise to mature sclerotia in 3–7 days (1,24). Sclerotia may form on the surface of the host, in the pith, or under decaying plant parts on the soil surface. Sclerotia may not develop and mature if the food base from the infected tissue is insufficient (24). With the formation of sclerotia, the disease cycle is complete.

Histology of disease resistance. Unfortunately, very little information is available on the nature of interactions between Sclerotinia and disease-resistant tissue. *Phaseolus coccineus* (Scarlet Runner bean) is resistant to infection by *S. sclerotiorum* (11). The resistance is characterized by limitation of lesion size and the formation of small, brown lesions. Histological examination shows that several stages of infection differ strikingly from susceptible *P. vulgaris* infections. The differences in the resistant host include: (i) penetration of the cuticle of *P. coccineus* often is impeded; (ii) secondary infection cushions often develop beneath the cuticle and adjacent to the epidermis; and (iii) infection hyphae in *P. coccineus* are often small, distorted, and not subcuticular. These differences suggest that the resistant tissue acts as a physical barrier or the middle lamella is not readily degraded to allow rapid penetration and infection.

PATHOGENESIS

Pathogenesis is a complex, dynamic process involving the pathogen's inherent capabilities and multiple factors that govern penetration and infection of a host plant. The host plant has an array of defense mechanisms that must be breached, inactivated, or annulled before disease can develop. This interaction between host and pathogen also is dependent on the surrounding environment and on time. The pathogen's battery of attacking mechanisms includes cell wall- and middle lamella-degrading enzymes, toxins, enzymes to degrade host tissue and defense substances, and rapidity of infection. The host can resistant attack by the presence of physical barriers, resistant cell wall materials, preformed antifungal compounds, infection-stimulated antifungal compounds, and inadequate nutrients for pathogen growth and development. If the host responds slowly or inadequately with one or more of these defense mechanisms, disease ensues, and death results.

Because the process of pathogenesis is complex, with multifaceted processes contributing to compatibility (virulence and susceptibility) and incompatibility (avirulence and resistance) there is no simple answer to the question of what is responsible for successful disease development. The combination of factors on either side of the balance determines whether disease will ensue or whether resistance will prevail. Whichever set of factors overpowers the other determines the outcome of the interaction.

An understanding of the sequence of events that occurs during the host-parasite interaction may enable us to exploit weaknesses in the process to our advantage to control disease caused by *Sclerotinia* spp.

Biochemistry of disease development. Much needs to be learned about the factors responsible for disease development; however, we do know that cell wall-degrading enzymes, enzymes capable of destroying cellular components, and production of oxalic acid are associated with disease development. The exact role of these components in pathogenesis is not clearly defined, but the pieces are beginning to fit together.

Penetration. Studies are lacking on the involvement of cutin-degrading enzymes in penetration of the host. DeBary (9) stated "The power of infecting is shown by the power of penetrating the membranes [cuticle] which are evidently dissolved at the point of penetration. Hence it is very probable that this power depends on the presence of a substance which can dissolve a membrane, a ferment [enzyme] in fact..." In addition, Prior and Owens (31) mentioned possible enzymatic action on the cuticle in association with infection cushions on clover leaves. However, Purdy (32) and Boyle (5) were unable to discern any alteration of host tissue due to the diffusion of substances before penetration. Until the possibility of enzymatic action on host tissue prior to penetration is demonstrated experimentally, ingress into the host must be considered a mechanical process as based on histological evidence.

Pectinases. Pectolytic enzymes always are associated with diseases caused by Sclerotinia (3,6,12,13,16,23,25–27, 34). Qualitative and quantitative information is rather limited, but these enzymes have been associated with quantitative decreases in the pectic substance content of diseased tissue (13) and with histochemical and structural changes in host cell middle lamellae (6,23). In addition, pectolytic enzyme activity has been localized in infected tissue (23).

