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DEVELOPMENT OF AN ASSAY FOR RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* INFECTION OF SOYBEAN AND INVESTIGATIONS INTO THE CAUSE OF THE VARIABILITY WITHIN A GENOTYPE FOR TOLERANCE TO THE FUNGUS

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ABSTRACT

DEVELOPMENT OF AN ASSAY FOR RESISTANCE TO SCLEROTINIA SCLEROTIORUM INFECTION OF SOYBEAN AND INVESTIGATIONS INTO THE CAUSE OF THE VARIATION WITHIN A GENOTYPE FOR RESISTANCE TO THE FUNGUS

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Soybeans [Glycine max (L.) Merr.] exhibit variability within a cultivar for resistance to Sclerotinia sclerotiorum (L.) De Bary. The cause of the variability was investigated. Stem sections were cut from greenhouse plants and inoculated in the laboratory with mycelium plugs. The resulting lesions were measured. Inoculations on the intact cuticle resulted in escapes and delayed infections which contributed to residual variability. These problems were minimized by inoculating on wounded tissue. The wound inoculation technique detected significant differences in lesion lengths among cultivars. The results matched field resistance and the occurrence of restricted lesions as detected by inoculations made on sites where cuticular waxes were removed. Attempts to increase the expression of resistance and the sensitivity of the method by lowering the duration of inoculation or the nutritional content of the

inoculum medium failed. Investigations into the cause of the residual variability of the wound inoculation technique implicated the growth environment of the plants.

This thesis and my all my efforts to complete my Masters degree are dedicated to my wife Sharon for without her help and support I probably would have never returned to school to pursue these endeavors.

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INTRODUCTION

The fungus Sclerotinia sclerotiorum (Lib.) De Bary can infect a multitude of plant hosts prompting Purdy (103) to describe it as "among the most nonspecific, omnivorous, and successful of plant pathogens". Adams (10) reported a host range of 64 families, 225 genera, and 361 species. The Compositae, Cruciferae and Leguminosae each have over 17 genera and 30 species which can host the pathogen.

In soybeans, [Glycine max (L.) Merr.], S. sclerotiorum causes Sclerotinia stem rot. In 1946, Weiss (134) reported the presence of the disease in New York, Maryland, Iowa, and Virginia. It has since been reported in Illinois (26, 27), Minnesota (45), New Jersey (53), Maryland (11), Wisconsin (47), Virginia (98), Arizona (56), Michigan (67), Ontario (23, 55), South Africa (124), France (112) and Brazil (136). It is primarily a localized problem, often occuring in fields previously planted with a more susceptible host (45, 56, 98). Two recent reports indicate that the disease incidence is increasing as soybean production expands into areas where more susceptible hosts have been grown (27, 47).

Field observations of the disease's effect on yield vary from no effect (98) to severe (56). Grau and Radke (47) found a negative correlation between disease severity ratings and yield where soybean cultivars were planted in either narrow or wide rows in a disease nursery. Based on previous studies, narrow rows were expected to yield 21%

more than wide rows. Instead the narrow row soybeans developed higher disease severity indexes, yield was decreased 42 % as compared to the yeild of less diseased wide rows. Lockwood and Isleib (69) also reported that yield and disease incidence were negatively correlated (r = -.94).

S. sclerotiorum is also the cause of white mold in Phaseolus vulgaris L. This disease is very similar to Sclerotinia stem rot of soybeans. White mold is by far the more studied of the two diseases. In all cases where sufficient research on Sclerotinia stem rot exists, it appears to parallel white mold research. This review will include white mold research to emphasize and fill in voids in Sclerotinia stem rot research.

EPIDEMIOLOGY

While the disease is often found in soybeans rotated with more susceptible hosts, it can also spread to previously uninfected fields by means of windblown ascospores (6). Ascospores can occasionally travel up to 10 kilometers from the point of release (25). Another method of long distance transmission is through contaminated seed. From 1969 to 1970, Nicholson et al.(93) found S. sclerotiorum internally seedborne in 28 of 37 lots of 'Lee 68' soybeans. In 1971, 1% of 'Cutler' lots and 2% of the 'Amsoy' and 'Beeson' lots were similarly contaminated. The fungus could still be recovered from the infected lots 18

months later. The plantings from these seed lots developed Sclerotinia stem rot though it was not shown that the infections originated from the infected seeds. In P. vulgaris, Steadman (118) found that 48% of the infected fields produced seed infected with the fungus. The infected seed produced healthy plants so it was concluded that the seedborne fungus was only important in disease dissemination. Soybean seed can also be contaminated with sclerotia which can spread the disease (98, 124). S. sclerotiorum has also been found in the seeds of certain crucifers (90) and S. minor has been found contaminating peanut (Arachis hypogea L.) seed (130).

Other methods of dissemination include movement of soil contaminated with mycelium or sclerotia (6), contaminated manure or straw (22, 115) and irrigation water (119).

Sclerotia give rise to the infectious forms of the disease, mycelia and ascospores. Increased sclerotium numbers in a field increases the amount of inoculum that can be produced. With leaf drop disease in lettuce, caused by S. minor, disease incidence is correlated to the number of sclerotia per unit area of soil (39). Imolehin and Grogan report similar results (63). Sclerotia numbers in a field are primarily increased in a susceptible host, though sclerotia have been shown to increase in the soil (30, 135). Adams (9) counted up to 1000 sclerotia of S. minor formed on a single lettuce plant. This would translated into an increase of 50 sclerotia per kg of soil. This is a dramatic

illustration of the potential buildup of inoculum in a field planted to a susceptible host. Sclerotium levels were lowered when all infected lettuce plants were removed over three years (97). The number of sclerotia of S. sclerotiorum in a bean field appears to range from 0-7 sclerotia per kilogram of soil (1, 108, 134). Abawi and Grogan (1) obtained surface counts of 16.1 and 5.4 sclerotia / 30 cm^2 in bean fields that had been severely infected. After plowing only 0.2 and 0 sclerotia / 30 cm^2 were found. A concentration of only 0.2 sclerotium per kg of soil was enough to infect 46 % of a dry bean canopy (110).

Inoculum can also be produced outside of the field. Apothecia were found in fence rows and wooded areas (1) and this was considered to be an important source of inoculum. S. sclerotiorum can also infect many weedy hosts including Xanthium pensylvanicum Gandoger.(11), Ambrosia artemisiifolia L.(40), Capsella bursa-pastoris (L.) Medic. (85), Barbarea vulgaris R. Br. (85), Sonchus arvensis L. (86) and Cirsium arvense L. (24, 86). All these are weeds commonly found in soybean fields.

S. sclerotiorum survives from year to year primarily as sclerotia. Cook et al.(30) reported that 75% of sclerotia buried for 3 years at depths from 5 to 20 cm germinated. They also found that the fungus could overwinter as mycelia in bean seeds but not in crop residues. Adams (9) found that sclerotia could survive for 15 months when buried up to 30.4 cm but that survival at 61 cm was poor. Merriman et

al.(82) reported that more sclerotia survived at the soil surface than when buried. Plowing reduced disease by burying the sclerotia.

Soil moisture level is an important factor in sclerotium survival with several reports indicating that survival is greater in drier conditions (7, 63, 83). Sclerotia also survive better in soils that have never been infected before (7, 63). This is apparently due to the absence of sclerotia parasites. Coniothyrium minitans (126, 125) has been shown to lower sclerotium survival as has Gliocladium roseum (87) and Trichoderma spp. (57, 63, 64, 78).

While rotations with non-host crops have been reported to be an effective method of controlling the disease (115, 137), it must be a long rotation. A three year rotation to a non-host crop in Nebraska following susceptible dry beans produced no significant reduction in sclerotium numbers (110). Surprisingly the growing of susceptible dry beans for three years did not increase sclerotia levels. Adams and Ayers (10) concluded that the sclerotia of S. sclerotiorum may remain viable in the soil for 4-5 years.

S. sclerotiorum can infect a host either as mycelium or as ascospores. Sclerotia can produce mycelia and apothecia. The apothecia then produce and release ascospores. Much of the research determining which form of the pathogen is the primary form of inoculum has been done in P. vulgaris and points to ascospores being the primary inoculum of

infections. Similar research with soybeans is lacking, though parallels can be drawn which indicate ascospores are also the primary inoculum in soybeans.

The growth of mycelium through the soil appears to be very restricted. Infections of beans by soil mycelium were only successful when an additional energy source was provided (1). Even then infections were sporadic if the inoculum was placed further than 1 cm from the plant. Mycelium growing from a food base failed to infect lettuce (Lactuca sativa L.) seedlings which were further than 2 cm from the food base (92). The sclerotium does not appear to supply the mycelium with the nutrition necessary for extended growth (1, 102). Purdy (102) showed that mycelium cannot infect a host unless it is supplied with an exogenous energy source and that the energy source acts as a bridge to the host. However, Abawi and Grogan (1) reported no infections accomplished in this way. Cook et al.(30) concluded that less than 10% of the infections in their study could be attributed to this process. It was also noted that mycelium did not to grow from the ground up on the outside of the stem to initiate above-ground lesions. Sclerotium produced mycelium can infect the host but it appears to be of minor importance except in certain situations where there is abundant senescent tissue and the tissue is in contact with both the soil and the host.

Infections and epidemics of S. sclerotiorum in soybeans and P. vulgaris predominately originate in the plant canopy

(1, 26, 30, 45, 46, 56, 89, 115, 124). Cook et al.(30) stated that above ground lesions can only be initiated by an airbourne propagule. This indicates that ascospores are the primary inoculum. The presence of ascospores has been positively correlated with disease development (1). When bean tissue was collected from various fields, disease symptoms only developed on the tissue from fields where ascospores were also found. Mycelium can infect plant debris which could become windborne and cause infections (89), but the nature of epidemics and the proven pathogenicity of ascospores (27, 30, 92, 102, 110, 134) indicate that ascospores are indeed the primary inoculum of S. sclerotiorum.

An exogenous energy source is required for any substantial infection to occur by either ascospores or mycelium. While infections can occur without the benefit of an energy source (92, 102, 121), they are generally restricted to a spotting or flecking of the surface. Ascospores failed to infect tomato leaves (Lycopersicon esculentum Mill.) without energy being supplied from either flower tissue or a nutrient solution (101). Grogan and Abawi (48) found that spore survival was closely correlated with the nutrient content of the medium upon which the spores were germinated. Spore survival was related to the amount of growth and formation of appressoria. The presence of the energy source appears to promote appressoria production (1, 2, 46, 102, 123). This allows the pathogen

to penetrate the host through the cuticle (2, 73, 102, 121). Purdy (102) placed ascospores in distilled water and noted that no appressoria formed. Infections of host tissue did not occur unless a carbon source was added. In the same study, mycelium formed appressoria without an energy source being supplied but were still unable to infect. Sutton and Deverall (121) noted infections of young soybean tissue by ascospores lacking an appressorium or an energy source. These infections were restricted to a few cells under the ascospore. While penetration was achieved without an energy source, colonization of the host tissue did not occur. The ascospores formed appressoria if supplied with pollen grains, but spreading infections only occurred when flower tissue, a more substantial energy source, was provided. This evidence along with the work of Purdy (102) indicates that while an exogenous energy source promotes the formation of appressoria from ascospores, it must also have a further role in promoting infection.

In P. vulgaris the apparent energy source used to fuel infections is senescent tissue, primarily flower blossoms (2). While wounded tissue can be infected at any time (1, 3, 92), epidemics only occur after flowering. In a New York study, Natti (89) found that white mold epidemics in beans occurred 8 to 14 days after flowering, regardless of the planting date. The infections appeared to originate in leaf and branch axils where blossoms were lodged. Other authors have also reported that lesions originated in branch axils

where spent bean blossoms were found (1, 30). Studies in other species also indicate that the presence of flower tissue is important for infection (66, 81, 101). In greenhouse studies of soybeans, Cline and Jacobson (27) found that plants were infected by ascospores only after the plants had flowered. Sutton and Deverall (121) reported that soybean blossoms were a suitable energy source for ascospore infections of the host.

The blossom tissue of several P. vulgaris and P. coccineus (111) lines was screened for resistance to colonization by ascospores or mycelium, but no differences were found.

Environmental factors influence the inoculum concentration and the infection process of S. sclerotiorum. One of the key factors is moisture. Grau and Radke (47) showed that increased moisture due to irrigation resulted in greater disease severity in soybeans. Similar effects of moisture have been noted by other researchers in soybeans (56, 112) and in P. vulgaris (34, 64, 84, 89, 110, 111).

For sclerotia to produce apothecia they must be in nearly saturated soil for approximately 10 days (6). Apothecia and ascospores may be produced throughout the growing season if the conditions are favorable (1, 6). Others have also observed increased apothecia production in wetter conditions (29, 34, 110). In lettuce fields, apothecia could only be found in isolated wet areas under the plant canopy (92). Laboratory studies have also shown

the importance of sufficient moisture for carpogenic germination (48, 62). The importance of sufficient moisture for apothecium germination in relation to white mold epidemology was shown by Natti (89). This research showed a positive correlation between disease severity and high July rainfalls before flowering, but no correlation between disease severity and rainfall during blossoming. The July rainfall favored carpogenic germination of sclerotia and ascospore production thus creating the primary inoculum for infections.

Apothecia formation has also been shown to be influenced by herbicides (105) and depth of burial. Singh and Singh (113) reported maximum carpogenic germination of sclerotia on the soil surface and that germination decreased with depth of burial. However, in another study germination was greatest when the sclerotia were buried (82). This would no doubt be influenced by the precipitation pattern and drainage of a particular site.

Ascospore survival is influenced by relative humidity. In an laboratory experiment, spore survival was highest in a low relative humidity (48). The longest survival time was 21 days at 7% relative humidity. In field studies in mhich ascospores were exposed to fluctuating relative humidity and temperature, survival ranged from 9 to 12 days. Thus ascospores may land on the host and remain viable for a period of time during which they may germinate when conditions are favorable.

Moisture is also critical for ascospore germination and host infection. In a field study on the effect of rainfall, temperature, relative humidity and leaf wetness on disease severity, only leaf wetness was positively correlated with disease (1). Weiss et al.(133) also found the duration of leaf wetness to be the most important factor determining disease incidence. Attempts to infect bean tissue with mycelium grown on agar were only successful when free moisture was present at the infection interface for 16 to 24 hours (1). Successful infections with colonized, dry blossom tissue or ascospores needed free moisture at the infection interface for 48 to 72 hours before host penetration was observed. Shorter durations of moisture may also support infections (19).

Lesion development is also influenced by moisture. Abawi and Grogan (1) found that lesion development ceased if the lesion and stem surface were dry. Even a relative humidity of 99 % was insufficient to maintain lesion growth. The arrested lesions required a 48 to 72 hour exposure to free moisture before they would resume growth. Growth of mycelial colonies on agar medium declined as the water potential of the medium drops below -20 bars (48), again illustrating slower growth with drier conditions.

Temperature also plays an important role in inoculum production and host infection. Apothecium production appeared to be optimum at 10° - 20° C (1, 110) though production has been noted at 25° C (1), indicating a broad

range of temperatures suitable for the production of ascospores. A broad range of temperatures (10 - 30° C) was also reported as favorable for ascospore germination with 25° C appearing optimal (1). Germ tubes were reported to grow fastest at 25° C. Lesion and mycelium growth in beans and soybeans appeared optimum at 20° C (1, 98) though 25° C also appeared adequate (133). Lesions did not appear to expand at 10° or 30° C (1, 133). The optimum temperatures are in the ranges expected in soybean and bean fields at the critical flowering period. Weiss et al.(133) reported favorable temperatures 82 % of the time in a regularily irrigated bean field in Nebraska. As such it is doubtful that temperature is a limiting factor in the disease epidemology in the areas where S. sclerotiorum is traditionally a problem.

HISTOLOGY

Upon germinating on the host, the pathogen can infect through the stomata (65), although penetration through the cuticle appears the most common form of infection (2, 75, 76, 102, 123). Generally ascospores will colonize a bean blossom and produce mycelium. The mycelium continues the infection process (2, 121).

Generally an appressorium-like structure (infection cushion) develops from a hypha, and penetration occurs by purely mechanical means (73, 75, 76, 123). This also appears to be true for other Sclerotinia species (76, 100,

114). The infection cushions vary in complexity by the number of hyphae but basically consist of several hundred parallel hyphal tips which are perpendicular to the host surface and some longer peripheral hyphae (76). The infection cushions form upon physical contact of the hyphae with the host tissue (2, 36, 41, 102). The hyphae then develop the dome-shaped infection cushions (2, 41, 73, 102). This is followed by the actual penetration, which may be accomplished by a penetration peg (2, 102). While penetration appears to be physical, there is evidence that some Sclerotinia species (100, 114) produce enzymes that precede penetration. Cell death was observed beneath the point of penetration prior to the actual penetration (76. 77).

Abawi et al.(2) found the complexity of the infection cushion to be associated with the nutrient level of the medium. Tariq and Jefferies report (123) that nutrition was important for formation of infection cushions but did not influence the complexity. They found that the physical resistance of the surface influenced the complexity of the resulting infection cushion with more complex infection cushions formed on the surface of P. coccineus leaves than on the softer P. vulgaris leaves.

Upon penetration the pathogen forms a vesicle (73, 102) from which infection hyphae form which continue the infection (73, 74, 102). In beans the infection hyphae grow radially from the vesicle and grow intercellularly (2, 73,

76, 102) between the cuticle and epidermal cell layer and in the cortex (73). The subcuticular hyphae branch, and the branches run parallel to each other, forming an infection front (73, 76). The subcuticular hyphae grow faster than the cortex hyphae though it is the later that girdle the stem (73). Up to this point all hyphal growth has been intercellular.

Ramifying hyphae form from the infection hyphae 12-24 hours after infection (73, 76). These hyphae form to the rear of the infection front and branch extensively (73). The ramifying hyphae grow both inter- and intracellularly in healthy and dead cells and invade the vascular tissue (73, 100). It is the ramifying hyphae which emerge from the stem through stomata or breaks in the cuticle (2, 73, 76).

The infection hyphae appear to be responsible for changes in the host's pectic material and for the death of host cells that occur in advance of any hyphae (73). These functions are aided by production of diffusible toxins and enzymes.

To colonize host tissue and move intercellularly the middle lamella must be degraded. S. sclerotiorum produces polygalacturonases which have pectolytic activity and accomplish this task (18, 42, 50, 74, 86). Lumsden (74) found that the enzymes can be produced as early as 12 hours after infection and the amount of pectic material decreases as the polygalacturonases increase (42, 74). Endo-polygalacuronase (Endo-pg) is produced first and has

been found both in vitro (18, 42, 50) and in vivo (50, 74). Endo-pg is localized in the advancing margins of lesions in the early stages of infection (24 hours) after which it quickly declines (74). Early reports claimed that no correlation existed between pathogenicity and enzyme acivity (54, 86). Held (54) did report that a degenerate strain of S. trifoliorum lacked the ability to produce the wilt symptoms. Lumsden (74) later found a positive relationship between Endo-pg production and virulence and postulated that the relationship was missed in the earlier reports due to the early inactivation (48 hrs.) of the enzyme.

