Phytophthora Rot of Soybean

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Phytophthora root and stem rot symptoms were first observed in Indiana in 1948 and in Ohio in 1951. The causal agent was identified in Ohio and North Carolina in 1954. Since that time, Phytophthora rot has been reported in Argentina, Australia, Brazil, Canada, the People's Republic of China, Hungary, Italy, Japan, the former Soviet Union, and throughout the soybean-growing regions of the United States.

The disease may cause plant stand losses and complete yield reductions in very susceptible soybean cultivars. The estimated reduction in yield in 1994 was 560,300 metric tons. The severity of losses depends on cultivar susceptibility, rainfall, soil type, tillage, and compaction. Phytophthora rot is most severe in poorly drained clay soils that are readily flooded. Plant loss can occur in lighter soils and on well-drained soils if they are saturated for an extended period of time when plants are young.

Soybean is the only important host of the causal fungus. It has been isolated from *Lupinus* spp. native to the United States. In greenhouse inoculation tests but not in the field, it has been found to be pathogenic to alfalfa, sweet clover, snap bean, and crane's-bill. For several years, *Phytophthora* isolates from alfalfa, soybean, and other hosts were considered to be the same species, leading to confusing reports on the host range of the species that infects soybean. There is one report of *P. parasitica*, a pathogen with a large host range, causing soybean stem rot.

Symptoms

Fig. 1. Delayed emergence and postemergence damping-off, early symptoms of Phytophthora rot. (Courtesy A. F. Schmitthenner)

Fig. 2. Postemergence damping-off and seedling root and stem rot caused by Phytophthora rot. (Courtesy A. F. Schmitthenner)

Phytophthora rot may be found in soybean at any stage of development. Early symptoms are seed rot and pre- and postemergence damping-off (Fig. 1), which may necessitate extensive replanting. These symptoms occur in soils flooded soon after planting and are commonly misidentified as water damage. Postemergence damping-off and seedling root and stem rot (Fig. 2) also occur under the same conditions and reduce stands.

Symptoms on older seedlings depend on the relative susceptibility or tolerance of the cultivar. In the most susceptible cultivars or in those with low tolerance, seedling stems may appear water soaked, leaves may turn yellow, and the plants wilt and die. In the least susceptible or highly tolerant cultivars, root rot may cause stunting but plants are not killed.

Older plants of cultivars with low tolerance may die throughout the season from infections that occur soon after planting. Symptoms include yellowing between veins and leaf margins and chlorosis of upper leaves followed by wilting. Leaves remain attached after the plants die. Foliar symptoms are preceded by destruction of the lateral roots, severe taproot rot, and girdling stem lesions that may progress up the stem as high as 10 nodes (Fig. 3) before the plants wilt (Fig. 4). The cortex and vascular tissues are discolored. In moderately susceptible plants, stem lesions consist of long, narrow, sunken, brown lesions that progress up one side of the stem (Fig. 5). Plants with unilateral stem lesions may not wilt.

Fig. 3. Girdling stem lesion caused by *Phytophthora* rot. (Courtesy A. F. Schmitthenner)

Fig. 4. Wilting caused by *Phytophthora* rot. (Courtesy A. F. Schmitthenner)

Fig. 5. Linear stem lesion characteristic of cultivars that are moderately susceptible or tolerant to *Phytophthora* rot. (Courtesy A. F. Schmitthenner)

In older plants of highly tolerant cultivars, the only symptoms are generally rot of the secondary roots and discoloration of the taproot. These plants are not killed by the fungus but may be stunted and slightly chlorotic, and symptoms may resemble those of mild nitrogen deficiency or flooding. Occasionally, a one-sided stem lesion may occur in such cultivars. These mild symptoms, referred to as hidden damage, may reduce yield by as much as 40%. Hidden damage can be seen and measured by comparing nontreated plants with those treated with a fungicide such as metalaxyl or by comparing near-isogenic lines with and without race-specific resistance.

Foliar blight has been reported after heavy rains and can be produced in a growth chamber on young leaves. Symptoms consist of progressive, light brown lesions with yellow margins that can form on young leaflets under continuous misting. Older leaves are resistant to foliar blight, a phenomenon referred to as age-related resistance.

Causal Organism

Fig. 6. Terminal sporangia of *Phytophthora sojae* and proliferation of new sporangia forming terminally or within the old sporangia. (Courtesy A. F. Schmitthenner)

P. sojae produces simple, indeterminate sporangiophores. Typically, terminal sporangia (conidia) are obpyriform ($32\text{--}53 \times 42\text{--}65 \mu\text{m}$) and nonpapillate (Fig. 6). Sporangia germinate indirectly by extruding fully formed zoospores into a thin, delicate, membranous, evanescent vesicle, which quickly expands and ruptures. Zoospores are sometimes trapped within sporangia and germinate there. The resulting germ tubes penetrate the sporangium wall. Empty sporangia commonly proliferate internally (Fig. 6), forming new sporangia terminally or within the old sporangia. Sporangia may also germinate directly, functioning as conidia. Hyphal swellings are commonly formed.