Several pectolytic enzymes are produced in diseased bean tissue and similar ones appear to be produced in cultures of *S. sclerotiorum* (23). Lumsden (23) detected a viscosity-reducing polygalacturonase (PG) as early as 12 hr after inoculation of bean plants. The activity reached a peak 24 hr after inoculation at about the time of irreversible establishment of the infection. Thereafter the activity decreased (up to 48 hr), until in maturing lesions, another peak of viscosity-reducing activity occurred.

The first peak of enzyme activity was identified as an endo-polygalacturonase (endo-PG), based on random hydrolysis of the pectin polymer substrate (13,23). The PG was adaptive; that is, glucose suppressed its formation when added to growth media (23,34). Apparently, hydrolysis products in diseased tissue also were suppressive, since the enzyme activity declined after the initial 24-hr period of disease incubation (23). The optimum pH for activity of the enzyme was between pH 4.5 and 5.5. In addition, the enzyme appeared to be more active on pectate than on pectin. The endo-PG was localized in advancing margins of infected bean hypocotyls during the early (up to 48 hr) stages of infection but not during the later stages and was not associated with mycelium in culture (23). The location of activity was determined in thin sections of diseased tissue by plating them on a pectate medium in

which opaque halos surrounded those tissue sections with intense enzyme activity.

In contrast to the intense reaction of the endo-PG, pectinase activity in older lesions produced a hazy reaction when tissue slices were placed on the pectate medium. This enzyme catalyzed the rapid release of reducing endgroups and completely hydrolyzed pectin substrate to galacturonic acid (23). Although possibly a mixture of exo-PG and endo-PG, the predominant activity was characteristic of exo-PG. The activity was associated with advancing margins of maturing lesions, with older portions of lesions, and with mycelium in older cultures. The enzyme activity is comparable to that described by Hancock (13) in 2- to 4-day-old infected sunflower and in other host tissues assayed for enzyme activity after several days of incubation (3,26,27). Production of this enzyme is not suppressed by glucose (23). The optimum pH for activity is in the range of pH 4.5 to 5.5 (12,13,23).

Pectin methylesterase (PME) also was detected early in pathogenesis in diseased bean tissue and was associated with the advancing margins of lesions throughout disease development (23). The fungal PME was clearly distinguishable from host PME on the basis of its lack of dependence on salt for activation and its much lower optimum pH (pH 5.0) for activity than the host PME (pH 8.0). The PME has been demonstrated in diseased tissue by others (3,13,26) and its action in the demethylation of host plant pectin has been clearly demonstrated (13,23). Pectin transeliminase is not produced by *Sclerotinia* spp. (3,13,23,26).

Cellulases and hemicellulases. These enzymes often have been associated with *Sclerotinia* spp. and pathogenesis (3,4,6,14,19,27). Their role in pathogenesis, however, has not been elucidated. Sequential degradation by *S. sclerotiorum* of native, insoluble cellulose was attributed to C_1 enzyme from *S. sclerotiorum*, soluble cellulose by C_x enzyme, and hydrolysis of cellobiose to glucose by β-glucosidase (19). The optimum pH for C_x enzyme activity was pH 3.0. If this series of enzymes is operational in the degradation of native cellulose, *S. sclerotiorum* appears to have the complete system and can utilize native cellulose as an energy source. Abundant cellulase is produced adaptively in diseased tissue (3,19). Moreover, the content of α-cellulose in diseased tissue declines substantially with the age of an infection (19) and alteration of cellulose structure in infected tissue has been observed (5,6). Similarly, the araban and galactan fractions of infected sunflower tissues were degraded extensively (14). Arabanase was associated with infected tissue, but galactanase activities were not measured. In contrast, xylanase was detected in infected tissues at concentrations capable of extensively degrading native xylan; however, xylan breakdown appeared to be restricted. This suggested that arabans and galactans were more accessible to enzymatic breakdown than xylans. Partially purified galactanase (4) readily solubilized carbohydrates, including the galactan component of sycamore and potato cell walls. It did not macerate potato tuber tissue, although galactose was released.