Exo-polygalacturonase (Exo-pg) is also produced and is believed to break down the substrate left from the Endo-pg digestion (74). It is found in mature lesions (74) and is correlated to the rapid growth phase of an infection. Both Exo- and Endo-pg have a peak activity at a pH between 4.5 and 5.5 (42, 50, 74).

Another key enzyme in pathogenesis is pectin methylesterase (PME). This enzyme occurs naturally in uninfected bean tissue but PME from the host and the pathogen can differentiated (74). PME is also produced early in the infection process (42, 50, 74) and is associated with the advancing margins of lesions. PME content increases with time (74). Hancock showed that the PME levels are two times higher in infected than in uninfected tissue and that as PME increases, methoxy group content decreases (50). PME functions by demethylating pectin to pectate (50, 74) which

is more rapidly degraded by the polygalacturonases (50). PME has not been correlated with pathogenicity (74, 86) which may be expected as the Endo-pg can degrade methylated pectin substances.

Both Endo-pg and PME can be found in the middle lamella approximately two cells in advance of the infection hyphae (74) suggesting that these enzymes are produced by the infection hyphae. The middle lamella was already altered one to two cells in advance of the S. sclerotiorum hyphae in apple (Malus spp.) infections indicating the role of these two enzymes in facilitating infections. In later stages of infection the PME probably works in concert with Exo-pg.

Cellulases and hemicellulases are also produced by S. sclerotiorum (18, 70, 74). Cellulase is abundant in diseased tissue and appears to provide the pathogen with nutrition via cellulose breakdown as well as to facilitate cell invasion (70). The disease severity of a tissue has been associated with the cellulase levels. Cellulose content of diseased tissue declines as the tissue is degraded (70).

Phosphatidase is another enzyme that is produced early in the infection process (71). It is capable of hydrolyzing phosphorus-containing components of the cell wall. The enzyme is induced by calcium and the optimum pH for activity is 4.0.

Oxalic acid has long been associated with S. sclerotiorum infections (35, 80, 96) and is believed to

cause many of the changes that occur in the host tissue during pathenogenesis. The pH of healthy bean tissue is approximately 6 to 7 while the margins of advancing lesions have a pH close to 4.0 (50, 72, 74, 80, 86). The drop in pH has been correlated with the presence of oxalic acid (80). The lower pH favors the activity of the polygalacturonases, cellulases, hemicellulases and phosphatidase (50, 51, 70, 71, 74).

A synergistic relationship between oxalic acid and polygalacturonases has also been found with Sclerotium rolfsii (15). Oxalic acid chelates calcium ions which otherwise would also inhibit the activity of both Endo- and Exo-pg (15, 50, 51) and tissue maceration. The removal of calcium from the calcium-pectate complexes renders them more susceptible to polygalacturonase degradation (15, 80). The lowering of the pH and the chelation of calcium ions may account for the cell death in advance of the pathogen (75). Calcium oxalate crystals have been found in xylem vessels (73) and may be associated with wilt symptoms (75). Oxalic acid also stimulates the activity of PME (75).

Maxwell and Lumsden (80) showed a positive correlation between the disease severity in bean tissue and the level of oxalic acid. An isolate of S. sclerotiorum which produced slow disease development produced less oxalic acid than an isolate which rapidly infected bean hypocotyls. The isolates were from different hosts.

Oxalic acid treatment of sunflower (Helianthus annuus L.) shoots produced wilt symptoms identical to those produced by S. sclerotiorum infections (94). Wilted leaves had 15 times more oxalic acid than healthy leaves. Oxalic acid moved through the xylem and the xylem sap pH was one unit lower than normal, 2 cm ahead of lesions. The fact that oxalic acid accumulates in the leaves indicates the mobility of this compound within the plant.

Physiological resistance to S. sclerotiorum has been noted in soybeans (23, 27, 45) and Phaseolus species (41, 109, 111) and is characterized by the development of restricted, reddish brown lesions. The interaction of pathogen produced enzymes and the structural components of the tissue has not been studied. Dow and Lumsden (41) found that penetration of the cuticle of resistant Phaseolus coccineus occurred less often and was more difficult than in more susceptible genotypes. Upon penetration the infection hyphae were smaller than normal and rarely developed beneath the cuticle where rapid growth normally occurs. They concluded that the resistant tissue slowed the infection process and thus allowed the plant to actively respond to the pathogen.

As polygalacturonases and oxalic acid have been correlated with virulence (74, 121), it is of interest to review evidence on whether tissue can be resistant to enzyme degradation and if such resistance could account for differences in host-pathogen compatibility.

Rhizoctonia solani Kuhn produces an enzyme complex similar to S. sclertiorum (14). In bean hypocotys infected with R. solani, calcium was found to accumulate within lesions and in the tissue surrounding a lesion (14). This indicates an active tissue alteration in response to the pathogen. The calcium inhibited polygalaturoanase activity and the oxalic acid chelated the calcium. Bateman (14) hypothesized that the calcium accumulation liberates the host PME from the cell walls. The PME then demethylates pectic substances exposing carboxyl groups which have a high affinity for calcium ions. The calcium ions become strongly complexed with the pectic materials and thus inhibit polygalacturonase activity and limit lesion growth.

The tissue of bean hypocotyls becomes resistant to R. solani with increasing age (16, 17). The older tissue is characterized by changes in cell wall polysaccharide composition (91), changes in the pectic materials (16) and higher calcium content (16). The older tissue was more resistant to maceration than younger tissue (16) and the older cell walls were more resistant to enzyme degradation than younger cell walls (17). This evidence shows that tissue can vary in resistance to the enzyme complex. The age-related changes have not been conclusively linked to the resistance of the older tissue though the changes would seem to play an important role in slowing the infection process. Bateman et al.(17) reported that young hypocotyls can also rapidly limit lesion growth which indicates a response

beyond the age-related changes. Phytoalexin accumulation was also found in response to the infections. The authors concluded that lesion limitation was probably the result of a combination of induced cell wall alterations and phytoalexin accumulation.

Resistance to R. solani is associated with cell wall maturity in bean hypocotyls. Within a species the sequence of events leading to maturity does not vary though the time needed to reach maturity can (91). Among three P. vulgaris cultivars the time required to attain maturity varied by four days. The slower maturing cultivars were more susceptible to R. solani. This was not a very large test but it does suggest that cultivars that mature faster may be more resistant. This resistance could be due to the age-related changes already covered.

Stockwell and Hanchey (120) found increased calcium levels in lesions caused by R. solani on P. vulgaris. More calcium accumulated in the older, more resistant tissue. The authors failed to find increased calcification of the cell walls at the lesion border. They concluded that the calcium accumulation may have been cytoplasmic and that an increase in phenolics in the lesion tissue may have been responsible for lesion limitation. Cultivar responses were not investigated.

Cell suspension cultures from sunflower cultivars resistant or susceptible to S. sclerotiorum have been tested for reaction to oxalic acid (94). The cultures derived from

resistant cultivars were also more resistant to cell lysis by oxalic acid. As the middle lamella had already been dissolved this indicates that the resistance was in the cell wall or the cytoplasm.

Calcium accumulations have been noted in other host-pathogen interactions. Hammond and Lewis (49) found calcium accumulations and ultrastructural changes in the lesions produced by Leptosphaeria maculans on B. napus stems. The increase in calcium was associated with an accumulation of a lignin-like material. The changes often occurred well ahead of the hyphal front. The calcium was mainly deposited on the cell walls. In the expanding phase of the infection the hyphae grew through these calcium-lignin barriers and even when lesion growth ceased the hyphae were still several cells away from the completely lignified cell walls. The authors concluded that while the changes may slow hyphal growth, they could not account for the cessation of growth. Since little calcium accumulated in the middle lamella, it is doubtful that it became more resistant to polygalacturonase degradation. They proposed that the changes were an attempt by the host to compartmentalize the infection and that the effect of the calcium could be the result of other metabolic functions.

In Hypomyces solani f. sp. cucurbitae infections of squash (Cucurbita spp.), calcium accumulated in the lesions and was deposited on the cell wall and the middle lamella

(52). Despite this, the calcium did not appear to slow the rate of tissue maceration.

Lignin accumulation in response to fungal infections is well documented (106, 129). Vance et al.(129) reported that heavy lignification is an induced host response to fungal pathogens in resistant reactions in Solanum tuberosum L., Daucus carrota L. var. sativa D.C., Triticum aestivum L. and Cucumis species. In general lignification is thought to impede hyphal growth. It makes tissue more resistant to compressive forces and enzyme degradation. Ride (106) hypothesized that the lignification may prevent the diffusion of pathogen produced toxins from the immediate infection site and that its phenolic precursors may be toxic to the pathogen. It is unclear whether lignification is the primary cause of resistance or a result of resistance.

The role of calcium and lignin in limiting S. sclerotiorum infections has not been studied. The fact that P. coccineus tissue can show a physical resistance to S. sclerotiorum infections (41) indicates that the tissue is somehow resistant to the pathogen. In this system is not known whether the resistance is due to a passive or induced tissue difference or due to some other host response. The evidence from other host-pathogen systems which involve similar enzymes suggests that tissue alterations occur in response to a pathogen and that these changes are more pronounced in incompatible reactions. The role of the alterations are unknown.

Soybeans and P. vulgaris produce phytoalexins in response to S. sclerotiorum infections (122). The type of phytoalexin produced varied with the inoculated tissue (leaves or hypocotyls) and with the inoculum type (ascospores or mycelia). Ascospore inoculation of bean and soybean leaves resulted in hypersensitive reactions. High concentrations of phaseollin or phaseollidin accumulated in the bean leaves while no phytoalexins were detected in the soybean leaves. In addition no inhibitors of fungal growth were detected in the soybean leaves using bioassays. There remains a possibility that the phytoalexins accumulated in only the few hypersensitive soybean cells whereas the assay was run using the entire leaf thus possibly diluting the phytoalexin to a concentration too low to detect.

Bean leaves infected with mycelium did not contained detectable phytoalexins and had spreading lesions. Bean hypocotyls contained only kievitone 24 hours after inoculation and the lesions maintained a steady growth rate over a 100 hour period at 18° C . The rate of growth was much slower at 28° C and it ceased after 42 hours. Phaseollin was detected and kievitone levels were significantly higher at 28° C. The concentrations of these two compounds further increased from 42 to 100 hours. Soybean leaves accumulated low levels of glyceollin but soybean hypocotyls were not tested. The soybean leaf lesions were similar to the bean leaf lesions. The various phytoalexins produced were tested for their effects on the

growth of S. sclerotiorum ascopores and mycelia in vitro. Phaseollin was the most toxic while glyceollin was the least toxic.

The levels of phaseollin and kievitone in the bean hypocotyls at 28°C would be expected to suppress fungal growth based on the in vitro assays. The glyceollin levels in the soybean tissues were never very high though the concentrations may have been higher in the cells adjacent to the infections. These higher concentrations could be toxic to the fungus. The role of the phytoalexin in resistance is uncertain. The concentration of phytoalexin at the hyphal tip must be shown to be high enough to cause the cessation of fungal growth.

Red clover (Trifolium pratense L.) produced phytoalexins in response to S. trifoliorum infections (37). Pterocarpans were found to accumulate in the lesions. The lesions on all the cultivars tested continued to spread but the rate was slower on the two cultivars which consistently accumulated higher pterocarpan levels.

Phytoalexin production has been associated with resistance to several fungal diseases (79). Resistance to Pytophthora megasperma var. sojae in soybeans has been associated with glyceollin accumulation. This association was found whether resistance was due to a gene- for-gene response, a light-induced response or an age- related response.

Sutton and Deverall (122) showed that soybeans and beans produce phytoalexins in response to S. sclerotiorum infections that are fungitoxic and that in beans, these phytoalexins accumulate to toxic concentrations. Both crops produce phytoalexins in response to other pathogens (17, 79). The evidence indicates that the phytoalexins play an important role in limiting infections. Differences in phytoalexin accumulation among cultivars of a species may be a determining factor in differences in compatability. It is probable that tissue alterations and phytoalexins work in concert in response to pathogen invasions. The nature of the interaction in unknown.

AVOIDANCE AND RESISTANCE

To minimize the losses from S. sclerotiorum researchers have attempted to identify resistant genotypes which could be employed in a breeding program. There have been two different approaches to this problem. One approach has been to develop genotypes that avoid becoming infected by S. sclerotiorum. The other approach is the development of genotypes that are physiologically resistant or tolerant to infections.

There have been many reports of disease avoidance in Phaseolus species. The principle of avoidance is that certain plant architectures create a microclimate which is not conducive to the infection processes. White mold severity was found to be lower in wide rows versus narrow

rows and in determinate versus indeterminate Great Northern isolines (116). This indicated that the microclimate could be controlled to reduce disease severity. Coyne et al.(31) noted that mechanical or chemical alteration of a cultivar's architecture influenced the disease severity. They found that dense, compact canopies resulted in a higher disease severity than upright, open plant canopies. Plants with dense canopies developed more humid microclimates which favored the disease. An ideotype was proposed for avoidance. The ideal type would be upright, determinate or short indeterminate, fewer main stems, long internodes, fewer and shorter branches and small trifoliate leaves. These traits enhance air movement through the foliage and lead to conditions that are unfavorable for disease development.

Their observations of the effects of the more open canopy on the microclimate were confirmed in later studies that showed that temperatures were cooler and that leaves remained wet for longer periods of time when plant canopies are dense and compact (19, 132). Plant architecture can also influence the production of primary inoculum. Steadman et al.(110) found fewer apothecia beneath open canopied bush beans than under viney, dense canopied beans.

Later studies have changed the ideotype somewhat. Determinacy in itself does not appear to be very important as a determinate pinto bean, 'Ouray', remained susceptible (13, 32). Further tests on nearly isogenic determinate and

indeterminate lines showed no difference in apparent susceptibility (32). Schwartz et al.(111) suggested that the height of the canopy from the soil was a key component to avoidance. Other researchers have found that early maturing lines avoided the disease better than late maturing lines (32, 43). This suggests that a plant can avoid infection by either plant architecture, maturity or a combination of both.

It is difficult to study and determine the extent of disease avoidance in the field because of the confounding factors such as avoidance through plant maturity or architecture and physiological resistance. For example 'Black Turtle Soup' bean cultivar has a dense, viney indeterminate growth habit and as such should be quite susceptible to white mold. But 'Black Turtle Soup' apparently possesses a physiological trait that confers resistance (13, 32, 33). To further complicate field tests of avoidance, it has been found that the plant architecture of the adjoining rows influences the disease severity in any given row (43). These problems as well as an apparently low heritability for disease avoidance (narrow sense =.12) (12) indicate that selecting for this trait in a breeding program will not be very efficient.

In soybeans there have been no studies on disease avoidance. Results of row width studies (47, 136) on disease development suggests that an avoidance ideotype could be effective. But with the trend to plant soybeans in narrow

rows it is doubtful whether this strategy will be accepted in soybean production.

The second approach is to find sources of physiological resistance and incorporate them through a breeding program. This resistance would be expressd as an ability to prevent or limit infections to non-lethal proportions. This could be accomplished by preventing any of the steps in the infection process after the inoculum arrives at a suitable site.

In both soybeans and Phaseolus species there have been mentions of physiological resistance from field studies. In soybeans, Grau and Bissonette (45) noted differences in susceptibility among seven cultivars. In later studies cultivar differences were again found significant (46, 47). Among the cultivars that were tested over more than one year, 'Gnome' appeared susceptible while 'Corsoy' and 'Hodgson' and 'Hodgson 78' appeared tolerant to the disease. Lockwood and Isleib (68, 69) have also found significant differences among cultivars. Of the cultivars tested in over years, Gnome and 'Weber 84' were susceptible while 'Corsoy 79', 'Hardin', Hodgson 78, 'Pella' and 'Evans' were tolerant. Studies from Virginia (98) and Brazil (136) have also noted differences in resistance among soybean cultivars.

The field studies suggest differences in physiological resistance, but other factors may be confounded in the results such as maturity and plant architecture avoidance,

interrow effects (43) and inoculum distribution. Lockwood and Isleib (68) noted that the disease was variable in locations within a test. The distribution of inoculum of S. minor was shown to have a significant effect on the severity of leaf drop in lettuce (39, 63). Grau and Bissonette (45) noted that Corsoy, and to a lesser extent 'Chippewa 64', could restrict infections to localized, reddish brown lesions which indicated a physiological reaction to an infection. The localized lesions did not result in the death of the portions of the plant above them.

Laboratory and greenhouse research can remove many of the confounding factors of field studies. In an early greenhouse study, 14-day-old soybean seedlings were inoculated on wounded cotyledons with mycelia grown on potato dextrose agar (45). Steps were taken to keep the inoculations moist. All cotyledons were destroyed but in some cultivars the infections did not spread beyond the cotyledon in all plants. In general the results matched their field results and Corsoy even developed red, restricted lesions as in the field.

A different technique was employed by Lockwood and Isleib (67, 68). Stem sections were cut from greenhouse plants and inoculated in the laboratory by placing mycelial plugs on unwounded tissue at the apiacl end of the stem. The length of the resulting lesion was then measured. There were escapes and variability within a cultivar, but despite this, significant differences were found among genotypes.

Gnome and Weber 84 developed longer average lesion lengths than Corsoy. This techique and a modification of it has been used to screen plant introductions and several have been identified as being more resistant than Corsoy (68, 69).

Cline and Jacobson (27) compared several greenhouse inoculation techniques. Ascospores were used as inoculum and were either sprayed on flowering plants or on flower blossoms that were then placed in leaf axils. In other techniques, mycelium was grown on carrot root discs or celery petioles. The carrot discs were placed on the leaves while the celery pieces were placed on the stem of the soybean plants between the cotyledon and first trifoliate node. The celery pieces were then removed after 24 hours. In all experiments the environment was controlled to maintain conditions optimal for infection. The celery petiole technique, called limited term inoculation (LTI) and originally developed by Hunter et al.(60), produced the most uniform results. The ascospore inoculations were quite variable as infected blossoms often fell off. Placing the flower blossoms in the leaf axils reduced the number of escapes and increased disease severity. The LTI method produced significant differences among the cultivars tested. 'Elf', 'Evans' and 'Wells II' developed more severe symptoms than Corsoy, 'Williams' and 'Union'. Gnome also developed more severe symptoms than the more resistant cultivars though the difference was not significant. All three of the

resistant cultivars developed restricted, reddish brown lesions though not on all of the tested plants of a given cultivar. The cause of the variation within a cultivar was not determined. The disease severity was influenced by the age of the plants and the light intensity of the growth envirorment.

The LTI technique was used by Boland and Hall (23) to evaluate soybean cultivars. They obtained variable results and concluded that several trials were needed to make solid conclusions. They noted variability within a cultivar and the appearance of the reddish brown, restricted lesions. The restricted lesions were more common in the more resistant cultivars.

In all the above-mentioned assays there remains variation in the ratings of resistance including variation within a cultivar. There is also a possibility of escapes. Nevertheless the results matched, to some extent the field observations of resistance, indicating that these techniques may be useful indicators of physiological resistance.

Physiological resistance has also been noted in Phaseolus species in field studies. Black Turtle Soup (13, 32, 33), 'Charlevoix' (33, 109), a red kidney bean and 'Valentine' (109), a snap bean, have shown physiological resistance in the field. All appear to restrict infections to reddish brown lesions though this ability was diminished in Valentine (109). The restricted lesions were rarely found in the most susceptible genotypes. Black Turtle Soup,

Valentine and Charlevoix all produced the resistant reaction in growth room conditions (111). Several P. coccineus lines have been noted to exhibit physiological resistance in the field (33, 115, 117).