The optimum temperature for direct germination is 25°C , for indirect germination 14°C , and for zoospore production 20°C . Zoospores are ovoid, bluntly pointed at one or both ends, and flattened on the sides. They have two flagella, a short one directed anteriorly and the other, four to five times as long, directed posteriorly. At the end of the motile period, which may last up to several days, zoospore movement becomes sluggish and jerky and encystment occurs. Encystment may be triggered prematurely by agitation or by bivalent or trivalent cations. The cysts frequently germinate immediately and directly, producing germ tubes, which generally swell to form appressoria when they contact a solid surface. After a short time, normal growth of mycelia is resumed from the appressoria. Cysts sometimes germinate by producing secondary zoospores, leaving the cyst membrane behind. Rarely, a miniature sporangium is formed at the tip of the germ tube. Cysts germinate more vigorously in nutrient solutions than in distilled water.

Fig. 7. Oospores of *Phytophthora sojae* produced in culture. (Courtesy A. F. Schmitthenner)

P. sojae is homothallic. Antheridia and oogonia develop in abundance on corn meal, lima bean, V8, or potato dextrose agar. The antheridia are diclinous and generally paragynous, although amphigynous antheridia may be found. The oogonia (29–58 μm) are thin walled and spherical or subspherical. An oospore develops after an antheridium fertilizes an oogonium. Dormant oospores (Fig. 7) have thick, smooth inner and outer walls, fine-grained cytoplasm, a spherical, refractive body in the center, a well-developed reserve granule, and a pair of pellucid bodies at the outer edge of the cytoplasm. Germination may occur after the pellucid bodies merge and fuse. At germination, the pellucid bodies are no longer detectable, the smooth inner wall erodes, the central refractive body is absorbed, and the spore has the appearance of a sporangium. When the oospore germinates, the inner wall is absorbed and the germ tube produces either a sporangium or mycelium.

Oospores may germinate about 30 days after formation. Germination occurs in distilled water and is increased by low levels of nutrients and root exudates. Light increases the percentage of germination but inhibits subsequent formation of sporangia. This inhibition of sporangial formation is reversed by root exudates. Oospore germination is initiated within 2 days of separation from mycelium but is nonsynchronous and may continue for 30 days or more. The optimum temperature for formation and germination of oospores is 24°C.

Sporangia develop in dilute extracts of lima bean or other plant material. They can also be induced to form on solid agar if washed repeatedly with water. Sporangia will form abundantly in young cultures grown on lima bean extract that are washed repeatedly with Chen-Zentmyer salt solution. The medium and age of the culture affect oospore size and sporangial shape and size.

Mycelium is coenocytic when young and becomes septate with age. It branches mostly at right angles, and there is a slight constriction at the base of each branch. Hyphae are 3–9 μm wide and frequently slightly curled. The optimum temperature for growth of most isolates is 25–28°C. Isolates of *P. sojae* vary widely in cultural characteristics, morphology, and virulence to specific resistance genes. There are 37 described races of the pathogen and also many isolates not yet described that do not fit any race designations. Races can be distinguished by differential reactions on eight near-isogenic lines, each with a different *Rps* resistance gene. These races are identified by a number or by a list of the resistance genes that they can defeat (virulence phenotype) (Table 1). The latter method is preferred. Additionally, five *Rps* genes have been used to further describe the races, and many lists have been generated from various sources. Many races no longer exist or differ from their original descriptions.

Three common selective media used for isolating *P. sojae* from plants are P10VP, PARPH, and PBNIC. The first two have a cornmeal agar base and include pimarinic (10 mg/l) and quintazone (100 mg/l) for selective inhibition of nonpythiaceous fungi. The first utilizes vancomycin (200 mg/l) and the second ampicillin (250 mg/l) and rifampicin (10 mg/l) for bacterial control. The PBNIC medium utilizes benomyl (10 mg/l), quintazone (40 mg/l), and iprodione (20 mg/l) for control of nonpythiaceous fungi and neomycin sulfate (100 mg/l) and chloramphenicol (10 mg/l) for bacterial control. Hymexazol (20 mg/l) can be used in all three media for partial control of *Pythium* spp. *P. sojae* cannot be isolated directly from soil. A semiquantitative baiting method has been described in which soybean leaf disks are floated on water flooded over soil for 2 hr and then plated on selective media.

Disease Cycle and Epidemiology

Fig. 8. Oospores of *Phytophthora sojae* produced in a hypocotyl of a soybean seedling 1 week after inoculation. (Courtesy A. F. Schmitthenner)

Fig. 9. Sporangia of *Phytophthora sojae* produced in water 12 hr after hypocotyl lesions were surface sterilized and placed in sterile distilled water. (Courtesy A. F. Schmitthenner)

P. sojae persists in soils as oospores either in crop residues or free in soil after residues decompose. Many oospores are formed in roots and stems of susceptible and tolerant soybean cultivars (Fig. 8). Oospores can survive for many years without a host. Factors controlling dormancy and germination in soil have not been determined.