Miscellaneous enzymes. Besides cell wall-degrading enzymes, a few other enzymes have been studied in relation to pathogenesis. Phosphatidase B, which is capable of hydrolyzing phosphatide components of cell membranes, is produced abundantly in culture and is detectable early in disease development in bean (20,27). The enzyme is inductive, extracellular, activated by calcium, and has an activity optimum at pH 4.0.

Proteolytic enzyme activity, potentially responsible for degradation of host protoplasm and possibly cell wall constituents, was detected in *S. sclerotiorum* and *S. minor* cultures (18), and in infected tissue (18,27). In diseased celery, cucumber, and carrot extracts, notable protease activity was detected at 2 days after inoculation and increased to a maximum at 10 days (18). The optimum pH for protease activity was pH 3.0.

Oxalic acid in diseased tissue. Oxalic acid first was associated with Sclerotinia infection by deBary (8). Infected carrot tissue showed a strong acidic reaction, and nonvolatile acids were implicated in the pH change. He determined that 0.319% of the carrot tissue was oxalate, mostly as the calcium salt. More recently, Maxwell and Lumsden (25) detected 1.1, 31.4, and 48.3 mg of oxalate per gram dry wt of tissue at 0, 2, and 4 days after inoculation of bean tissue with *S. sclerotiorum*. These reports, as well as others (12,15,27–29), strongly implicate oxalic acid and perhaps other organic acids in pathogenesis.

Physiology of disease development. Our knowledge of the histopathology and individual biochemical components of the host-parasite interaction gives indirect insight into the physiological processes that occur during pathogenesis. The actual study of the physiological processes provides the direct evidence necessary to establish the mechanisms of pathogenesis.

Colonization of tissue. Exclusive intercellular penetration of infection hyphae through tissue (24) is enhanced by enzymes capable of degrading the middle lamella of host cells. The three pectolytic enzymes produced by *Sclerotinia* spp. would serve the pathogen in this capacity. The endo-PG (23) undoubtedly is essential for successful advance of the pathogen during the very early stages of pathogenesis. After demethylation of pectin by PME, endo-PG probably is responsible for hydrolysis of the middle lamellae of cells, thus enabling the fungus to move rapidly through tissues in an intercellular manner. Indirect evidence also suggests an important role for endo-PG. The ability to produce large quantities of the enzyme in vitro was associated with isolates of *S. sclerotiorum* that were most virulent on bean (23). In addition, the endo-PG readily macerated susceptible bean and cucumber tissue (23) but not resistant (12,16) potato tuber tissue (23). The early appearance and subsequent inactivation of this enzyme in diseased tissue may account for the lack of correlation of PG with virulence reported in other studies (16,26,27).

The PME probably is essential for rapid action by endo-PG. These enzymes work together to degrade highly methylated pectin. The PME demethylates pectin in the middle lamella, forming pectate, which is the preferred substrate for Sclerotinia exo- and endo-PG (13,23). Correlation of PME with virulence of Sclerotinia isolates has not been possible however (23,26).

PME also is active during the later stages of pathogenesis at which time the exo-PG is most active. Exo-PG hydrolyzes pectate more readily than pectin, and, therefore, exo-PG and PME also would work in concert to degrade middle lamellar pectin. The production of exo-PG is correlated with growth of *Sclerotinia* (23) and also may play a role in the nutrition and development of the pathogen in invaded tissue.

Nutrition during pathogenesis. The nutrition of *Sclerotinia* spp. during all stages of disease development is probably the most important factor in determining success or failure in the establishment of disease in the host. Even before infection, the availability of a food base is usually a prerequisite for successful infection (1,9,24,32). During infection, the fungus organizes into specialized infection hyphae, which must require a considerable amount of energy, and in turn, an abundant, readily available source of nutrients. The nutrition provided by the food base may determine whether or not disease occurs on a potential host. Infection hyphae of *S. sclerotiorum* can be induced in culture when the fungus is grown on cellophane placed on an appropriate agar medium (22). Production of the inflated, parallel infection hyphae depends on the nutrient status of the medium and physical contact with the surface of the cellophane film. For example, cellophane-covered bean stem-extract medium, induced a greater amount of parallel hyphal arrangement and more inflated hyphae than a cellophane-covered cornmeal agar. In general, media made from host plant tissues induce a greater amount of infection hyphae formation than nonhost media (Lumsden, *unpublished*).