An early greenhouse study was conducted by Adams et al.(8). Cultivars were tested by inoculating 10 day old bean plants with mycelium infected oat seeds. Of the 180 genotypes tested nine appeared to exhibit resistance. All P. coccineus lines were resistant.

Abawi et al.(5) sprayed flowering plants with ascospores and placed the plants in a mist chamber. Resistance was noted and the inheritance investigated utilizing ascospore inoculation. The F_1, F_2, BC_1, BC_2 and parents from a cross of P. vulgaris cultivar 'Bush Blue Lake' x P. coccineus were evaluated for resistance. The data pointed to resistance being controlled by a single dominant gene. The authors stated that the resistance was associated with the blossom tissue though no reasons were given. P. coccineus is also resistant to infections from mycelial inoculations (4, 8) which implicates some factor involving stem tissue.

In a study comparing various inoculation techniques the method of Abawi et al.(5) produced inconsistent results (59). This was attributed to differences in the ages of flowers and to flowers falling off the plant. The technique was modified by removing one-day-old blossoms, spraying them with ascospores and placing them in leaf axils. The results

of this method were still variable with escapes occurring. The results were more consistent than before and several lines previously considered resistant by Abawi et al.(5) appeared susceptible. This clouds the conclusion that resistance may be controlled by a single dominant gene (5).

Further modifications led to the development of the limited term inoculation (LTI) technique (60). Mycelium infected celery pieces were attached to the stems of four week old soybeans between the cotyledonary and first trifoliate node. The best results were achieved when the inoculum was removed after 24 hours. During inoculation the bean plants were kept in a growth chamber under conditions favorable for infection. The plants were then placed on a greenhouse bench and rated for disease severity four days later. This technique was less variable than using ascospores and significant variation among cultivars for disease severity was detected. Some variability within cultivars remained and escapes were possible. The results were similar to field observations with Bush Blue Lake being susceptible and P. coccineus lines being resistant. Plant age and the light intensity of the growth environment had an effect on disease severity. A similar comparison of techniques in soybeans produced the same results (27).

The LTI technique was used to evaluate plant introductions for resistance to white mold in P. vulgaris (60). A line was considered resistant if 50% of the plants

survived. This illustrates the variability of the assay. Despite this 13 of 310 lines exhibited resistance.

Dickson et al.(38) screened three segregating populations for resistance to white mold. Population 1 consisted of 19 F_2 families generated from 4-way crosses among susceptible P. vulgaris lines. Population 2 was produced from eight crosses between intermediate resistant x intermediate resistant and intermediate resistant x resistant P. vulgaris and P. coccineus lines. Population 3 was generated from 21 combinations of 10 P. coccineus lines resistant to white mold. In all populations F_2 families were tested in the F_3 generation. In Populations 1 and 2 the survival rates when subjected to LTI treatment were only 0.8% and 2.0% respectively. Population 3 had a 3.8% survival rate. The survivors of Populations 1 and 2 were advanced to the F_4 and retested. The combined survival rate of the two populations was 17%. Population 3 was not tested in the F_4. The authors concluded that repeated selection increased the resistance of the population. This probably occurred through selection for and accumulation of additive resistance genes from the various susceptible lines. While not tested they felt that the heritability of this trait was high enough for efficient selection based on the increase in the percentage of survivors in one generation.

The inheritance of the physiologial resistance has been investigated in several field trials. Tests of the

segregating progeny from a cross between a resistant, late maturing, viney Black Turtle Soup bean and a susceptible, early, viney Great Northern bean indicated the the inheritance is quantitative with a low heritability (33). Early maturing, resistant recombinants were recoverd. Limited populations derived from selfed plants in BC_1 and BC_2 of a cross between P. vulgaris and P. coccineus segregated in a pattern indicative of a single dominant gene controlling resistance (33). Escapes could have affected these results.

In another study, a half diallel was constructed between three resistant and three susceptible Phaseolus vulgaris lines (44). Fifteen F_2 populations and the six parent lines were tested in the greenhouse, where inoculum was evenly distributed, and in the field where the effect of the adjacent row (43) was eliminated with control rows. The results showed no cross x environment effect or parent line x environment effect. There was less disease development in the field which was attributed to possible avoidance. This has also been noted elsewhere (109). Avoidance was not a factor in the greenhouse. The results were the same in both environments with GCA effects being highly significant indicating additive gene action. Heterosis only accounted for 5% of the genetic variation. There was a suggestion of partial dominance in one of the resistant lines. While the results were the same in both environments the authors noted that this does not indicate that the same genes are involved

in both environments as the field resistance could be due to avoidance. It is interesting that the three resistant lines may have different genes controlling resistance.

Resistance in P. vulgaris appears to be a quantitative trait (33, 38, 44) while resistance in P. coccineus appears to be controlled by a single dominant gene (5, 33). While some of the results have been questioned (59), it remains possible for the two species to have different genetics of resistance. Walker (131) reported that in some cabbage (Brassica oleracea L.) lines, resistance to Fusarium oxysporum is controlled by a single dominant gene while in other lines resistance is quantitatively inherited.

There have been several attempts to use crude filtrates of Sclerotinia sclerotiorum cultures or oxalic acid in potential screening methods. 'Ex Rico 23' a cultivar of white beans that had been reported to be resistant to white mold in the field (127) had a lower disease incidence and smaller lesions including a smaller water soaked area than susceptible cultivars. Oxalic acid has been noted to produce the typical wilt symptoms of a S. scerotiorum infection (35, 39, 94, 96). By immersing the petioles of excised leaves in [^{14}C]-labeled oxalic acid, the rate of oxalic acid diffusion through the leaf was followed (128). Oxalic acid diffused slower in the resistant Ex Rico 23 than in the susceptible cultivars. Furthermore the labeled oxalic acid was restricted to the main veins in Ex Rico 23

whereas a uniform distribution was found in the suceptible cultivars. No other resistant cultivars were checked.

A crude enzyme filtrate of a S. sclerotiorum culture was used to test sunflowers for resistance to infection (58). The filtrate mimicked field symptoms and cultivar differences were detected for reaction to the filtrate. There was variability within a cultivar for the reaction. The results were not compared with any field study so the method's ability to predict field reactions is unknown.

Sunflower cell suspension cultures have been tested for tolerance to oxalic acid (94). Cultures were generated from cultivars that had been rated as tolerant or susceptible based on field and greenhouse studies. The cultures from the resistant cultivars showed a higher tolerance to oxalic acid than the cultures from the susceptible cultivars as measured by cell lysis. The tissue had already been macerated to release the cells so the tolerance must be due to the properties of the cell wall or the cytoplasm, not the middle lamella.

Blanchette and Auld (21) used a heat stable element from a crude culture filtrate and oxalic acid to induce field symptoms of S. sclerotiorum. Non-host crops did not developed symptoms. The ability of the technique to distinguish cultivar differences was not reported. Resistance of alfalfa (Medicago sativa L.) to S. trifoliorum has been tested by spraying seedlings with oxalic acid (107). While the spray induced symptoms of the disease it

was concluded that reaction to oxalic acid was not correlated to field reactions to the pathogen. The other oxalic acid assay mentioned in this review were performed by immersing stem or petiole ends in an oxalic acid solution. It is possible that spraying oxalic acid on the leaf surface did not simulate the way the plant normally encounters oxalic acid (i.e. via subcuticular production from hyphae) and therefore did not test for resistance to the normal infection process.

This author has tried to cover primarily S. sclerotiorum in soybeans and Phaseolus species. But it is still interesting to note that sources of resistance have been found in other crops such as alfalfa (107), peanuts (28, 99), lettuce (77), safflower (Carthamus tinctorius L.)(88), peas (Pisium sativum L.)(20) and sunflowers (94, 95, 104).

SUMMARY

S. sclerotiorum causes Sclerotinia stem rot in soybeans and white mold in P. vulgaris. Both diseases can cause considerable crop loss. Sclerotinia stem rot usually appears in soybeans when they follow a more susceptible crop in a rotation. The disease spreads from field to field mainly by windblown ascospores and infected seed. The main reason for the recent reports of an increase in the incidence of Sclerotinia stem rot appears to be the spread of soybean cultivation into areas where the environment

favors the disease and where susceptible crops are traditionally grown.

The disease overwinters as sclerotia that survive in the soil and in the stalks of diseased plants. Survival is influenced by the depth of burial, soil moisture, and the presence of microparasites. Sclerotium numbers in a field increase primarily by means of their production inside an infected host.

Sclerotia undergo carpogenic germination in the spring and early summer when the temperature and moisture conditions are favorable. The sclerotia produce apothecia which in turn produce ascospores. The ascospores are ejected into the air and may land on the plant surfaces. Ascospores are the primary form of inoculum and can survive on the plant surfaces for approximately two weeks.

Mycelium from germinating ascospores colonize senescent tissue, primarily blossom tissue. The pathogen needs to colonize this intermediary tissue as an energy source so it can infect stem or leaf tissue. The energy is necessary for the formation of infection cushions and successful cuticle penetration which apparently occurs by mechanical means. Ascospore germination and host penetration require free moisture for extended periods of time. Cooler temperatures (20^{0} C) also favor infections.

Upon entering the host, the pathogen produces infection hyphae which grow intercellularly between the cuticle and the epidermis to form an infection front. Behind the front

the infection hyphae give rise to smaller ramifying hyphae which grow inter- and intra-cellularly. The pathogen produces enzymes and oxalic acid which precede the infection hyphae by one or two cells. This enzyme complex alters and degrades the pectic materials of the middle lamella and cell walls and facilitates fungal growth. Other enzymes are later produced which digest cellulose and cell contents. Oxalic acid reproduces disease symptoms when applied alone to host tissue, and tolerance to oxalic acid has been correlated with S. sclerotiorum field resistance. The quantitative production of polygalacturonase and oxalic acid has been correlated with the virulence of an isolate.

Plants escape lethal S. sclerotiorum infections through avoidance of the disease and/or by physiological means. In P. vulgaris avoidance has been attributed to differences in maturity and through modified plant architecture which alters the microclimate so it is less favorable for the infection process. Physiological resistance has been found in P. vulgaris, P. coccineus and soybeans and is expressed by restricted, reddish brown lesions. This trait has been studied in the field and with greenhouse and laboratory techniques. Field studies of resistance can produce results which are confounded with avoidance, escapes, uneven inoculum distribution and interrow effects.

The inheritance of physiological resistance has been studied in P. vulgaris and P. coccineus. In P. vulgaris the

resistance appears to be polygenic while the resistance in P. coccineus appears controlled by a single dominant gene.

Physiological resistance in P. coccineus is expressed by a resistance to infection and the ability of the host to restrict hyphal growth. Phytoalexins are produced in soybeans and P. vulgaris in response to S. sclerotiorum. At the present there have not been any specific studies performed on the actual causes of resistance to S. sclerotiorum.

PROPOSED RESEARCH

For a resistance assay to be of use to a breeder it must produce repeatable results, correlate with field resistance and be sensitive to difference among cultivars. Many of the reports of laboratory and greenhouse studies on resistance covered in this review report variation within a cultivar for resistance to S. sclerotiorum (20, 26, 41, 45, 58, 60, 61, 67, 94). This variation contributes to the error term and lowers the sensitivity of the test to genotypic differences. The cause of the variability has not been determined. Except for the filtrate and oxalic acid tests the variation could arise from escapes. Other possible causes could reside in the techniques themselves, in the growth environment of the materials to be tested and residual genetic heterogeneity within a cultivar for resistance to S. sclerotiorum.

The studies reported hereafter were conducted to develop an improved screening technique which lowers the variability in response to the disease within a cultivar. An improved assay would increase the selection efficiency of a resistance breeding program. The cause of the residual variability was investigated so that it could be eliminated or reduced.

The starting point for modification of the assay was the technique of Lockwood and Isleib (69). It was familiar to cooperative researchers and had the advantage over other assays of ease, speed, controlled environment and a quantitative assessment of disease development.

LIST OF REFERENCES

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1. Abawi, G.S. and R.G. Grogan. 1975. Sources of primary inoculum and effects of temperature and moisture on infection of beans by Whetzelinia sclerotiorum. Phytopath. 65:300-399.

2. Abawi, G.S., F.J. Polach and W.T. Molin. 1975. Infection of bean by ascospores of Whetzelinia sclerotiorum. Phytopath 65:673-678.

3. Abawi, G.S., D.C. Provvidenti, R.G. Grogan and J.E. Hunter. 1975. Predisposition of beans to infection by ascospores of Whetzelinia sclerotiorum prior to blossoming. (Abstr.) Proc. Am. Phytopathol. Soc. 2:61.

4. Abawi, G.S., R. Provvidenti and J.E. Hunter. 1975. Evaluating germplasm for resistance to Whetzelinia sclerotiorum. Proc. Am. Phytopathol. Soc. 2:50.

5. Abawi, G.S. and R. Provvidenti. 1978. Inheritance of resistance to white mold disease in Phaseolus coccineus. Jour. Hered. 69:200-202.

6. Abawi, G.S. and R.G. Grogan. 1979. Epidemiology of diseases caused by Sclerotinia species. Phytopath. 69:899-903.

7. Abawi, G.S., R.G. Grogan and J.M. Dunlway. 1985. Effect of water potential on the survival of sclerotia of Sclerotinia minor in two California soils. Phytopath. 75:217-221.

8. Adams, P.B., C.J. Tate, R.D. Lumsden amd J.P. Meiners. 1973. Resistance of Phaseolus species to Sclerotinia sclerotiorum. Rep. Bean Improv. Comm. 16:8-9.

9. Adams, P.B. 1975. Factors affecting the survival of Sclerotinia sclerotiorum in the soil. Plant Dis. Rep. 59:599-603.

10. Adams, P.B. and W.A. Ayers. 1979. Ecology of Sclerotinia species. Phytopath. 69:896-899.

11. Adams, P.B. 1983. Cocklebur: A new host for several Sclerotinia species. Plant Dis. 67:484-485.

12. Agbo, F.M.O. and D.R. Wood. 1979. Inheritance of resistance to white mold in Pinto x Aurora crosses. Rep. Bean Improv. Comm. 22:26-27.

13. Anderson, F.N., J.R. Steadman, D.P. Coyne and H.F. Schwartz. 1974. Tolerance to white mold in Phaseolus vulgaris dry edible bean types. Plant Dis. Rep. 58:782-784.

14. Bateman, D.F. 1964. An induced mechanism of tissue resistance to polygalacturonase in Rhizoctonia -infected hypocotyls of bean. Phytopath. 54:438-445.

15. Bateman, D.F. and S.V. Beer. 1965. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by Sclerotium rolfsii. Phytopath.55:204-211.

16. Bateman, D.F and R.D. Lumsden. 1965. Relation of calcium content and nature of the pectic substances in bean hypocotyls of different ages to susceptibility to an isolate of Rhizoctonia solani. Phytopath. 55:734-73.

17. Bateman, D.F., H.D. Van Etten, P.D. English, D.J. Nevins and P. Albersheim. 1969. Susceptibility to enzyme degradation of cell walls from bean plants resistant and susceptible to Rhizoctonia solani Kuhn. Plant Physiol. 44:641-648.

18. Bauer, W.D., D.F. Bateman and C.H. Whalen. 1977. The purification of an endo-B-1,4 galactanase produced by Sclerotinia sclerotiorum on isolated plant cell walls and potato tissue. Phytopath. 67:862-868.

19. Blad, B.L., J.R. Steadman and A. Weiss. 1978. Canopy structure and irrigation influence on white mold disease and microclimate of dry edible beans. Phytopath. 68:1431-1437.

20. Blanchette, B.L., and D.L. Auld. 1978. Screening field peas for resistance to white mold. Crop Sci. 18:977-979.

21. Blanchette, B.L. and D.L. Auld. 1979. A rapid screening technique to determine plant resistance to Sclerotinia sclerotiorum. (Abstr.) Phytopath 69:193.

22. Blodgett, E.C. 1946. The Sclerotinia rot disease of beans in Idaho. Plant Dis. Rep. 30:137-144.

23. Boland, G.J. and R. Hall. 1986. Growthroom evaluation of soybean cultivars for resistance to Sclerotinia sclerotiorum. Can. J. Plant Sci. 66:559-564.

24. Bronsten, J.L., and T.G. Sands. 1986. Field trial of Sclerotinia sclerotiorum to control Canada thistle (Cirsium arvense). Weed Sci. 34:377-380.

25. Brown, J.G. and K.D. Butler. 1936. Sclerotiniose of lettuce in Arizona. Ariz. Agric. Exp. Sta. Bull. 63:475-506.

26. Chamberlain, D.W. 1951. Sclerotinia stem rot of soybeans. Plant Dis. Rep. 35:490-491.

27. Cline, M.N. and B.J. Jacobson. 1983. Methods of evaluating soybean cultivars for resistance to Sclerotinia sclerotiorum. Plant Dis. 67:784-786.

28. Coffelt, T.A. and D.M. Porter. 1982. Screening peanuts for resistance to Sclerotinia blight. Plant Dis. 66:385-387.

29. Coley-Smith, J.R. and R.C. Cooke. 1971. Survival and germination of fungal sclerotia. Annu. Rev. Phytopathol. 9:65-92.

30. Cook, G.E., J.R. Steadman and M.G. Boosalis. 1975. Survival of Whetzelinia sclerotiorum and initial infection of dry edible beans in western Nebraska. Phytopath 65:250-255.

31. Coyne, D.P, J.R. Steadman and F.N. Anderson. 1974. Effect of modified plant architecture of Great Northern dry bean varieties (Phaseolus vulgaris) on white mold severity, and components of yield. Plant Dis. Rep. 58:379-382.

32. Coyne, D.P., J.R. Steadman and H.F. Schwartz. 1977. Reaction of dry bean germplasm to Sclerotinia sclerotiorum. Plant Dis. Rep. 61:226-230.

33. Coyne, D.P., J.R. Steadman and H.F. Schwartz. 1977. Genetic variation, inheritance and breeding strategy for resistance to Sclerotinia sclerotiorum in beans (Phaseolus vulgaris L.). Rep. Bean Improv. Comm. 20:19-21.

34. Dana, B.F and E.K. Vaughan. 1949. Etiology and control of Sclerotinia sclerotiorum on Bush Blue Lake beans. Phytopath 39:859.

35. De Bary, A. 1886. Ueber einge Sclertinien und Sclerotienkranheiten. Bot. Z. 44:377-474.

36. De Bary, A. 1887. In: Comparitive morphology and biology of the fungi. pp. 380-382, Clarendon Press, Oxford, England.

37. Debnam, J.R. and I.M. Smith. 1976. Changes in the isoflavones and pterocarpans of red clover on infection with Sclerotinia sclerotiorum and Botrytis cinerea. Physiol. Plant Path. 9:9-23.

38. Dickson, M.H., J.E. Hunter, M.A. Boettger and J.A. Cigna. 1982. Selection for resistance in Phaseolus vulgaris L. to white mold caused by Sclerotinia sclerotiorum (Lib.) De Bary. J. Amer. Soc. Hort. Sci. 107:231-234.