P. sojae cannot be recovered directly from soil incubated for an extended period of time at 3°C or lower (conditions comparable to overwintering). The soil must be incubated for at least 1 week at 25°C before *P. sojae* can be isolated by using soybean seedlings or leaf-disk baits. Little oospore germination occurs until soil temperatures exceed 15°C, and germination is delayed at that temperature. From such observations, it is assumed that in the spring, oospores germinate whenever the temperature is suitable for forming sporangia. The sporangia accumulate until the soil is flooded, at which time zoospores are released. Sporangia also form on the surfaces of infected roots (Fig. 9), providing secondary inoculum. Soils must be flooded or saturated with water for zoospores to be produced. Zoospores can swim short distances (1.0 cm or less) in saturated soil but are disseminated primarily by moving floodwater. Phytophthora rot is essentially a monocyclic disease; plants become more tolerant with age and secondary inoculum does not increase loss.

Zoospores are attracted to seeds and roots by genistein and other isoflavonoid exudates. They encyst on the root surfaces and immediately germinate and penetrate the roots. Hyphae grow intercellularly in root tissues. Intracellular growth has been reported in hypocotyls. Globular and fingerlike haustoria penetrate host cells in susceptible cultivars. Cell necrosis is more prevalent in resistant than in susceptible cultivars. Colonization of the cortex is similar in susceptible and resistant cultivars for about 14 hr. Colonization then ceases in resistant

cultivars, and the fungus fails to reach the vascular tissue.

Oogonia and oospores are formed in infected root and stem tissues of susceptible, tolerant, and resistant cultivars. However, more oospores are formed in susceptible and tolerant cultivars than in resistant cultivars. Oospore formation in resistant cultivars is not associated with root rot. Leaf infection, which is rare, results in more severe foliar symptoms than does root infection. Leaf infection occurs when soil particles containing the pathogen are splashed onto leaves during rainstorms. If the weather remains misty and cloudy, leaves can become severely infected and the fungus grows toward the petioles and stems.

Phytophthora root rot is most common in heavy, tightly compacted, fine (clay) soil or soils with an impervious, shallow hardpan that are subject to saturation and flooding. Flooding rains within a week after planting create conditions most favorable for disease development. Highly tolerant cultivars may escape severe damage if they emerge and reach the first trifoliolate stage without conditions becoming suitable for zoospore release. Less tolerant cultivars can be damaged from root rot that develops from midseason infection periods. Damage caused by *P. sojae* can be reduced by tile drainage to remove excess soil water and reduce the duration of saturation and flooding. Damage increases in areas of reduced tillage, especially no-till, because these areas absorb more rainfall and are more easily saturated. Population densities of *P. sojae* in no-till areas with fine-textured soils are higher than in no-till areas with moderately textured soils. Monocropping of soybean increases damage, but rotation with nonhosts may not reduce disease severity. Application of high levels of fertilizer (KCl) just before planting may increase damage.

Management

Race-specific resistant cultivars should be used. Thirteen genes at seven loci control race-specific, whole-plant resistance. Resistance results in an incompatible interaction in which the fungus fails to colonize tissue beyond a hypersensitive fleck-type lesion. Cultivars with the *Rps1c*, *Rps1d*, *Rps1k*, or *Rps3a* gene and several with two genes are available.

A second type of race-specific resistance has been named "root resistance." In some cultivars, the hypocotyls are susceptible but the roots may be partially resistant. This phenomenon has not been clearly defined, and no specific genes have been described.

A third type of reaction is a cultivar-specific quantitative difference in disease that is a type of non-race-specific, partial resistance and has been referred to as "field resistance," "rate-reducing resistance," or "tolerance." Tolerant cultivars include Asgrow A3127, Conrad, Elgin, Jack, Ripley, and Zane.

It is important to know which virulence phenotypes are present in the field before selecting a cultivar with specific resistance. In the Lake Erie Basin, for example, *Rps1k* and *Rps3a* or the gene stack of *Rps1k* + *Rps6* are most effective. Elsewhere in the Midwest, *Rps1a* and *Rps1c* are still effective. With continued breeding for resistance, other gene stacks or as yet undescribed genes can be incorporated into soybean for future management through resistance.

Metalaxyl, an acylalanine fungicide specific for oomycetes, is often applied to tolerant cultivars as a seed treatment (Apron) or as an in-furrow spray or granule (Ridomil). Metalaxyl is more effective on highly tolerant cultivars. In general, metalaxyl applied to the soil is taken up by the roots and provides longer-lasting control than metalaxyl seed treatment.

Integrated control, which combines high tolerance, improved drainage, and tillage, is as effective as resistance or fungicides in most soil environments. Metalaxyl seed treatment may partially compensate for excess soil moisture resulting from no-till cropping. Crop rotation should be used to avoid increasing inoculum levels in the field, and the application of potash fertilizer or animal manure immediately before planting should be avoided. Very early planting when

soil temperatures are below 15°C may allow soybean to avoid significant damage but increases infection by *Pythium* spp. (see Pythium Damping-Off and Root Rot).

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