Cellulase (19), hemicellulases (4,14), exo-PG (23), phosphatidase (20,27), proteolytic enzymes (18,27), and other enzymes may play a nutritional role in pathogenesis. The action of these enzymes on cell walls and cell contents can provide an abundant carbon and nitrogen supply essential for the intensive metabolic activity of *Sclerotinia* spp. as the infection hyphae move rapidly through host tissue. Cell wall-degrading enzymes (4,14,19,23), possibly produced by the ramifying hyphae of *Sclerotinia* spp. (24), could be responsible for extensive degradation of cell walls and thus make abundant carbohydrates available. The ramifying hyphae which branch from infection hyphae well behind the hyphal tips, clearly are capable of intracellular colonization of host cells; thus,

cellulolytic enzymes capable of degrading cell walls must be produced to allow penetration by hyphae. Further evidence suggests a secondary or nutritional role of cell wall-degrading enzymes in pathogenesis. The decrease in α-cellulose content of infected tissue is slight 2 days after inoculation when disease is clearly established, but becomes extensive later in pathogenesis (19). Hemicellulose degradation also is extensive late in pathogenesis (14). In addition, examination of infected tissue reveals no alteration in the birefringence of infected host tissue early in pathogenesis or at the margin of lesions, but destruction of birefringence later in disease development (Lumsden, *unpublished*). This suggests alteration of the crystalline structure of cell walls in tissues after colonization by Sclerotinia infection hyphae.

Nutritional sources of nitrogen required for growth and extracellular enzyme production could be supplied by the action of phosphatidase (20,27) and proteases (18,27). A specific nutritional role for these enzymes cannot, however, be assigned until further work establishes such a role. The contents of invaded cells also would supply nitrogen. *Sclerotinia sclerotiorum* appears to utilize organic or ammoniacal forms of nitrogen more readily than nitrate (16).

Hydrolyzed plant material in the killed portions of invaded tissue is probably the primary source of nutrients. Thatcher (33), however, suggested that changes in cell permeability in advance of invading hyphae may satisfy food requirements during the initial period of infection before hydrolysis of cell wall material and death of protoplasts.

Permeability changes and water relationships. Increased permeability of infected host cells has been assumed since the classical work of Thatcher (33). Four-fold increases in permeability of infected tissue were detected in detached celery petioles. In addition, the permeability changes were noted "inches away" from any sign of necrosis. These changes in permeability were considered responsible for the water-soaking symptoms of infection and death of cells.

More recent results of Hancock (15) give another interpretation of permeability changes in Sclerotinia-infected tissue. His study revealed that permeability as indicated by influx and efflux of water and urea, and electrolyte leakage, are less in sunflower hypocotyl sections from above lesions caused by *S. sclerotiorum* than comparable sections from healthy plants. Decreased permeability of host cells above lesions was thought to be associated with changes in the nonlipid components of the plasmalemma. In addition to these findings, Newton (27) was unable to establish a cause-effect relationship of various hydrolytic enzymes, including PG, cellulase, phosphatidase, and protease, with electrolyte leakage from healthy tissue.

These apparent conflicts with Thatcher's (33) results can be resolved by Hancock's (15) finding that increases in permeability and electrolyte leakage did indeed occur in detached, senescing celery stalks as used by Thatcher but not in intact celery or sunflower in which permeability decreased. Decreased permeability probably would have little impact on *Sclerotinia* spp. but could adversely affect the host tissue by restricting growth, predisposing it to injurious effects, and adversely affecting resistance to pathogen invasion.

In view of these findings, water soaking of tissues and accumulation of copious fluids around infection hyphae (24,28) need reevaluation. Instead of resulting strictly from leakage from host cells (28,33), perhaps liquid accumulation is a result of the increased osmotic pressure of invaded tissue. Thatcher (33) suggested that the fungus is responsible for a flow of water from the lower plant parts to its own locality. The greater osmotic pressure of the pathogen hyphae and solutes in the fluid surrounding the hyphae could result in osmotic flow of water from other regions. This fluid could allow transport of nutrients from distant areas and act as milieu for enzyme reactions and for the diffusion of oxalic acid into noninvaded tissues several cells distant.