39. Dillard, H.R and R.G. Grogan. 1985. Relationship between sclerotial spatial pattern and density of Sclerotinia minor and incidence of leaf drop. Phytopath. 75:90-94.

40. Dillard, H.R. and J.E. Hunter. 1986. Association of common ragweed with Sclerotinia rot of cabbage. Plant Dis. 70:26-28.

41. Dow, R.L. and R.D. Lumsden. 1975. Histology of resistance of Phaseolus coccineus (Scarlet Runner bean) to Sclerotinia sclerotiorum. Proc. Am. Phytopath. Soc. (Abstr.) 2:122.

42. Echandi, E. and J.C. Walker. 1957. Pectolytic enzymes produced by Sclerotinia sclerotiorum. Phytopath. 47:303-306.

43. Fuller, P.A., D.P. Coyne, J.R. Steadman and R.F Mumm. 1984. Inter- and intra-row inter-genotypic competition influences selection for avoidance of white mold disease in dry edible beans. J. Amer. Hort. Sci. 109:567-572.

44. Fuller, P.A., D.P. Coyne and J.R. Steadman. 1984. Inheritance of resistance to white mold disease in a diallel cross of dry beans. Crop Sci. 24:929-933.

45. Grau, C.R. and H.L. Bissonette. 1974. Whetzelinia stem rot of soybeans in Minnesota. Plant Dis. Rep. 58:693-695.

46. Grau, C.R., V.L. Radke and F.L. Gillespie. 1982. Resistance of soybean cultivars to Sclerotinia sclerotiorum. Plant Dis. 66:506-508.

47. Grau, C.R. and V.L. Radke. 1984. Effects of cultivars and cultural practices on Sclerotinia stem rot of soybean. Plant Dis. 68:56-58.

48. Grogan, R.G. and G.S. Abawi. 1975. Influecence of water potential on growth and survival of Whetzelinia sclerotiorum. Phytopath. 65:122-138.

49. Hammond, K.E. and B.G. Lewis. 1986. Ultrastructural studies on the limitation of lesions caused by Leptosphaeria maculans in stems of Brassica napus var. oleifera. Physiol. Molecul. Plant Path. 28:251-265.

50. Hancock, J.G. 1966. Degradation of pectic substances associated with pathenogenesis by Sclerotinia sclerotioruum in sunflower and tomato stems. Phytopath. 56:975-979.

51. Hancock, J.G. 1967. Hemicellulose degradation in sunflower hypocotyls infected with Sclerotinia sclerotiorum. Phytopath. 57:203-206.

52. Hancock, J.G. and M.E. Stanghellini. 1968. Calcium localization in Hypomyces-infected squash hypocotyls and the effect of calcium pectate lyase activity and tissue maceration. Can. J. Bot. 46:405-409.

53. Held, V.M. and C.M. Haenseler, 1953. Cross-inoculations with New Jersey isolates of Sclerotinia sclerotiorum, S. minor and S. trifoliorum. Plant Dis. Rep. 37:515-517.

54. Held, V.M. 1955. Physiological differences between a normal and a degenerate strain of Sclerotinia trifoliorum. Phytopath 45:39-42.

55. Hildebrand, A.A. 1948. Soybean diseases in Ontario. Soybean Dig. 10:16-17.

56. Hine, R.B. and J.E. Wheeler. 1970. The occurrence of some previously unreported diseases in Arizona. Plant Dis. Rep. 54:179-180.

57. Huang, H.C. 1976. Biological control of Sclerotinia stem wilt in sunflowers. pp.69-72. in Rep. Annu. Conference Manit. Agron. 1976.

58. Huang, H.C. and D.G. Dorrell. 1978. Screening sunflower seedlings for resistance to toxic metabolites produced by Sclerotinia sclerotiorum. Can. J. Plant Sci. 58:1107-1110.

59. Hunter, J.E. and M.H. Dickson. 1979. Screening for resistance to white mold. Rep. Bean Improv. Comm. 22:18-22.

60. Hunter, J.E., M.H. Dickson and A. Cigna. 1981. Limited-term inoculation: A method to screen bean plants for partial resistance to white mold. Plant Dis. 65:414-417.

61. Hunter, J.E., M.H. Dickson and M.A. Boettger. 1982. Evaluation of plant introductions of Phaseolus species for resistance to white mold. Plant Dis. 66:320-322.

62. Imohelin, E.D., R.G. Grogan and J.M Dunlway. 1980. Effect of temperature and moisture tension on growth, sclerotial production, germination and infection by Sclerotinia minor. Phytopath. 70:1153-1157.

63. Imohelin, E.D. and R.G. Grogan. 1980. Factors affecting survival of sclerotia, and effects of inoculum density, relative position and distance from the host on infection of lettuce by Sclerotinia minor. Phytopath 70:1162-1167.

64. Jones, D. and D. Watson. 1969. Parasitism and lysis by soil fungi of Sclerotinia sclerotiorum (Lib.) De Bary, a phytopathenic fungi. Nature 224:287

65. Jones, D. 1976. Infection of plant tissue by Sclerotinia sclerotiorum: A scanning microscope study. Micron 7:275-279.

66. Kruger, W. 1975. The influence of some environmental factors on the development of apothecia and ascospores of Sclerotinia sclerotiorum (Lib.) De Bary. Z. PflKrankh. PflSchutz. 82:101-108.

67. Lockwood, J.L. and T.G. Isleib. 1983. Resistance of soybeans to white mold. Annual Report to the Soybean Promotion Committee of Michigan 1983-84.

68. Lockwood, J.L. and T.G. Isleib. 1984. Resistance of soybeans to white mold. Annual Report to the Soybean Promotion Committee of Michigan. 1984.

69. Lockwood, J.L. and T.G. Isleib. 1985. Resistance of soybeans to white mold. Annual Report to the Soybean Promotion Committee of Michigan. 1985.

70. Lumsden, R.D. 1969. Sclerotinia sclerotiorum infection of bean and the production of cellulase. Phytopath. 59:653-657.

71. Lumsden, R.D. 1970. Phosphatidase of Sclerotinia sclerotiorum produced in culture and in infected bean. Phytopath. 60:1106-1110.

72. Lumsden, R.D. 1972. Localization of pH changes in bean hypocotyls infected with Sclerotinia sclerotiorum. (Abstr.) Phytopath. 62:774.

73. Lumsden, R.D and R.L. Dow. 1973. Histology of Sclerotinia sclerotiorum infection of bean. Phytopath. 63:708-715.

74. Lumsden, R.D. 1976. Pectolytic enzymes of Sclerotinia sclerotiorum and their localization in infected bean. Can. J. Bot. 54:2639-2641.

75. Lumsden, R.D. 1979. Histology and physiology of pathogenesis in plant diseases caused by Sclerotinia species. Phytopath. 69:890-896.

76. Lumsden. R.D. and W.P. Wergin. 1980. Scanning-electron microscopy of infection of bean by species of Sclerotinia. Mycologia 72:1200-1209.

77. Madjid, A., S. Honma and M.L. Lacey. 1983. A greenhouse method for screening lettuce for resistance to Sclerotinia sclerotiorum. Scientia Hort. 18:201-206.

78. Makkonen, R. and O. Pohjakallio. 1960. On the parasites attacking the sclerotia of some fungi pathogenic to higher plants and the resistance of these sclerotia to their parasites. Acta Agric. Scand. 10:105-126.

79. Mansfield, J.W. 1982. The role of phytoalexins in disease resistance. In: Phytoalexins. pp. 253-288. eds. Bally, J.A. and J.W. Mansfield. Blackie, Glasgow and London.

80. Maxwell, D.P. and R.D. Lumsden. 1970. Oxalic acid production by Sclerotinia sclerotiorum on infected bean and in culture. Phytopath 60:1395-1398.

81. Mc Lean, D.M. 1958. Role of dead flowers parts in the infection of certain crucifers by Sclerotinia sclerotiorum (Lib.) De Bary. Plant Dis. Rep. 42:663-666.

82. Merriman, P.R., M. Pywell, H. Harrison and J. Nancarrow. 1979. Survival of Sclerotinia sclerotiorum and effects of cultivation practices on disease. Soil Biol. Biochem. 11:567-570.

83. Moore, W.D. 1949. Flooding as a means of destroying the sclerotia of Sclerotinia sclerotiorum. Phytopoath. 39:920-927.

84. Moore, W.D. 1955. Relation of rainfall and temperature to the incidence of Sclerotinia sclerotiorum in vegetables in south Florida during the years 1944-1954. Plant Dis. Rep. 39:470-472.

85. Morgan, O.D. A study of four weed hosts of Sclerotinia species in alfalfa fields. Plant Dis. Rep. 55:1087-1089.

86. Morrall, R.A.A., L.J. Duczek and J.W. Sheard. 1972. Variations and correlations within and between morphology, pathogenicity, and pectolytic enzyme activity in Sclerotinia from Saskatchewan. Can. J. Bot. 50:767-786.

87. Mueller, J.D., M.N. Cline, J.B. Sinclair and B.J. Jacobson. 1985. An in vitro test for evaluating efficacy of mycoparasites on sclerotia of Sclerotinia sclerotiorum. Plant Dis. 69:584-587.

88. Mundel, H.H., H.C. Huang and G.C. Kozub. 1985. Sclerotinia head rot in safflower: Assessment of resistance and effects on yield and oil content. Can. J. Plant Sci. 65:259-265.

89. Natti, J.J. 1971. Epidemiology and control of bean white mold. Phytopath. 61:669-674.

90. Neergaard, P. 1958. Mycelial seed infection of certain crucifers by Sclerotinia sclerotiorum (Lib.) De By. Plant Dis. Rep. 42:1105-1106.

91. Nevins, D.J., P.D. English and P. Albersheim. 1968. Changes in cell wall polysaccharides associated with growth. Plant Physiol. 43:914-922.

92. Newton, C.H. and L. Sequeira. 1972. Ascospore as the primary infective propagule of Sclerotinia sclerotiorum in Wisconsin. Plant Dis. Rep. 56:798-802.

93. Nicholson, J.F., O.D. Dhingra and J.B. Sinclair. 1972. Internal seedbourne nature of Sclerotinia sclerotiorum and Phompsis sp. and their effect on soybean seed quality. Phytopath. 62:1261-1263.

94. Noyes, R.D and J.G. Hancock. 1981. Role of oxalic acid in the Sclerotinia wilt of sunflower. Physiol. Plant Path. 18:123-132.

95. Orellana, R.G. 1975. Photoperiod influences on the susceptibility of sunflowers to Sclerotinia stalk rot. Phytopath. 65:1293-1298.

96. Overall, B.T. 1952. A toxin in culture filtrates of Sclerotinia sclerotiorum. Aust. J. Sci. 14:197-198.

97. Patterson, C.L. and R.G. Grogan. 1985. Differences in epidemiology and control of lettuce leaf drop caused by Sclerotinia minor and Sclerotinia sclerotiorum. Plant Dis. 69:766-770.

98. Phipps, P.M. and D.M. Porter. 1982. Sclerotinia blight of soybeans caused by Sclerotinia minor and Sclerotinia sclerotiorum. Plant Dis. 66:163-165.

99. Porter, D.M, M.K. Beute amd J.C. Wynne. 1975. Resistance of peanut germplasm to Sclerotinia sclerotiorum. Peanut Sci. 2:78-80.

100. Prior, G.C., and J.H. Owen. 1964. Pathological anatomy of Sclerotinia trifoliorum on clover and alfalfa. Phytopath 54:784-787.

101. Purdy, L.H. and R. Bardin. 1953. Mode of infection of tomato plants by the ascospores of Sclerotinia sclerotiorum. Plant Dis. Rep. 37:361-362.

102. Purdy, L.H. 1958. Some factors affecting penetration and infection by Sclerotinia sclerotiorum. Phytopath 48:605-609.

103. Purdy, L.H. 1979. Sclerotinia sclerotiorum: History, diseases and symptomology, host range, geographical distribution, and impact. Phytopath. 69:875-880.

104. Putt, E.D. 1958. Notes on differences in susceptibility to Sclerotinia wilt in sunflowers. Can. J. Plant Sci. 38:380-381.

105. Radke, V.L. and C.R. Grau. 1896. Effects of herbicides on carpogenic germination of Sclerotinia sclerotiorum. Plant Dis. 70:19-23.

106. Ride, J.P. 1978. The role of cell wall alterations in resistance to fungi. Ann. Appl. Biol. 89:302-306.

107. Rowe, D.E. and R.E Welty. 1984. Indirect selection for resistance to Sclerotinia crown and stem rot on alfalfa. Can. J. Plant Sci. 64:145-150.

108. Schwartz, H.F. and J.R. Steadman. 1977. Sclerotinia sclerotiorum inoculum production in western Nebraska. Bean Improv. Ann. Rep. 20:69-70.

109. Schwartz, H.F., J.R. Steadman and D.P. Coyne. 1977. Resistance of Charlevoix and Valentine to infection by Sclerotinia sclerotiorum. Rep. Bean Improv. Comm. 20:71-71.

110. Schwartz, H.F. and J.R. Steadman. 1978. Factors affecting sclerotium populations of, and apothecium production by, Sclerotinia sclerotiorum. Phytopath. 68:383-388.

111. Schwartz, H.F, J.R. Steadman and D.P. Coyne. 1978. Influence of Phaseolus vulgaris blossoming characteristcs and canopy structure upon reaction to Sclerotinia sclerotiorum. Phytopath 68:465-470.

112. Signoret, P.A., P.C. Bernaux and B. Poinso. 1975. Soybean diseases in France. Plant Dis. Rep. 59:616-617.

113. Singh, U.P. and R.B. Singh. 1983. The effect of soil texture, soil mixture, soil moisture and depth of soil on carpogenic germination of Sclerotinia sclerotiorum. ZPP 90:662-669.

114. Smith, V.L., Punja, Z.K. and S.F. Jenkins. 1986. A histological study of infection of host tissue by Sclerotium rolfsii. Phytopath 76:755-759.

115. Starr, G.H., H.J. Walters and G.H. Bridgemon. 1953. White mold (Sclerotinia) of beans. Wyo. Agric. Exp. Sta. Bull. 322.

116. Steadman, J.R, D.P. Coyne and G.E. Cook. 1973. Reduction of severity of white mold disease on Great Northern beans by wider row spacing and determinate plant growth habit. Plant Dis. Rep. 57:1070-1071.

117. Steadman, J.R., D.P. Coyne and H.F. Schwartz. 1974. Field reaction of beans to severe white mold infection. Rep. Bean Improv. Comm. 17:84-85.

118. Steadman, J.R. 1975. Nature and epidemiological significance of infection of bean seed by Whetzelinia sclerotiorum. Phytopath. 65:1323-1324.

119. Steadman, J.R., C.R. Maier, H.F. Schwartz and E.D. Kerr. 1975. Pollution of surface irrigation water by plant pathogenic organisms. Water Resour. Bull. 11:796-804.

120. Stockwell, V. and P. Hanchey. 1982. Cytohistochemical techniques for calcium localization and their applications to diseased plants. Plant Physiol. 70:244-251.

121. Sutton, D.C and B.J. Deverall. 1983. Studies on infection of bean (Phasleolus vulgaris) and soybean (Glycine max) by ascospores of Sclerotinia sclerotiorum. Plant Path. 32:251-261.

122. Sutton, D.C. and B.J. Deverall. 1984. Phytoalexin accumulation during infection of bean and soybean by ascospores and mycelium of Sclerotinia sclerotiorum. Plant Path. 33:377-383.

123. Tariq, V.N. and P. Jefferies. 1984. Appressorium formation by Sclerotinia sclerotiorum: Scanning electron microscopy. Trans. Brit. Mycol. Soc. 82:645-651.

124. Thompson, A.H. and G.C.A. Van Der Westhuizen. 1979. Sclerotinia sclerotiorum (Lib.) De Bary on soybean in South Africa. Phytophylactica 11:145-148.

125. Tribe, H.T. 1957. On the parasitism of Sclerotinia trifoliorum by Coniothyrium minitans. Trans. Brit. Mycol. Soc. 40:489-499.

126. Trutmann, P., P.J. Keene and P.R. Merriman. 1980. Reduction of sclerotial inoculum of Sclerotinia sclerotiorum with Coniothyrium minitans. Soil Biol. Biochem. 12:461-465.

127. Tu, J.C. and W.D. Beversdorf. 1982. Tolerance to white mold (Sclerotinia sclerotiorum (Lib.) De Bary) in Ex Rico 23, a cultivar of white bean (Phaseolus vulgaris L.). Can. J. Plant Sci. 62:65-69.

128. Tu, J.C. 1985. Tolerance of white bean (Phaseolus vulgaris) to white mold (Sclerotinia sclerotiorum) associated with tolerance to oxalic acid. Physiol. Plant Path. 26:111-117.

129. Vance, C.P., T.K. Kirk and R.T. Sherwood. 1980. Lignification as a mechanism of disease resistance. Ann. Rev. Phytopathol. 18:259-288.

130. Wadsworth, D.F. 1985. Potential for transmission and spread of Sclerotinia minor by infected peanut seed and debris. Plant Dis. 69:379-381.

131. Walker, J.C. 1969. Plant Pathology Third edition. McGraw Hill, New York.

132. Weiss, A., L.E. Hipps, B.L. Blad and J.R. Steadman. 1980. Comparison of within-canopy microclimate and white mold disease (Sclerotinia sclerotiorum) development in dry edible beans as influenced by canopy structure and irrigation. Agri. Meterol. 22:11-21.

133. Weiss, A. and E.D. Kerr. 1980. Temperature and moisture influences on development of white mold disease (Sclerotinia sclerotiorum) on Great Northern beans. Plant Dis. 64:757-759.

134. Weiss, F. 1946. Check list revision. Plant Dis. Rep. 30:130-137.

135. Williams, G.H. and J.H. Western. 1965. The biology of Sclerotinia trifoliorum Erikss. and other sclerotia forming fungi II. The survival of sclerotia in the soil. Ann. Appl. Biol. 56:261-268.

136. Yorinori, J.T. and M. Homechin. 1985. Sclerotinia stem rot of soybeans, its importance and research in Brazil. p. 582-588. in: World soybean conference III proceedings. ed. Richard Shibles. Westview Press Boulder.

137. Young, P.A. and H.E. Morris. 1927. Sclerotinia wilt of sunflowers. Mont. Agric. Exp. Sta. Bull. 208.

DEVELOPMENT OF AN ASSAY FOR RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* INFECTION OF SOYBEAN

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) De Bary is the cause of Sclerotinia stem rot in soybean [Glycine max (L.) Merr.]. This disease has been reported in many soybean producing states (5, 6, 11, 13, 17, 31). The disease is not widespread and is more prevalent in cool, wet areas (13, 14, 32) and when soybeans follow a more susceptible host in a crop rotation (11, 14, 24). The disease can be quite devastating and causes considerable yield losses (13, 14, 19). Cultivars that are resistant to Sclerotinia stem rot could minimize the losses from this disease.