Oxalate in host tissues and pH changes. The effect of oxalic acid in diseased tissue may be manifold. During the early stages of disease development and at advancing margins of lesions, oxalic acid may work synergistically with pectotylic enzymes as demonstrated for other similar diseases (25). Oxalic acid is a very strong chelator of calcium and other cations. In this capacity, oxalic acid would tie up mono- and divalent cations that inhibit maceration of tissue (13) through inhibition of the action of endo- and exo-PG. Oxaic acid in vitro stimulates the degradation of pectic substances by endo-PG, exo-PG, and PME (Lumsden, *unpublished*).

Oxalic acid also affects the pH of infected tissues. Changes in pH occur in infected tissue (13,18,21,23,25,26) and have been localized at the advancing margins of lesions (21). Values decreased from pH 5.0 to 4.0 at advancing margins of lesions on bean hypocotyls as determined by microspectrophotometric methods (21). Increased acidity in the developing lesions would favor the activity of endo- and exo-PG (13,23), cellulase (19), hemicellulase (14) and other hydrolytic enzymes (18,20), with pH optima for activity well below the pH of healthy host tissue and cell sap. Moreover, drastic pH changes would have severe direct effects on cell viability and ability to respond to pathogen invasion. Increased acidity would also favor rapid growth of the fungus (25).

Toxicity to host cells (8,15,16,29,33) resulting in death, may be due to drastic pH changes or the cation chelation properties of oxalic acid. Oxalate at concentrations and pH values detected in lesions (25) alone is sufficient to kill sunflower cells (15). It has been noted, however, that a portion of the killing factor in lesions is heat labile (8,27). Although not examined, this suggests the possibility that pectolytic enzymes are involved in the killing action as with other diseases (15). Overell (29) discounted oxalic acid as a component of toxins secreted by *S. sclerotiorum* in plant tissues. This was based solely on the observation that oxalic acid was associated only with aging cultures. However, the pH, buffering capacity, and glucose concentration in cultures of *Sclerotinia* spp. are very important in determining the production and the quantity of oxalate produced (25).

Oxalic acid also may be responsible for wilting symptoms usually associated with disease caused by *Sclerotinia* spp. (16,30). Crystals identified as oxalate (Lumsden, *unpublished*) have been observed occluding xylem vessels (24). Vascular plugging (30) may be responsible for wilting or there may be a direct effect of oxalate on the water relationships in lamellar tissue.

Virulence and resistance. Virulence of *Sclerotinia* spp. on the one hand and susceptibility or resistance of the host on the other determine the outcome of the host-parasite interaction. However, correlation of various enzymes or toxic substances with virulence has been difficult to demonstrate. Although not definitive studies, several reports indicate positive correlations of enzyme and metabolite production with virulence. Endo-PG (23), protease (18), and toxic metabolite (16) have been positively correlated with virulent isolates of *Sclerotinia* spp. PME was associated with all isolates tested on bean, but the amount produced was not correlated with virulence (23). Similarly, others have reported no correlation of virulence with phosphatidase (20,27), protopectinase (16), pectin methylesterase (26), and polygalacturonase (26,27). Oxalic acid has not been directly correlated with virulence. However, a conductivity assay for measuring virulence was devised that was presumed to measure the alteration of host cell permeability and subsequent leakage of electrolytes from disks of infected tissue (28). Some electrolyte leakage probably can be attributed to leakage from dead host cells, but most of the increased conductivity may be attributable to oxalic acid produced by the pathogen.

The physiology of Sclerotinia disease resistance has not been studied adequately and, in fact, disease resistance among many susceptible genera of dicotyledonous plants has not been found. Monocots generally are immune or very resistant. The topic of general disease resistance will be covered in the symposium paper on control.

Three general types of resistance reactions to *Sclerotinia* spp. have been briefly described. These are: resistance of tissue to breakdown, possibly associated with nutrition of the fungus; presence of preformed antifungal materials; and formation of phytoalexins. Resistance of Scarlet Runner bean to *S. sclerotiorum*, already discussed, appears to be due in part at least to

a physical barrier to infection or middle lamellae of host cells that greatly impede penetration and infection (11). Resistance of clover to *S. trifoliorum* has been postulated to be due to more efficient use of food reserves by certain clover varieties, resulting in a resistant middle lamella that is less easily hydrolyzed by enzymic action (16). In addition, failure of nonhosts to induce infection hyphae formation suggests a nutritional basis for resistance (22).

Preformed antifungal materials have been examined in clover leaves infected with *S. trifoliorum* (10). Several isoflavones released from glycosidic combination on infection exhibited little activity toward *S. trifoliorum*. Antifungal activity toward *S. trifoliorum* has been shown for 7-hydroxy-4'-methoxy-isoflavone from clover (35). Other preformed materials have been associated with resistance of onion and potato to *S. sclerotiorum* infection (12). Unidentified substances from resistant potato tissue inhibited maceration of susceptible radish, cucumber, and carrot tissue by extracts from *S. sclerotiorum* cultures. Immune onion tissue extracts completely prevented maceration.

Phytoalexins have been associated with resistance in Sclerotinia diseases. Two clover cultivars resistant to *S. trifoliorum* consistently accumulated more phytoalexin than the susceptible ones, but the final overall concentration in the resistant cultivars would not have inhibited *S. trifoliorum* in vitro (10). In addition, Cruickshank and Perrin (7) suggested that *S. sclerotiorum* should not be pathogenic to bean on the basis of the quantity of phaseollin producted (18μg/ml). Perhaps the success of *Sclerotinia* spp. depends on speed of attack, before the host can respond by producing phytoalexins. The role of phytoalexins in pathogenesis and resistance needs further investigation.

CONCLUSIONS

Pathogenesis by *Sclerotinia* spp. is a complex phenomenon that is not adequately understood. The enzyme systems do not appear to be greatly different from other similar soilborne plant pathogens or even saphrophytic microorganisms. Oxalic acid is a common metabolite of many fungi. The success of *Sclerotinia* spp. as pathogens appears, therefore, to be dependent on a complex combination of factors that can overwhelm the host plant by rapidly acting before the host can respond. These factors include: production of infection cushions or appressoria to enable mechanical penetration of the host cuticle; formation of specialized, organized infection hyphae that are capable of rapid, intercellular development beneath the host cuticle and in the cortex; elaboration of appropriate pectolytic enzymes and oxalic acid to degrade the middle lamella of host cells, chelate cation inhibitors of enzyme activity, change the pH of host tissue to a range more favorable for enzymic action, and toxify host cells, making them less responsive to invasion; and production of degradative enzymes capable of hydrolyzing cell wall and protoplasmic constituents and providing a constant, abundant supply of nutrients for rapid growth and development of infection hyphae. This sequence of events needs verification and may not be complete. It does, however, provide a logical explanation of the behavior of *Sclerotinia* spp. in a susceptible host-pathogen relationship.

The opposing system, the resistance response, is much less well understood. There is an unlimited area of potentially very fruitful research. We need research in three general areas to explain resistant reactions. These areas are: physical barriers that may impede penetration and development of the pathogen; the presence or absence of nutritional factors or preformed inhibitors that may prevent *Sclerotinia* spp. from developing within the hosts; and changes in the host induced by the host-parasite interaction.

A more thorough understanding of the mechanism of pathogenesis with *Sclerotinia* spp. and elucidation of the mechanisms by which crop plants resist infection hopefully will enable us to develop more effective methods for detecting resistant germplasm. Simple biochemical tests that identify resistance, coupled with modern breeding methods for manipulating resistance in germplasm, can give us a reliable tool for obtaining our goal—control of the devastating crop losses caused by *Sclerotinia* spp.

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