Resistance has been reported among soybean cultivars in the field (4, 11, 18, 24, 32) and in greenhouse and laboratory experiments (4, 6, 11, 17, 18). In field studies the expression of physiological resistance or susceptibility can be confounded with disease avoidance (27), inoculum distribution (8, 16), and interrow effects (10). Greenhouse and laboratory studies minimize these problems but may require considerable space, growth and/or mist chambers, may be labor intensive and escapes are still possible. The laboratory method of Lockwood and Isleib (17) offers the advantages of requiring little space, having a quick and easy inoculation procedure, a constant inoculation and incubation environment between experiments, and offers a quantitative assessment of disease development. At present the method lacks the sensitivity to distinguish the difference between the closely related lines that would need

to be screened in the advanced stages of a resistance breeding program.

The following experiments were undertaken to improve the technique of Lockwood and Isleib (17) by identifying the causes of the residual variability and either eliminating them or accounting for them in the experimental procedures.

MATERIALS AND METHODS

All the plant material was grown in the greenhouse under natural and supplemental light (16 hrs. day, 8 hrs. night). The plant age varied by experiment as some experiments required longer stems. The ages of the plants used in the experiments are summarized in Table 1 along with the experimental design used in the experiments. The plants were grown in 20 cm diameter clay pots containing a 5:3:2 mixture of sandy loam soil, sphagnum peat and torpedo sand. In the experiments where the plants were grown for more than 45 days, each pot received a fertilizer treatment consisting of 4 gm Rapid-Gro Plus fertilizer (23-19-17) in 1000 ml of water. Four plants were grown in each pot. Each pot was randomly assigned one genotype. All pots were randomly placed 130 cm below the light source on the greenhouse bench such that the pot rims touched each other.

The assay was performed on stem sections inoculated in controlled laboratory conditions. In Experiments 1, 2, 3, 6 and 22 the stem sections were prepared from the top 15 cm of the main stem. The leaves and petioles were then cut from

Table 1. The experimental design used in Experiments 1 through 22.

Exp. no.	Design†	No. of reps.	Stems per trtmnt/rep combination	Inoculations per stem	Plant age	Treatments
					-- days --	
1	RCBD	3	4	1	30	16 genotypes
2	RCBD	3	4	1	30	9 genotypes
3	RCBD	3	4	1	30	2 x 9 factorial
			(2 inoculation site preparations by 9 genotypes)			
4	RCBD SP	3	6	3	74	4 genotypes
5	RCBD SP	2	2	7	79	5 genotypes
6	RCBD	3	4	1	39	9 genotypes
7	CRD	unequal	1	1	50	3 genotypes
8	CRD	21	1	1	40	5 genotypes
9	RCBD	3	12	1	38	5 genotypes
10	RCBD	3	12	1	38	5 genotypes
11	RCBD	3	12	1	38	5 genotypes
12	RCBD	3	5	1	37	5 x 3 factorial
			(5 durations of inoculation by 3 genotypes)			
13	RCBD	2	4	1	58	5 genotypes
14	CRD	unequal	1	1	60	4 genotypes
15	RCBD	3	unequal	1	47	5 genotypes
16	RCBD SP	2	3	5	60	2 genotypes
17	CRD	4	1	4	42	2 genotypes
18	RCBD SP	2	4	4	58	5 genotypes
19	CRD	unequal	1	4	36	4 genotypes
20	CRD	5	1	4	72	4 genotypes
21	RCBD	2	5	1	60	2 x 2 factorial
				(2 media concentrations by 2 genotypes)		
22	CRD	unequal	1	1	50	4 genotypes

† RCBD = randomized complete block design, CRD = completly randomized design, RCBD SP = randomized complete block design with a split plot.

this 15 cm stem section. The sections were then placed in plastic bags and transported to the laboratory. In Experiments 6 and 22 the top 0.5 cm was removed to create a wound. In Experiments 4, 5 and 17 the uppermost 24 cm, 51 cm and 38 cm respectively were cut and trimmed for study. In Experiments 7 through 15 and Experiment 21, the stem sections were prepared as follows: one cut was made 1.5 cm above the node bearing the uppermost fully expanded trifoliate leaf. The second cut was made 15 cm below this node, thus producing a 16.5 cm section. In the laboratory, immediately before inoculation, a final cut was made perpendicular to the length of the stem through the node which bore the uppermost trifoliate leaf. The final cut produced a 15 cm stem section for testing. The stem sections were inoculated on this freshly cut surface. The stem sections for Experiment 16 and Experiments 18 through 20 were prepared as follows: The top 24 cm of the main stem was removed and discarded. A second cut was made below this cut to produce stem sections of 51 cm for Experiments 16, 19 and 20, and stem sections of 38 cm for Experiment 18.

All the stem sections were inoculated and incubated in clear plexiglass boxes (56 x 53 x 8 cm), to maintain a humid environment for disease development. A 4 cm layer of medium grade vermiculite was placed in each box. The vermiculite was then moistened with 1500 ml of distilled water. The stem sections were then randomly assigned to locations within the box.

All experiments were performed in a room cooled to approximately 20° C. The temperature did flucuate upwards to 24° C during the day.

The inoculum for all experiments was grown on 2% millet seed medium. The millet seed was ground in a wiley mill with a 20 mesh screen. The inoculum for an experiment was started by placing a .5 by .5 cm plug of medium and mycelium from another culture in the center of a 15 cm diameter petri dish of medium. The dish was then sealed with Parafilm and incubated for 5 days in darkness at ambient room temperatures (approximately 24° C). Plugs of inoculum were cut with a 0.4 cm cork borer at a distance of 4 to 6 cm from the center of the dish. Approximatly 60 plugs were prepared per dish. Before removalof the plugs, the agar surface was covered with a 0.3% water agar solution. The plugs of inoculum were removed from the dish with a dissecting needle and were placed mycelium side down on a particular location on a stem section. In Experiment 21, 0.3% millet seed media was tested.

In Experiments 1 through 4 and on half the stems in Experiment 5, the inoculations were made on intact tissue. In Experiments 1, 2 and 3 the inoculum was placed on the area of the stem just below the uppermost node. In Experiments 4 and 5 multiple inoculations were made per stem section. In Experiment 4, three inoculations were made approximately 6.2, 12.0 and 18.0 cm from the apical end of the stem section. In Experiment 5 seven inoculations were

made approximately 6, 12, 19, 25, 32, 38 and 45 cm from the apical end of the 51 cm stem section. In Experiments 6 through 15 and Experiments 21 and 22 the inoculum was placed on the freshly cut apical end of the 15 cm stem section.

In Experiments 16 through 20 and on half of Experiment 5, all inoculation sites were first coated with clear nail polish. The nail polish was allowed to dry and then carefully peeled from the site with a dissecting needle and fingers. This was termed the wax removal (WR) technique. In Experiments 16 through 20 there were multiple inoculations per stem section. In Experiments 17 and 18 four inoculations were made approximately 7, 16, 24 and 32 cm from the apical end of the 38 cm stem section. In Experiments 19 and 20 four inoculations were made approximately 10, 22, 32 and 44 cm from the apical end of the 51 cm stem section. In Experiment 16 there were 5 inoculations approximately 8, 18, 25, 34 and 44 cm from the apical end of the 51 cm stem section. No inoculations were made on nodal tissue in any of the multiple inoculation experiments.

Lesion lengths was measured from the point of inoculation to the point of the lesion where the epidermal tissue was no loner easy to peel off with the edge of a plastic ruler. In the multiple inoculation experiments the lesions grew in both directions on the stem. In these experiments lesion length was measured as the distance between the two leading edges of the lesion.

Two different methods of inoculation were used in Experiment 3. Half of the stems were inoculated on intact tissue. The inoculation sites of the other half of the stems were first treated with chloroform. The inoculation site (same site as described earlier for experiment 3) received two quick wipes with a cotton swab soaked in chloroform. The site was then wiped with distilled water and allowed to dry. The inoculum was then placed on the site as before.

RESULTS

Inoculation on Intact Stem Tissue

Inoculations on intact tissue (Experiments 1 - 5) often failed to produce infections. The escapes were given a value of zero, resulting in a high error term. In one experiment the lesion lengths ranged from 0 to 11.5 cm for 'Weber 84' and from 0 to 7 cm for 'Gnome'. The percentage of inoculations that resulted in infections varied among experiments and among cultivars within an experiment (Table 2).

The difference among experiments in the percentage of inoculations producing infections was highly significant ($P < .001$, $X^2 = 107.9$, 4 df). The experiment totals could be influenced by the cultivars involved in a particular experiment. Only Experiments 4 and 5 involved the same set of cultivars and as such were directly comparable.

Table 2. The incidence of infections from Experiments 1 through 5.

Experiment	1		2		3		4		5		
Date	6/10/85		8/21/85		10/2/85		4/5/86		4/10/86		% of
Plant age	30 days		30 days		30 days		74 days		79 days		total
	I †	NI	I	NI	I	NI	I	NI	I	NI	infected
Gnome	4	8	11	1	7	5	19	35	1	27	35.6
PI 297514	0	12	5	7	3	9	19	35	0	28	22.9
Corsoy	1	11	7	5	6	6	12	42	0	28	22.0
Weber 84	11	1	8	4	5	7	8	46	0	28	27.1
Wells II	2	10	4	8	9	3	-	-	-	-	41.7
Century	4	8	9	3	6	6	-	-	-	-	52.8
Corsoy 79	4	8	4	8	9	3	-	-	-	-	47.2
PI 358314	3	8	7	5	3	9	-	-	-	-	36.1
Evans	2	10	-	-	8	4	-	-	-	-	41.7
Ozzie	-	-	9	3	-	-	-	-	-	-	75.0
% of total infected	28.7		59.3		51.9		26.9		0.9		32.4

† I = Number of inoculations resulting in infections. NI = Number of inoculations not resulting in infections.

Experiment 5 was conducted with plants left over from Experiment 4 and which were five days older. The space between the individual plants was increased in Experiment 5 as compared to Experiment 4 which increased the light intensity on the stems and may have resulted in a thicker cuticle (23). Experiment 5 had fewer infections than Experiment 4 which could have been due to the thicker cuticle being a more effective barrier to infection.

As not all cultivars appeared in all experiments, the cultivar totals in Table 2 could be influenced by the varing conditions of the experiments in which a cultivar appeared. By creating a subset of the data in Table 2, unequal effects were removed from the margin totals which allowed a more extensive analysis of experiment and cultivar effects on the incidence of infection (Table 3). The results of Experiment 5 were so different from the other four experiments that it was excluded from the data subset.

There was a highly significant effect of experiments ($P < .001$, $X^2 = 26.4$, 3 df) on the incidence of infection. Experiments 1, 2 and 3 varied only by the time of year when the plants were grown, suggesting that the growth environment influenced the incidence of infection. In Experiment 4 there were multiple inoculations per stem with some inoculations made on older tissue than in the other three experiments. The older tissue may have had a thicker cuticle than the younger tissue which could account for the

Table 3. Disease incidence in four cultivars over four experiments.

	Experiment					X^2 for differences among experiments within a genotype
	1	2	3	4	Total	
	% infected					
Corsoy	8.3	58.3	58.3	22.2	30.0	11.1 *
Gnome	33.3	91.7	58.3	35.2	45.5	14.2 **
PI297514	0.0	41.7	25.0	35.2	30.0	6.6
Weber 84	91.7	66.7	41.7	14.8	35.5	31.5 **
Total %	33.3	64.6	45.8	26.8	35.3 %	
	27.7**	6.8	3.0	8.3*	X^2 for differences among cultivars within an experiment	

* and ** denote significance at the .05 and .01 alpha levels respectively

low infection rate of Experiment 4. Experiment 4 used 74-day-old plants, while 30-day-old plants were tested in the other three experiments. Despite this age difference the incidence of infection was not significantly different ($P > .05$, $X^2 = .79$, 1 df) between Experiments 1 and 4.

In Experiments 1 and 4 there was a significant cultivar effect on the incidence of infection. However, the two experiments were different with regard to the ranking of the cultivars. Weber 84 had the highest incidence of infection in Experiment 1 and the lowest incidence of infection in Experiment 4. The differences in the ranking of cultivars between experiments suggested a cultivar by experiment interaction. This interaction seemed to prevent a repeatable cultivar influence on the incidense of infection. A Chi-square test on cultivar totals, pooled over

experiments, showed that the differences in the infection rates among the cultivars was no greater than expected by chance alone (X^2 = 6.9, 3 df) .

The second problem with the technique of inoculating on the intact cuticle was that the resulting infections were not all initiated at the same time. On the second day of Experiment 3, 6.5% of the stems had become infected. By the fifth day, 51.9 % of the stems were infected. At the end of the experiment the stems that were infected on the second day had longer lesions than the stems which became infected afterwards (Table 4). This contributed to variability within a cultivar. Only four of the nine cultivars in Experiment 3 had infected stems on day 2 and are included in Table 4.

Table 4. Comparison of the final average lesion lengths from Experiment 3 (10/2/85) between stems infected by the second day and stems which developed lesions after the second day.

	Average lesion length for stems with lesions on day 2	Average lesion length for stems developing lesions after day two	Difference
	cm		
Weber 84	7.2	3.9	3.3
PI 297514	7.0	1.3	5.7
Corsoy 79	8.0	3.8	4.2
PI 358314	8.0	3.0	5.0

In Experiment 4, data were collected on lesion development for ten days after inoculation. The data for a

particular day was pooled over all cultivars and the average lesion length of a particular day was calculated only from the lesions that were initiated on that day. Not all cultivars were equally represented in the average lesion of each day as not all cultivars developed a new lesion on each day. New infections were initiated on each of ten days. While the average lesion length for each day was influenced by the cultivars developing infections on that day, there was still a strong trend of longer lesions with earlier infection (Fig. 1).

Inoculation on Sites Where Either the Cuticle or the Cuticular Waxes Were Removed

The role of the cuticle in preventing and delaying infections was investigated in Experiments 3 and 5. In Experiment 3, a total of 108 stems from nine cultivars were inoculated on the intact cuticle. Another 108 stems from the same cultivars were treated with chloroform to remove the cuticle (M. Bukovac, personal communication). The chloroform treatment also wounded the underlying tissue as shown by scarring of uninoculated control stems treated with chloroform.

Only 51.9% of the 108 untreated stems became infected five days after inoculation while 100% of the treated stems developed lesions. After two days, 93.5% of the stems treated with chloroform were already infected.

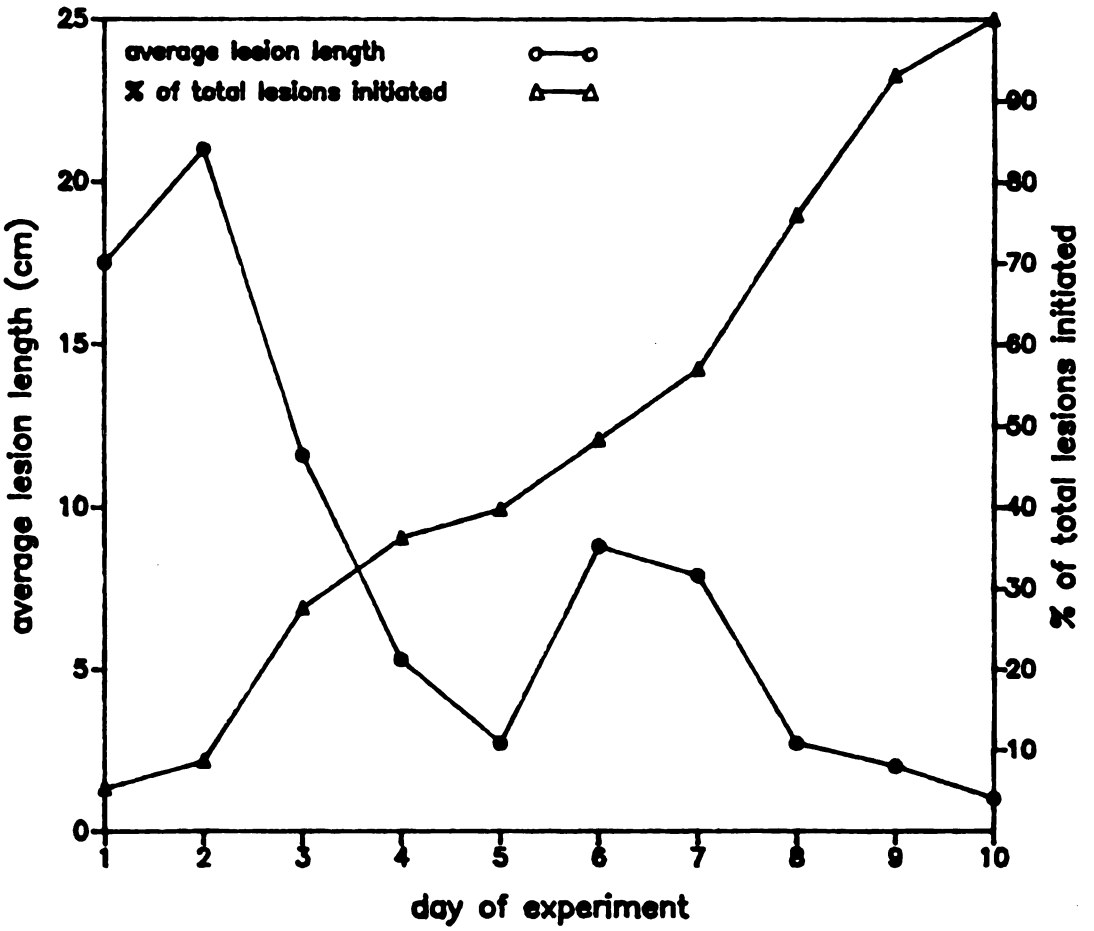

Figure 1: Cumulative percent of lesion initiation and average lesion length of lesions initiated on a particular day of Experiment 4.

In Experiment 5, half of the inoculations were made on the intact cuticle and the other half were made on sites where only the cuticular waxes had been removed with clear nail polish (M. Bukovac, personal communication). This treatment also caused a slight wounding of the underlying tissue by removing the trichomes. This wounding was considerably less than caused by the chloroform treatment. Of the 140 inoculations on the intact cuticle only 1 caused an infection while 129 of 140 (92.1 %) inoculations of treated tissue caused an infection.

Inoculation on Wounded Stem Tissue

The chloroform treatment of the stems created a wound and resulted in a more uniform incidence of infection. An inoculation technique concurrently under study by Dr. J. L. Lockwood produced wounds and was adapted as it was a faster and easier method of circumventing the cuticle barrier. Experiments 5 and 22 were performed using this technique. The apical bud was removed from the stem section and the inoculations were made on this site. Infection rates of 100% were achieved utilizing this technique.

Lesions were measured every 24 hours (Table 5). All stem sections were infected after 48 hours. Variation in average lesion lengths at 48 hours was not large either among genotypes or among the stems within a genotype with the exception of Weber 84 in the Experiment 22. This indicated that uniform infection occurred.

Table 5. Summary of lesion length data 48 hours after inoculation from Experiment 22 (10/28/85) and Experiment 6 (11/15/85).

	Experiment 6			Experiment 22		
	Range	Average lesion	Standard deviation	Range	Average lesion	Standard deviation
	----- cm -----			----- cm ----		
Gnome	.5 -.9	.7	.12	.6 -1.0	.8	.13
PI297514	.2 -.8	.5	.18	.3 - .8	.6	.13
PI358314	.3 -.8	.6	.13	.2 -1.1	.7	.25
Weber 84	.6 -.9	.7	.13	.7 -4.4	1.7	1.05
Corsoy	.3 -.9	.5	.17			
Corsoy 79	.4 -.7	.5	.08			
Wells II	.5 -.8	.7	.09			
Century	.5 -.7	.6	.09			
Ozzie	.5 -.9	.7	.11			

Further disease development was variable. Some of the lesions on the young tissue ceased growing and dried out, often at the first node encountered. This led to variability within a cultivar. The inoculation technique was altered by making the final cut through the middle of the node which bore the uppermost fully expanded trifoliate leaf and then immediately inculating on this cut. This avoided the young meristem tissue and provided a large surface on which to place the inoculum. This technique was termed wound inoculation (WI).

Lesion initiation with the WI technique was uniform as in Experiments 5 and 22. Further growth continued in a fairly uniform fashion within a cultivar but significant differences in average lesion lengths developed among cultivars. Several experiments were conducted to determine

if there were repeatable differences among cultivars in their ability to limit or slow lesion growth (Table 6). Only data from stems inoculated via the WI technique and with inoculum grown on the standard medium is included in Table 6. The experiments varied by size, date (and thus the environment in which the plants were grown), plant age, and the length of the experiment as shown by the duration of incubation.

The cultivars Gnome and Weber 84 were tested because they were reported to be susceptible to S. sclerotiorum in field trials (12, 13, 18, 19) and in previous greenhouse and laboratory tests (6, 18). Corsoy was selected because it had expressed resistance in field trials (12, 13, 19) and in greenhouse and laboratory tests (6, 11, 18). The plant introductions were selected because both had exhibited greater resistance than Corsoy in laboratory trials (18).

Several trends were apparent from these experiments. The most obvious was that Gnome developed the longest average lesion in eight of the nine tests. PI 297514 developed the shortest average lesion in six of the nine tests.

A more general trend was a grouping of the cultivars as "susceptible" and "resistant". The more susceptible cultivars were Gnome and Weber 84. The more resistant cultivars were Corsoy, PI 297514 and PI 358314 which always developed a shorter average lesion than Gnome or Weber 84. The difference was significant ($P < .05$) except for the

Table 6. Summary of average lesion length data from experiments utilizing the wound inoculation (WI) technique.

Experiment	7	8	9	10	11	12	13	14	15
Date	11/25/85	12/31/85	1/24/86	1/30/86	2/23/86	2/23/86	6/2/86	6/18/86	5/22/86
Plant age	50 days	40 days	39 days	39 days	38 days	37 days	57 days	60 days	47 days
Duration of inoculation	5 days	7 days	5 days	5 days	5 days	6 days	7 days	7 days	5 days
	cm								
Gnome	9.8 a	11.2 a	9.7 a	10.7 a	12.6 a	10.1 a	11.2 a	11.6 a	8.3 a
PI 297514	6.7 b	8.5 b	7.6 c	7.2 d	10.5 c	7.2 b	7.1 d	7.2 c	6.9 b
Weber 84	8.7 a	11.0 a	8.8 ab	10.6 a	11.4 a	10.0 a	9.7 b	-	8.7 a
Corsoy	-	-	8.4 bc	9.1 b	9.5 d	-	8.0 cd	4.0 d	8.2 a
PI 358314	-	-	8.1 bc	8.2 c	8.8 d	-	8.4 c	9.6 b	6.6 b
CV (%)	16.6	9.16	15.23	7.94	17.72	15.71	11.93	15.14	17.79
L.S.D. (0.05)	1.70	1.00	1.03	.52	.80	.32	1.15	1.54 †	.80 †
Range (cm)	3.1	2.7	2.1	3.5	3.7	2.9	4.2	7.6	2.1
Range / L.S.D.	1.8	2.7	2.0	6.7	4.6	9.1	3.7	4.4 ‡	2.6 §
Gnome-PI 297514	3.1	2.7	2.1	3.5	2.2	2.9	4.2	4.4	1.4
‡‡297/Gnm x 100	68.37	75.89	78.35	67.29	83.3	71.29	62.83	62.07	83.13
(Gnm-297)/L.S.D.	1.8	2.7	2.0	6.7	2.7	9.1	3.7	2.7 ††	1.8 ††

† Experiment had unequal replications. This L.S.D. is calculated from the rep. x cultivar line of the ANOVA.

‡ The L.S.D. used in this calculation is the appropiate one for comparing Gnome and Corsoy.

§ The L.S.D. used in this calculation is the appropiate one for comparing Gnome and PI 358314.

†† The L.S.D. used in this calculation is the appropiate one for comparing Gnome and PI 297514.

‡‡ Gnm = Gnome, 297 = PI 297514.

comparison between Gnome and Corsoy in Experiment 15. In Experiment 9, the difference between PI 358314, Corsoy and Weber 84 was not significant. In Experiment 15 the difference between Corsoy and Weber 84 was not significant. PI 297514 always developed a significantly shorter average lesion than Gnome or Weber 84.

The rankings within the resistant group varied by experiment. PI 297514 had the lowest average lesion length when data from all the tests were pooled and always developed a significantly shorter average lesion than the two susceptible cultivars. This indicated that PI 297514 was probably the most resistant cultivar tested. Table 7 contains the average values of the statistics in Table 6 pooled across all experiments. All five cultivars appeared in the same experiment five times (Experiments 9, 10, 11, 13, 15). The average lesion lengths from these five experiments are also included in Table 7.

Table 7. Averages of the statistics in Table 6 pooled across all experiments.

			Average lesion	
			all experiments	experiments 9, 10, 11, 13, 15
			---- cm ----	
Range	3.1 cm	Gnome	10.4	10.5
$L.S.D._{0.05}$	.82	Weber 84	9.9	9.9
Range / L.S.D.	3.73	Corsoy	8.6	8.9
PI 297514 / Gnome	75.6 %	PI 358314	8.1	8.0
Gnome-PI297514	2.50 cm	PI 297514	7.9	8.0
(Gnome-PI 297514)/L.S.D.	3.04			

An assay's ability to detect significant differences among cultivars is determined by an assay's ability to discriminate among cultivars (i.e. range of values) and its sensitivity to cultivar differences (L.S.D. or C.V.). The value in Tables 6 and 7, calculated by dividing the range by the L.S.D. expresses the range in units of significant difference. This value incorporates the discrimination and sensitivity of an experiment in one descriptive term. The value would be influenced by the the technique used in an experiment, the conditions of the experiment and the material tested. The range/L.S.D. showed that Experiment 12 was the most sensitive test of cultivar differences even though it did not produce the lowest coefficient of variability or the widest range of values. PI 297514 and Gnome appeared in all tests so the range between these cultivars divided by the L.S.D. was presented as a common check for all experiments. This value again showed Experiment 12 to be the most sensitive test.

The WI method only measured a stem's ability to slow lesion growth. Resistance was not expressed in any stem of any cultivar as no lesions stopped growing and all the lesions would have been lethal to a plant.

The WI method was modified in an attempt to increase the sensitivity of the WI method and allow for an increased expression of resistance.

Limited Duration of Inoculation

One change was the duration of inoculation. An adapted form of limited term inoculation (6, 15) was used in Experiment 12 where plugs of inoculum were removed after a specific time and replaced with plugs of 2% water agar. The control treatment was inoculum left on for the duration of the experiment (146 hours). In a later study it was found that the 2% water agar plugs had no effect on lesion development.

Durations of inoculations of 32, 46 and 146 hours essentially behaved the same as reflected in the number of escapes, the average lesion lengths and the sensitivity of the tests (Table 8). Durations of inoculations of 10 and 23 hours were characterized by stems escaping infection and higher error rates. The incidence of escapes among the cultivars at 10 and 23 hours was not significantly different (X^2 = .48, 2 df). When escapes were omitted from the data set, the average lesion length for 10 and 23 hours were similar to those of the longer durations. While there was a significant difference between the average lesion lengths omitting escapes among durations of inoculations, this significance was not be repeated in another experiment.

Shorter durations of inoculations resulted in more escapes but did not increase the range between the susceptible and resistant cultivars. Shorter durations of inoculations resulted in a reduced sensitivity to cultivar

Table 8. Summary of the effect of duration of inoculation on lesion development, Experiment 12 (2/23/86).

	Duration of inoculation (hours)					Overall average lesion
	10 A.L. †	23 A.L.	32 A.L.	46 A.L.	146 A.L.	
	cm					
Gnome	4.9 a	10.2 a	10.1 a	10.5 a	9.7 a	11.7
Weber 84	4.9 a	10.0 a	10.3 a	10.3 a	9.3 a	10.0
PI 297514	4.3 a	5.2 b	7.5 b	7.8 b	6.3 b	7.0
Overall average lesion	4.7	8.5	9.3	9.5	8.4	
CV (%)	94.5	23.3	11.4	11.1	20.9	
L.S.D.0.05	4.6	2.8	.8	1.0	1.5	
Range (cm)	.6	5.0	2.8	2.7	3.4	
Range / L.S.D.	.1	1.8	3.4	2.8	2.3	
NO. of escapes	19	3	0	0	0	
Average lesion omitting escapes						
	----- cm -----					
Gnome	8.1 b	10.2 a	-	-	-	
Weber 84	10.5 a	10.0 a	-	-	-	
PI 297514	6.4 c	6.4 b	-	-	-	
Overall average lesion omitting escapes	8.1 b ‡	9.1 a	9.3 a	9.5 a	8.4 b	

Means within a column with the same letter are not significantly different at alpha = .05.

† A.L. = average lesion length

‡ Means within this row with the same letter are not significantly different at alpha = .05.

differences. The incidence of escapes was not influenced by cultivar and therefore did not appear to be an increased expression of resistance between cultivars. There was no significant interaction of cultivar by duration of inoculation on average lesion length.

Lower Concentration of Nutrients in the Inoculum Medium

The concentration of the inoculum media was lowered in an attempt to weaken the pathogen. In Experiment 21 inoculum was grown on the standard 2% millet seed medium and on a medium containing only .3% millet seed powder (Table 9).

Table 9. Summary of the effects of inoculum media nutrient concentration on the incidence of infection and on lesion length, Experiment 21 (4/30/86).

	Average lesion per medium concentration		Cultivar average lesion	Average lesion with escapes omitted		Cultivar average lesion
	2 %	.3 %		2 %	.3 %	
	----------	----------		cm	----------	
PI 297514	4.9	2.9	3.9	5.4	5.4	5.6
Gnome	7.6	4.0	5.8	7.6	6.4	7.1
average lesion	6.2 a	3.5 b		6.5 a	5.9 a	

	Number of escapes	
	1	5
PI 297514	0	4
Gnome		

Means within a row with the same row with the same letter are not significantly different at alpha = .05.

The lower nutrient concentration of the medium produced a significantly lower incidence of infection than the 2%

standard medium (X^2 = 8.5, 1 df) indicating that the more dilute medium may have weakened the pathogen and allowed the host to better resist infection. PI 297514 had six escapes while Gnome had four though this difference was not significant (X^2 = .53, 1 df). When the escapes were omitted from the data set, the average lesion lengths resulting from the two concentrations were not significantly different. There was no significant interaction of cultivar by concentration on average lesion length.

Inoculation on Stem Tissue Where the Cuticular Waxes Have Been Removed

In Experiment 5, the wax portion of the cuticle was removed from the inoculation sites. Inoculations on these sites often resulted in restricted lesions. The restricted lesions were initiated at approximately the same time as normal spreading lesions but ceased growing at a length of approximately 1 cm or less. Often a restricted lesion would not spread beyond the 0.5 cm diameter scar caused by the wax removal. The restricted lesions ranged in color from a light buff to a rusty red, became dry by the end of an experiment, did not girdle the stem and would not have been lethal to the upper portions of an intact plant.

Several experiments were performed to investigate the potential of the wax removal (WR) technique as an improvement over the WI method (Table 10).

Table 10. Summary of the wax removal experiments.

Experiment	5	16	17	18	19	20
Date	4/10/86	4/30/86	5/12/86	6/2/86	6/20/86	6/11/86
Plant age	74 days	60 days	42 days	58 days	72 days	60 days
Duration of Inoculation	4 days	6 days	4 days	4 days	5 days	7 days
	----------	----------	----------	cm	----------	----------
Gnome	7.7 a	5.3 a	8.2 a	5.0 a	5.7 a	10.5 a
PI 297514	4.2 bc	.6 b	6.0 b	3.6 b	.6 b	4.1 b
Corsoy	3.2 cd			2.6 bc	4.8 a	2.0 b
PI 358314	2.0 d			1.9 c	.4 b	3.1 b
Weber 84	4.7 b			3.7 b		
CV (%)	58.4	82.3	21.3	47.9	93.4	47.9
$L.S.D._{0.05}$	1.36	1.26	1.09	1.24	1.70	3.18 *
Range (cm)	5.7	4.8	2.1	3.1	5.3	8.5
Range/L.S.D.	4.2	3.8	2.0	2.5	3.1	2.7
Gnome-PI297514	3.5	4.8	2.1	1.4	5.1	6.4
PI297514/Gnome	54.5%	10.5%	73.7%	72.0%	10.5%	39.1%
Gnm-297/L.S.D. †	2.6	3.8	2.0	1.1	3.0	2.0 §

Means within the same column with the same letter are not significantly different at alpha = .05.

† Gnm = Gnome, 297 = PI 297514,

* Experiment 20 had unequal replications.This L.S.D. is calculated using the rep X genotype line from the ANOVA.

§ The L.S.D. is the appropriate one for comparing Gnome to PI 297514.

The ranking of the cultivars by average lesion length under WR conditions was similar to that produced by the WI method. Gnome and Weber 84 always developed longer average lesions than the three "resistant" cultivars (Corsoy, PI 297514 and PI 358314). Differences between of average lesions of the two plant introductions and Gnome were always significant. The difference between the average lesion lengths of Corsoy and Gnome was not significant in Experiment 19.

Weber 84 appeared in only two of the six WR tests and the difference between the average lesion length of Weber 84 and any of the three resistant cultivars was only significant when comparing Weber 84 with Corsoy and PI 358314 in Experiment 5 and with PI 358314 in Experiment 13. Weber 84 had a significantly shorter average lesion than Gnome in both tests. The results of the WR method would place Weber 84 in the resistant group though Weber 84 was not extensively tested.

Despite the high coefficients of variability of the WR experiments, the results were repeatable with the trends established in the WI method, with the exception of Weber 84, being repeated (Table 11).

A direct comparison of the two methods is not valid due to unequal representation of cultivars between methods and the different environmental conditions in which the plants were grown for each experiment. The following observations are made under the assumption that the confounded factors

Table 11. Results of the wound inoculation (WI) and wax removal (WR) methods of inoculation pooled across all experiments.

	Wound inoculation	Wax removal	Wax removal, lethal lesions only	
CV	14.40 %	59.17 %		
Range (cm)	3.1	5.0		
L.S.D. 0.05	.82	1.63		
Range / L.S.D.	3.73	3.09		
PI 297514/Gnome X 100	75.60 %	43.26 %		
Gnome - PI 297514	2.50	3.74		
Gnome-PI 297514 / L.S.D.	3.04	2.30		
	Average lesion			
	---------- cm ----------			
Gnome	10.4	6.7	7.4	(138) †
Weber 84	9.9	4.2	5.2	(48)
Corsoy	8.6	3.1	4.2	(67)
PI 358314	8.1	1.9	3.8	(55)
PI 297514	7.9	3.0	5.3	(86)
Susceptible ‡ (Gnm, W84)	10.2	6.0		
Resistant (Cor, 297, 358)	8.2	2.7		
Difference	2.0	3.3		

† The number in parentheses is the number of lethal lesions that developed across all experiments.

‡ Susceptible or resistant based on WI results. Gnm = Gnome, W84 = Weber 84, Cor = Corsoy, 297 = PI297514, 358 = PI 358314.

did not greatly influence the statistics in Table 11. Due to the occurrence of restricted lesions, the WR method produced a larger coefficient of variability than the WI method and was less sensitive to differences among cultivars despite producing a wider range of values between the average lesions of the susceptible and resistant groups.

The frequency and distribution of restricted lesions from the WR experiments are summarized in Table 12. Experiment 17 was omitted as it did not produce restricted lesions. The absence of restricted lesions may have been due to the young, succulent plant material. Four of the five experiments had a significant cultivar effect on the distribution of restricted lesions at alpha = .05 and all were significant at alpha = .08. The cultivar totals in Table 12 contain unequal experiment contributions and are not comparable. An analysis of cultivar and experiment effects was performed on a subset of data from Table 12 which omitted data from Experiment 16 and Weber 84 (Table 13).

The effect of cultivars on the incidence of restricted lesions was significant in Experiments 5, 19 and 20. A Chi square test of the distribution of restricted and non-restricted lesions among cultivar totals pooled over all experiments was highly significant (X^2 = 40.2, 3 df) while a Chi-square test among the three resistant genotypes was not, indicating that it was the difference between the three resistant genotypes and Gnome that was responsible for the

Table 12. Summary of the incidence of restricted and non-restricted lesions in the wax removal (WR) experiments.

Experiment	5		16		18		20		19		
Date	4/10/86		4/30/86		6/2/86		6/20/86		6/11 86		
Plant age	74 days		60 days		58 days		72 days		60 days		
Duration of inoculation	4 days		6 days		4 days		5 days		7 days		Total
	R †	NR	R	NR	R	NR	R	NR	R	NR	% R
Gnome	0	28	6	24	5	27	3	17	0	16	11.1
Weber 84	1	27	-	-	11	21	-	-	-	-	25.0
Corsoy	7	21	-	-	7	25	8	12	11	9	33.3
PI 297514	3	25	25	5	10	22	18	2	8	16	47.8
PI 358314	8	20	-	-	14	18	17	3	14	14	48.6
Total % R	13.5		51.7		29.4		57.5		37.5		33.4
X2 for cultivar effects within an experiment	15.5 **		24.1 **		7.2		32.1 **		14.3 **		

* and ** denote significance at the .05 and .01 alpha levels respectively.

† R = the number of restricted lesions NR = the number of non-restricted lesions

Table 13. Summary of the incidence of restricted and non-restricted lesions of a subset of the data in Table 11.

Experiment	5		18		20		19		Total	X^2 for experiment effects	
	R †	NR	R	NR	R	NR	R	NR	% R		
Gnome	0	28	5	27	3	17	0	16	8.3	7.2	
Corsoy	7	21	7	25	8	12	11	9	33.3	7.5	
PI297514	3	25	10	22	18	2	8	6	37.5	32.8	**
PI358314	8	20	14	18	17	3	14	14	49.1	15.4	**
% R	15.9		28.1		57.5		37.5		32.6		
X^2 for genotype effects	10.9 *		7.1		32.1 **		14.8 **				

* and ** denote significance at the .05 and .01 alpha levels respectively.

† R = The number of restricted lesions, NR = The number of non-restricted lesions

significance in the overall Chi-square test. This indicated that there was a relationship between the WI average lesion lengths and the ability to limit an infection to non-lethal dimensions.

Inoculation on Stem Tissue Varing in Age

Through the use of multiple inoculations per stem, the effect of tissue age on the nature of an infection was studied. The position of an inoculation on the stem had a significant effect on the incidence of restricted lesions and on lesion length in all WR experiments. Older tissue, as designated by higher position numbers, developed shorter average lesion lengths (Fig. 2) even when the data from restricted lesions was removed from the data set (Fig. 3). Experiments 18, 19 and 20 were comparable when the data for Weber 84 were removed. In these experiments Gnome developed a longer average lesion than the three resistant genotypes at all four positions on the stems (Fig. 4).

The position of the inoculation had a significant effect on the appearance of restricted lesions within the resistant cultivars but not with Gnome (Table 14). The sample size for Gnome may have been too small to detect significance. Of the Gnome stems, 77.7% developed a lethal lesion at the lower positions while only 57.0 % of the resistant stems did so.

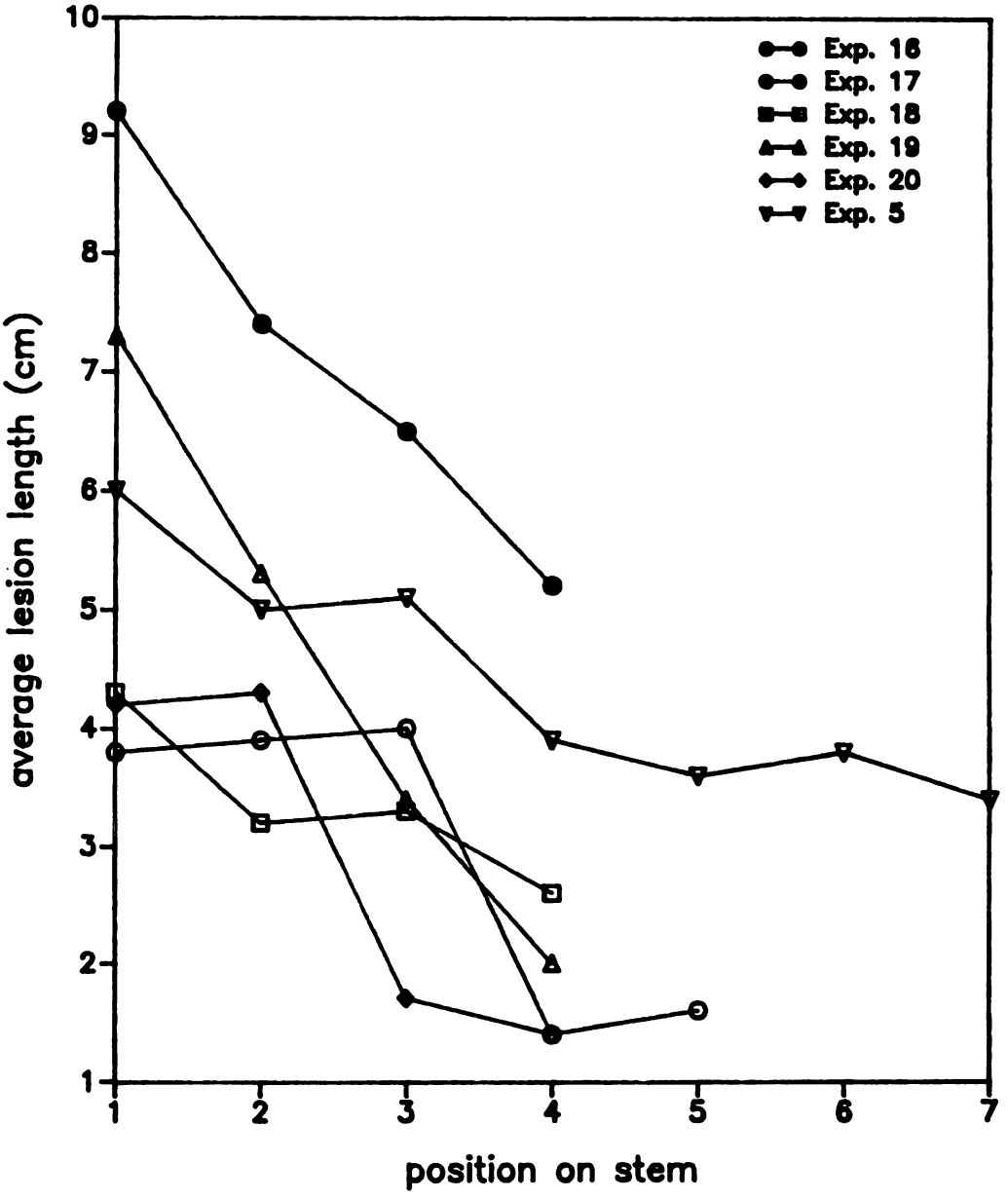

Figure 2: Average lesion length by position.

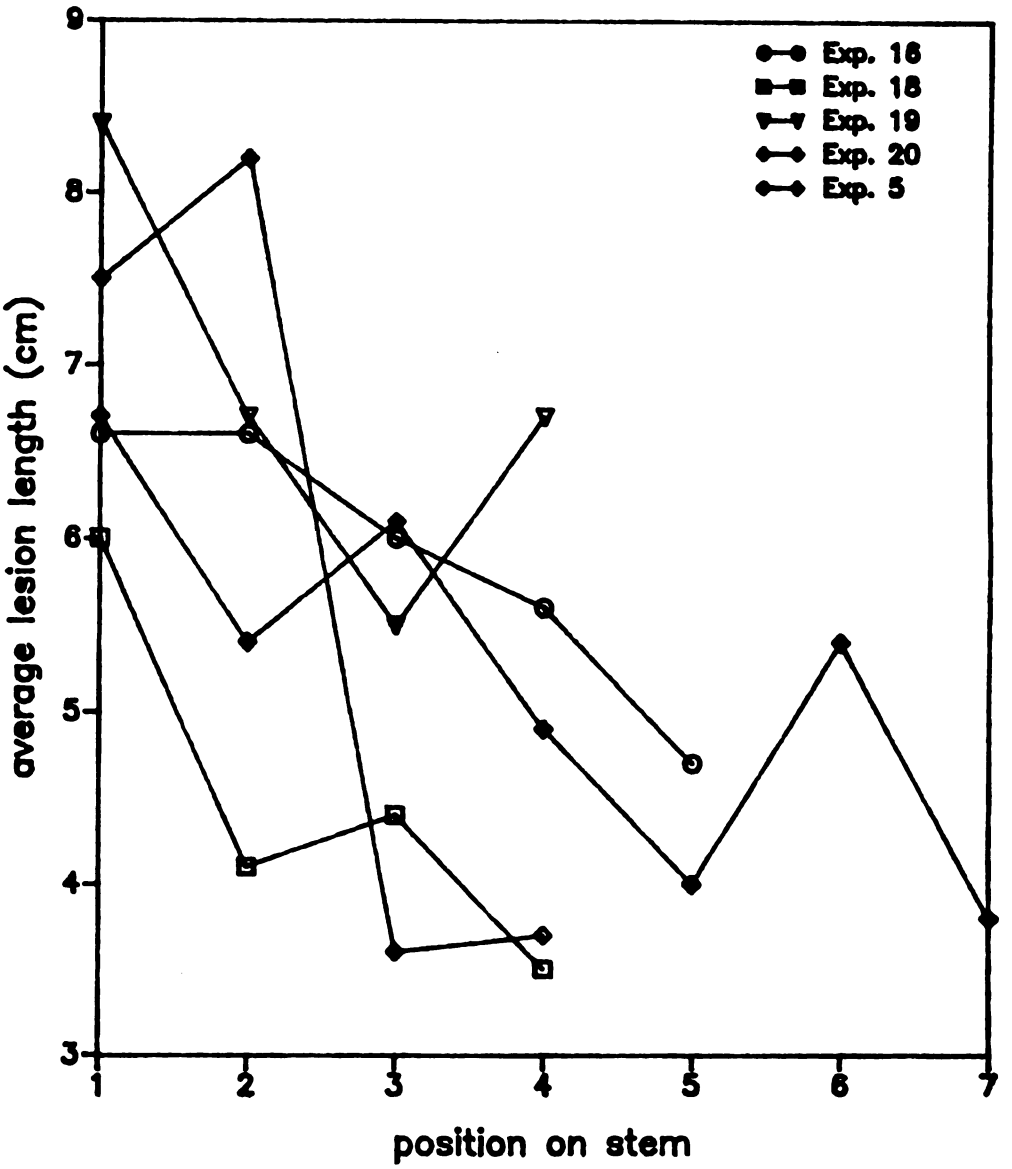

Figure 3: Average lesion length with restricted lesions omitted from the data set.

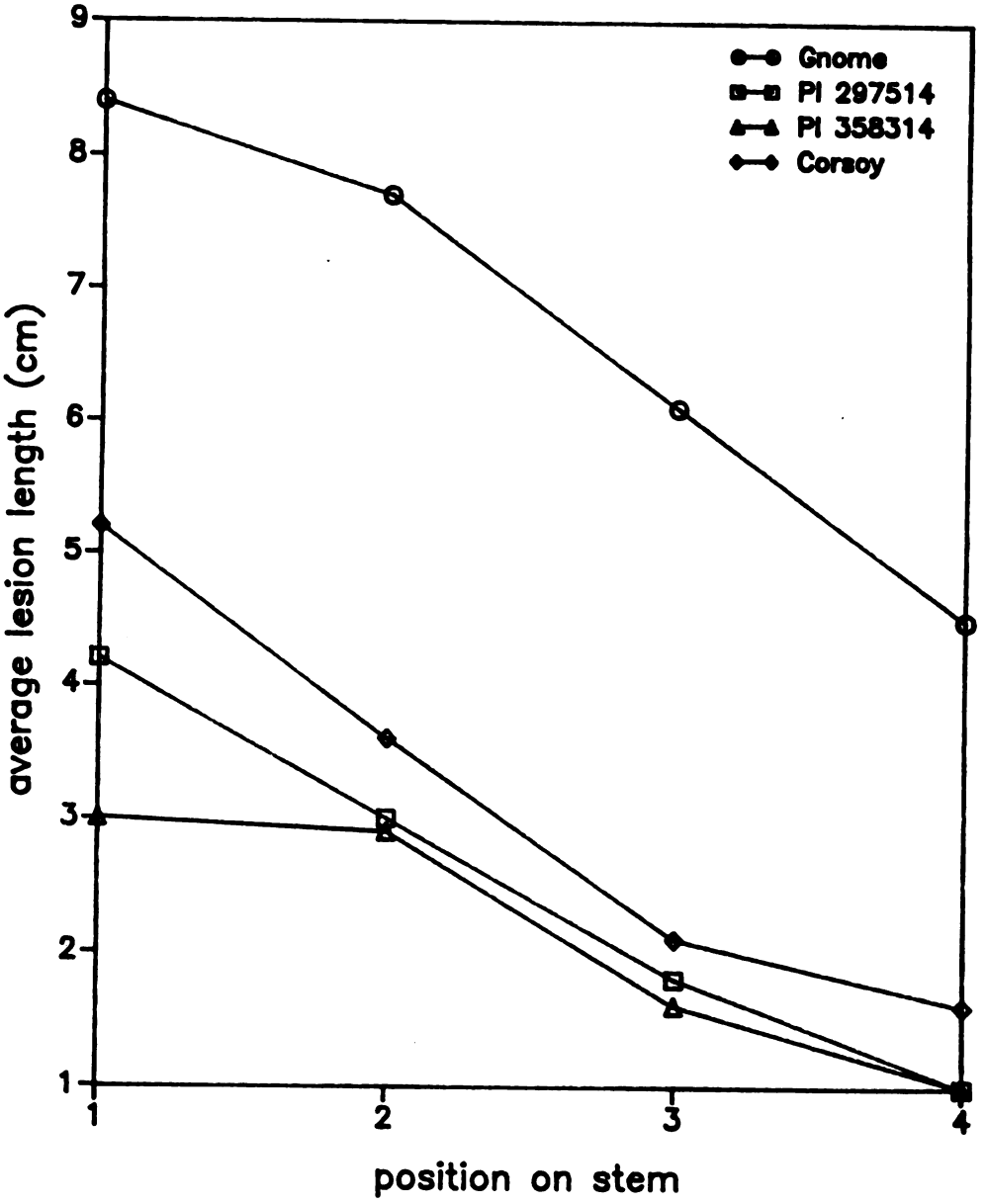

Figure 4: Average lesion length by cultivar from Experiments 18, 19 and 20

Table 14. Summary of the incidence of restricted lesions by position from data combined from Experiments 18 - 20.

		Gnome	Resistant cultivars †
Percentage of inoculations resulting in restricted lesions		11.8	46.9
Percentage of inoculations resulting in restricted lesions analyzed by position	Position 1	5.9	36.8
	Position 2	5.9	36.8
	Position 3	11.8	43.9
	Position 4	23.5	70.1
X^2 for position effects on the distribution of restricted lesions		3.4	13.2 **
Percentage of stems with lethal lesions at either positions 3 or 4		77.7	57.0

† The resistant genotypes are Corsoy, PI 297514 and PI 358314.

** denotes significance at alpha = .01 level

DISCUSSION

The results indicated two reasons for the low sensitivity of the assay when inoculating on intact tissue. The first problem was that not all inoculations resulted in infections. In two experiments, genotype had a significant effect on the incidence of infection but the pattern could not be repeated. The cause of the interaction between cultivars and experiments is unknown. The interaction prevented a repeatable cultivar effect the incidence of infection and contributed to error both within and between experiments.

The second problem was that infections were not uniformly initiated when inoculations were made on the intact cuticle. The later an infection was initiated, the shorter the resulting lesion was. Delayed infections and escapes led to variability within a genotype thus raising the error term of the experiment and lowering the sensitivity of the assay to differences among cultivars.

The cuticle was a barrier to infections, though not necessarily the cause of resistance. Circumventing the cuticle allowed for 100 % infection rates and a highly uniform initiation of lesions among all stems. Removal of only the cuticular waxes resulted in a significant increase in infection rates, suggesting that the wax components of the cuticle may play a key role in preventing infections. These conclusions and particularly the role of the wax

components must be tempered by the fact that the removal of the whole cuticle or just the wax layers was always confounded with some degree of wounding of the underlying epidermal tissue which in itself would facilitate infection. However, histological studies (20, 21) indicate that unwounded epidermal tissue would not be likely to resist an infection.

The development of a cuticle is influenced quantitatively by the greenhouse environment with high light intensity, high temperatures and low humidity favoring the formation of a thicker cuticle and wax portion of the cuticle (23). Variation in these factors probably account for the experiment-by-cultivar interaction observed when inoculating on the cuticle. Whether cuticle and/or wax differences could account for variability for resistance to infection within a species is debated (22). Variation in cuticle and wax properties exist between species (3, 20) but the variation within a species has not been extensively studied.

There appears to be differences among Phaseolus species for resistance to penetration by S. sclerotiorum with P. coccineus showing greater resistance than P. vulgaris (9, 29) though not necessarily a lower incidence of infection. The greater resistance was thought to be the result of a physical characteristic of the tissue. Even the ability to delay an infection could be important. As environmental factors must be favorable to initiate the infection process

(2), delays in the completion of an infection could allow for environmental changes to occur which may be less conducive to infection or for the depletion of the nutrients supporting infection. Evidence from this study indicated that the ability to resist infection was influenced by the environment. It may be worth further study to develop more sensitive methods for evaluating the effect of the cuticle and wax components on infections to see if there exists useful genetic variation among soybean cultivars for the ability to resist or delay infections.

The WI technique corrected the problems associated with inoculating on intact tissue. Inoculating on the node which bore the uppermost fully expanded trifoliate leaf and trimming the stem immediately before inoculation minimized the problems of inoculation site desication and inoculum plugs falling off the stem sections that occurred with inoculations on the apical bud wound. Also most infections in the field occur low in the plant canopy (5, 12, 26), so inoculations on a lower node were more representative of the tissue likely to become infected than inoculations on the apical end of the stem. The WI method resulted in uniform initiation of infections and increased variation among lesion lengths over time would be due to variation in the growth of the pathogen through the stem tissue.

The grouping of cultivars by average lesion length was repeated in nine WI experiments conducted over a six month period on plants grown in various environments indicating

that the WI technique produced reliable results. The WI technique grouped Gnome and Weber 84 as "susceptible" and Corsoy, PI 297514 and PI 358314 as "resistant". This corresponds to field observations of the susceptiblity of Gnome (4, 12, 13, 18) and Weber 84 (18, 19) and the resistance of Corsoy (12, 13, 19). The results also match those of other greenhouse and laboratory experiments for Gnome (6, 18) and Corsoy (6, 11, 18). The behavior of Weber 84 was variable in a previous laboratory test (18). The plant introductions have not been tested in field conditions but had been found to be more resistant than Corsoy in previous laboratory experiments (18). Thus the WI technique appeared to be an accurate predictor of field resistance. More genotypes should be tested in the field and by the WI method to confirm this relationship. It should be noted that field ratings of disease severity may not always be accurate indicators of physiological resistance due to the confounding factors of avoidance and inoculum distribution. Few soybean cultivars have been thouroughly tested for resistance in trials conducted over years and with consideration to the confounding factors that may be confused with the expression with physiological resistance.

The WI method only measured a cultivar's ability to slow lesion growth. Check cultivars must be used with this method as the significance of the results were relative to a susceptible and resistant cultivar included in each test. Complete resistance was not expressed as all the lesions

would have been lethal to a plant. The conditions of the WI test favored the pathogen in the host-pathogen interaction and may have precluded the expression of complete resistance. Under the WI conditions it is possible that a moderate or strong resistance is overcome leaving the slower lesion growth as a manifestation of its expression.

Through the WR technique, restricted lesions developed. Similar lesions have been noted in soybean (6, 4, 11), P. vulgaris (26, 27) and P. coccineus (9, 26) genotypes resistant of S. sclerotiorum infections. The incidence of restricted lesions was influenced by genotype. The cultivars rated resistant by the WI method developed significantly fewer lethal lesions than Gnome. Thus the WI results appeared to relate not only to field results but also to an ability to restrict an infection to non-lethal porportions. Despite the increased frequency of restricted lesions in the resistant cultivars, all cultivars developed some lethal lesions. The increased levels of resistance in some cultivars appeared to only lead to a lower probability of a lethal lesion. Resistance was influenced by the environment as shown by differences among experiments in the incidence of restricted lesions.

Inoculations made on older tissue resulted in fewer restricted lesions than inoculations made on younger tissue and the spreading lesions that did occur were shorter than the spreading lesions on younger tissue. The reduction in the incidence of restricted lesions on older tissue was more

pronounced in the resistant cultivars. This indicated that the resistant cultivars were not only less apt to develop lethal lesions but also that the lethal lesions were less likely to occur low on the stem where they would result in more damage. More extensive testing is needed to see if there is genetic variability for this ability to minimize damage from a lethal infection.

Attempts to further improve the sensitivity of the WI test failed. Shorter durations of inoculation or inoculum grown on weaker medium both resulted in a higher incidence of escapes. The lesions that did occur were no different than those achieved via the standard WI procedures. There was no cultivar effect on the incidence of escapes and therefore the modifications did not increase the ability of the assay to discriminate among cultivars. The modifications influenced the infection process, apparently favored the host in general, but they did not appear to increase the expression of resistance in the resistant cultivars and did not influence further lesion growth. The result of the modifications were a higher error term and a lower sensitivity to cultivar differences.

The lower concentration of nutrients in the medium resulted in significantly fewer infections probably by weakening the pathogen. Nutrition has been linked to the ability of S. sclerotiorum to successfully infect a host (1, 15, 28, 29). In the field the pathogen uses flower tissue to supply the nutrition for infection (2). This suggests

that if there existed genetic variation for the nutrient content of flower tissue, then this variation could be used to breed cultivars with flower tissue that could lower the incidence of infections. Schwartz et al.(27) found no difference among nine lines of P. vulgaris and P. coccineus for blossom resistance to ascospore colonization. The ability of the blossoms to support infections was not tested.

The WI method still developed a degree of unexplained variability between the stems of a cultivar as shown by the average coefficient of varibility of 14.4 %. This indicated that the test would not be accurate on single plants and will require the testing of several progeny to evaluate a line. In a breeding program this will require testing in later generations when a line produces more homogeneous progeny.

The WI method only measured a cultivar's ability to slow lesion growth which appeared to correspond to field resistance and the ability to restrict lesions to non-lethal dimensions. This is just one trait that could lead to the failure of an inoculation to result in a lethal lesion. The probability of a lethal lesion can be thought of as a function of the probabilities of the occurrence of the required steps. Any break in the chain of events that leads to a lethal lesion will result in resistance. Some factors other than slow lesion growth which may lead to a lower probability of a lethal infection by S. sclerotiorum are

suggested by this and other studies. A lower probability of an infection can result from disease avoidance. Modified plant architecture has been employed in P. vulgaris to avoid white mold (7). Flower tissue may be nutritionally unsuitable to support an infection therefore lowering the probability of an infection. Also the probability of an infection may be lowered if the cuticle layer is able to resist or delay penetration. Variation for the last two traits has yet to found. All the possible resistance mechanisms mentioned appear to be incomplete and be expressed as a lower probability of a lethal lesion.

LIST OF REFERENCES

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1. Abawi, G.S., F.J. Polach and W.T. Molin. 1975. Infection of bean by ascospores of Whetzelinia sclerotiorum. Phytopath 65:673-678.

2. Abawi, G.S. and R.G. Grogan. 1979. Epidemiology of diseases caused by Sclerotinia species. Phytopath. 69:899-903.

3. Baker, E.A and J.T. Martin. 1967. Studies on plant cuticle. X. The cuticles of plants of related families. Ann. Appl. Biol. 60:313-319.

4. Boland, G.J. and R. Hall. 1968. Growthroom evaluation of soybean cultivars for resistance to Sclerotinia sclerotiorum. Can. J. Plant Sci. 66:559-564.

5. Chamberlain, D.W. 1951. Sclerotinia stem rot of soybeans. Plant Dis. Rep. 35:490-491.

6. Cline, M.N. and B.J. Jacobson. 1983. Methods of evaluating soybean cultivars for resistance to Sclerotinia sclerotiorum. Plant Dis. 67:784-786.

7. Coyne, D.P, J.R. Steadman and F.N. Anderson. 1974. Effect of modified plant architecture of Great Northern dry bean varieties (Phaseolus vulgaris) on white mold severity, and components of yield. Plant Dis. Rep. 58:379-382.

8. Dillard, H.R and R.G. Grogan. 1985. Relationship between sclerotial spatial pattern and density of Sclerotinia minor and incidence of leaf drop. Phytopath. 75:90-94.

9. Dow, R.L. and R.D. Lumsden. 1975. Histology of resistance of Phaseolus coccineus (Scarlet Runner bean) to Sclerotinia sclerotiorum. Proc. Am. Phytopath. Soc. (Abstr.) 2:122.

10. Fuller, P.A., D.P. Coyne, J.R. Steadman and R.F Mumm. 1984. Inter- and intra-row inter-genotypic competition influences selection for avoidance of white mold disease in dry edible beans. J. Amer. Hort. Sci. 109:567-572.

11. Grau, C.R. and H.L. Bissonette. 1974. Whetzelinia stem rot of soybeans in Minnesota. Plant Dis. Rep. 58:693-695.

12. Grau, C.R., V.L. Radke and F.L. Gillespie. 1982. Resistance of soybean cultivars to Sclerotinia sclerotiorum. Plant Dis. 66:506-508.

13. Grau, C.R. and V.L. Radke. 1984. Effects of cultivars and cultural practices on Sclerotinia stem rot of soybean. Plant Dis. 68:56-58.

14. Hine, R.B. and J.E. Wheeler. 1970. The occurrence of some previously unreported diseases in Arizona. Plant Dis. Rep. 54:179-180.

15. Hunter, J.E., M.H. Dickson amd A. Cigna. 1981. Limited -term inoculation: A method to screen bean plants for partial resistance to white mold. Plant Dis. 65:414-417.

16. Imohelin, E.D. and R.G. Grogan. 1980. Factors affecting survival of sclerotia, and effects of inoculum density, relative position and distance from the host on infection of lettuce by Sclerotinia minor. Phytopath. 70:1162-1167.

17. Lockwood, J.L. and T.G. Isleib. 1983. Resistance of soybeans to white mold. Annual Report to the Soybean Promotion Committee of Michigan 1983-84.

18. Lockwood, J.L. and T.G. Isleib. 1984. Resistance of soybeans to white mold. Annual Report to the Soybean Promotion Committee of Michigan. 1984.

19. Lockwood, J.L. and T.G. Isleib. 1985. Resistance of soybeans to white mold. Annual Report to the Soybean Promotion Committee of Michigan. 1985.

20. Lumsden, R.D. 1079. Histology and physiology of pathogenesis in plant diseases caused by Sclerotinia species. Phytopath. 69:890-896.

21. Lumsden. R.D. and W.P. Wergin. 1980. Scanning-electron microscopy of infection of bean by species of Sclerotinia. Mycologia 72:1200-1209.

22. Martin, J.T. 1964. Role of cuticle in the defense against plant disease. Annu. Rev. Phytopathol. 2:81-100.

23. Martin, J.T. and B.E. Juniper. 1970. The cuticles of plants. 347 pages. Edward Arnold Ltd. Edinburgh

24. Phipps, P.M. and D.M. Porter. 1982. Sclerotinia blight of soybeans caused by Sclerotinia minor and Sclerotinia sclerotiorum. Plant Dis. 66:163-165.

25. Purdy, L.H. 1958. Some factors affecting penetration and infection by Sclerotinia sclerotiorum. Phytopath 48:605-609.

26. Schwartz, H.F., J.R. Steadman and D.P. Coyne. 1977. Resistance of Charlevoix and Valentine to infection by Sclerotinia sclerotiorum. Rep. Bean Improv. Comm. 20:71-71.

27. Schwartz, H.F, J.R. Steadman and D.P. Coyne. 1978. Influence of Phaseolus vulgaris blossoming characteristcs and canopy structure upon reaction to Sclerotinia sclerotiorum. Phytopath 68:465-470.

28. Sutton, D.C and B.J. Deverall. 1983. Studies on infection of bean (Phasleolus vulgaris) and soybean (Glycine max) by ascospores of Sclerotinia sclerotiorum. Plant Path. 32:251-261.

29. Tariq, V.N. and P. Jefferies. 1984. Appressorium formation by Sclerotinia sclerotiorum: Scanning electron microscopy. Trans. Brit. Mycol. Soc. 82:645-651.

30. Thompson, A.H. and G.C.A. Van Der Westhuizen. 1979. Sclerotinia sclerotiorum (Lib.) De Bary on soybean in South Africa. Phytophylactica 11:145-148.

31. Weiss, F. 1946. Check list revision. Plant Dis. 30:130-137.

32. Yorinori, J.T. and M. Homechin. 1985. Sclerotinia stem rot of soybeans, its importance and research in Brazil. p. 582-588. in World soybean conference III proceedings. ed. Richard Shibles. Westview press. Boulder.

SOURCES OF VARIABILITY WITHIN A GENOTYPE FOR RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* INFECTION OF SOYBEAN

INTRODUCTION

Sclerotinia stem rot in soybean (Glycine max (L.) Merr.) is caused by the fungus Sclerotinia sclerotiorum (Lib.) De Bary. Though reported in the major soybean producing areas of the midwest (3, 4, 8, 10, 15, 21), the disease has not been a widespread problem. It is more likely to be a problem when soybeans are planted in rotations with more susceptible hosts (8, 11, 19) and in cool wet environments that favor disease development (10, 22). In these situations the disease can be devastating to whole fields or sections of them causing considerable yield reductions (10, 17). It would be desirable to have soybean genotypes that exhibit resistance Sclerotinia stem rot for those regions where the disease has been a problem.

To breed for resistance there must be a method to screen genotypes for their reaction to the pathogen. A method should be quick, easy, repeatable, sensitive to differences among cultivars and correlated with field resistance. The wound inoculation (WI) method developed by Lockwood and Isleib (17) and adapted by Sneller et al.(unpublished) meets the above mentioned criteria plus it has the advantage of a quantitative rating of disease development. The WI technique still produced unexplained variability among the stems of cultivar for reaction to the pathogen. This variation contributed to the error term and reduced the sensitivity of the test. In one WI experiment,

lesions on stems of 'Weber 84' ranged from 5.9 to 10.8 cm and PI297514 lesions ranged from 2.3 to 10.1 cm.

Variability within a cultivar for reaction to S. sclerotiorum is common in other assays and in other species and is reported in soybeans (2, 4, 8, 15), Phaseolus vulgaris L. (13, 14), Helianthus annuus L. (12, 18, 20) and Pisum sativum L. (1).

If the cause of the variability was known, steps could be taken to minimize it which would result in an increased sensitivity of a test at differentiating between cultivars. Because the WI assay has many desirable features the following study was undertaken to see if the source of the variation could be ascertained.

There are several possible explainations for the variation in lesion lengths among the stems of a cultivar. There could be residual genetic heterogeneity within a cultivar for reaction to S. sclerotiorum. Three preliminary studies by the author were inconclusive with two studies supporting this hypothesis and one rebutting it. Another source of variability could originate in the growth environment of the plants used for testing. Other sources of variability could arise from the inoculum, the inoculation technique and from variation in the microclimate of the actual testing environment.

MATERIALS AND METHODS

Twenty seven single plants were harvested from field plots of five cultivars in October of 1985. Each plant was threshed separately and the seed kept separate. In this fashion five sets of twenty seven single plant families (SPF) were developed, one set for each cultivar to be tested. The cultivars had appeared to be the most variable in previous tests (Sneller et al. unpublished) and had been reported to be resistance or susceptibility to S. sclerotiorum. The susceptible genotypes were Weber 84 (16, 17) and 'Gnome' (4, 9, 15, 16, 17). The resistant cultivars were 'Corsoy' (4, 10, 15, 17), PI297514 (16), and PI358314 (16).

The plants were grown in the greenhouse under artificial light (16 hour day, 8 hour night) in 20 cm diameter clay pots, four plants per pot. The soil was a 5:3:2 mix of sandy loam, sphagnum peat and torpedo sand. Each pot was randomly assigned a cultivar and then each quarter section of the pot randomly assigned a seed from one of the SPFs to be tested. Pots were randomly assigned to locations on the greenhouse bench and were spaced 130 cm below the lights such that the rims of the pots touched one another. The plants in Experiment 1 were 38 days-old while Experiment 2 was performed on 47 day-old-plants.

The WI method was employed to test the disease reaction of the plants. This involves the inoculation of 15 cm stem

sections in the laboratory. The stem sections were prepared by cutting the main stem 1.5 cm above the node bearing the upper most trifoliate leaf and making a second cut 15 cm below this node resulting in a 16.5 cm stem section. The leaves and petioles were then cut from the stem section The stem sections were then transported to the laboratory in plastic bags. Immediately prior to inoculation, a final cut was made through the middle of the described node perpendicular to the long axis of the stem, resulting in a 15 cm stem section. The inoculum was placed on the surface of the final cut.

The stems were inoculated and incubated in clear plexiglass boxes (56 x 53 x 8 cm). The boxes were filled with a 4 cm thick layer of medium grade vermiculite. The vermiculite was moistened with 1500 ml of distilled water. After inoculation the boxes were covered with plastic wrap and a clear plastic lid was put in place. All stems were randomly assigned to locations within the boxes.

Inoculum was grown on 2% Bacto agar medium supplemented with 2% millet seed powder and was cultured from mycelium grown on the same medium. The inoculum was grown in 15 cm diameter petri dishs in the dark for five days at ambient room temperature (approximately 24°C). A 0.3% percent water agar solution was poured in the dish of inoculum to cover the surface of the medium. Plugs of inoculum were cut with a 4 mm cork borer from a distance of 4.0 to 6.0 cm from the center of the dish, then removed with a dissecting needle

and placed mycelium side down on the final cut at the apical end of the stem section. One dish of inoculum was used per replication.

Lesion measurements were taken five days after inoculation. A lesion was measured from the point of inoculation to the area of the lesion where the epidermis could no longer be easily peeled off with the edge of a plastic ruler.

The experiments were conducted in a room cooled to approximately 20 $^{\circ}$ C though this temperature fluctuated upwards to 24 $^{\circ}$ C during the day.

In Experiment 1, 27 SPFs from each cultivar were tested. The experiment was conducted in three runs due to the space constraints of the boxes. Each run was a randomized complete block design with three replications (boxes) with each cultivar was tested in each run and in each box. Nine different SPFs from each cultivar were tested in each run. Within each run, each box contained three different SPFs from each cultivar. Thus the SPF's were nested within boxes, runs and cultivars. In each box, each SPF was represented by four stems.

Experiment 2 was conducted in one run. The number of SPFs tested was not equal among the five cultivars. This was due the failure of enough plants of some SPF's to grow to the proper length. Nine SPFs were tested from Gnome, Corsoy and PI 358314. Eight SPFs of Weber 84 and seven SPFs of PI 297514 were tested. The overall design was a

randomized complete block with three replications (boxes). One stem from each SPF of each cultivar was represented in each replication. Thus the SPFs are not nested in boxes as in Experiment 1.

RESULTS

The analysis of variance from Experiment one showed that the variation among the SPFs of a cultivar was not significantly greater than the variation among the stems within a SPF (Table 1).

Table 1. Analysis of variance for lesion length for SPFs of five cultivers, Experiment 1 (1/24/86, 1/30/86, 2/23/86).

Source	df	MS	F test
Run	2	197.52	**
Cultivar	4	142.30	**
Run x Cultivar	8	20.22	***
Box (Run)	6	5.96	*
Cultivar x Box (Run)	24	2.23	
SPF (Cultivar Box Run)	90	2.14	
Stem (SPF Cultivar Box Run)	405	1.91	

Coefficient of variability = 14.66%

*, **, and *** denote significance at the .05, .01 and .001 alpha levels respectively.

Due to the design of the experiment the interaction of runs or boxes with SPFs could not be calculated. The effect of runs was significant as was the run by cultivar interaction. It was then assumed that the run in which a particular SPF was tested probably influenced the resulting

average lesion length of a SPF. To eliminate the possible run effect on SPF lesion lengths, three ANOVA's were performed, one for each run (Table 2).

Table 2. Analysis of variance by runs for lesion length for the SPFs tested in Experiment 1.

	Date	1/24/86 Run 1		1/30/86 Run 2		2/23/86 Run 3	
	df	MS	F test	MS	F test	MS	F test
Box	2	7.5		.8		9.6	
Cultivar	4	21.8	*	81.2	***	79.8	***
Box x Cultivar	8	3.6		.9		2.1	
SPF (Box Cultivar)	30	1.8		.9	*	.4	
Stem (SPF Box Cultivar)	135	1.7		.5		3.5	
Coefficient of variability		15.2 %		7.9 %		17.7 %	

*, ** and *** denote significance at the .05, .01 and .001 alpha levels respectively.

Box effects and the box by cultivar interactions were not significant in any of the runs. The assumption was then made that box effects would not influence the comparisons of SPFs among the boxes within a run. Only in Run 2 was the variation among the SPFs significantly greater than the variation among the stems within a SPF which may be reflective of the better sensitivity or to the existance of a larger difference among the 45 SPFs tested in Run 2 than among the 45 SPFs tested in each of the other runs.

The SPF (Box Cultivar) line from the ANOVA of each run was partitioned into five lines, each measuring the

contribution of an individual cultivar's SPFs to the overall effect (Table 3).

Table 3. Mean squares by runs for the source of variation in lesion length within a cultivar.

	Run 1		Run 2		Run 3	
	SPF(Box)	Error †	SPF(Box)	Error	SPF(Box)	Error
Corsoy	2.28	2.00	1.08	.76	4.64	8.96
Gnome	2.28 *	.89	.36	.33	.94	1.00
Weber 84	1.21	3.05	1.63 ***	.30	3.59	2.00
PI 358314	1.88	1.60	.62	.72	2.62	1.55
PI 297514	1.10	.90	.96	.54	6.86	4.06

* and *** denote significance at alpha = .05 and alpha =.001 levels respectively. Significance was tested with the reported error and not with the overall experimental error of each run.

† Error = Stem (Box, Cultivar)

The mean square of box effects was significant only for Weber 84 in Run 1. Therefore it was assumed that box effects do not significantly contribute to the variation among the SPFs. The effect of SPFs on lesion lengths was only significant for Gnome in Run 1 ($P < .05$) and for Weber 84 in Run 2 ($P < .001$). Significance was only found in the two combinations of cultivar and run with the lowest error term. The fact that significance was only found in two of the fifteen cases may be due to significant differences among the SPFs randomly assigned to these two tests or to chance alone as two of fifteen populations deviating beyond 95 % of normal is not very different from the one out of

twenty expected by chance alone. This makes the results of Experiment 1 inconclusive.

The SPFs from each cultivar with the longest and shortest average lesion length in each run were retested in Experiment 2 to see if the results could be repeated.

The overall effect of the SPFs on lesion development was not significant (Table 4). Upon partitioning, only the effect of the PI 297514 SPFs was significant. Separate analyses of variance were performed for each cultivar and the effect of the SPFs were tested with the resulting errors. Analyzed in this way the effect of the SPFs of Weber 84 was found significant at $P < .058$.

Table 4. Analysis of variance for lesion length for SPFs of five cultivars, Experiment two (5/22/86).

Source	df	MS	F test
Box	2	14.05	**
Cultivar	4	22.02	**
Box x Cultivar	8	2.64	
SPF (Cultivar)	37	1.40	
Corsoy	8	.76	
Gnome	8	.41	
Weber 84	7	.78	
PI 297514	6	4.44	*
PI 358314	8	1.29	
Stem (SPF Cultivar Box)	74	1.91	
Coefficient of variability		17.79 %	

*, ** and *** denote significance at the .05, .01 and .001 alpha levels respectively.

Table 5 compares the average lesion lengths obtained for the SPF's of the cultivars tested in Experiment 1 and Experiment 2.

Table 5. Correlation of the average lesion lengths of the SPFs tested in Experiment 1 with the average lesion lengths obtained in Experiment 2.

	r	Significance
All SPFs	.158	
Corsoy SPFs	-.513	
Gnome SPFs	.695	*
Weber 84 SPFs	-.434	
PI 297514 SPFs	-.735	
PI 358314 SPFs	-.045	

* denotes significance the .05 alpha level.

While the effect of the SPFs of PI 297514 and Weber 84 were significant in Experiment 2, the average lesion lengths of these SPFs were not significantly correlated to the average lesion lengths from Experiment 1 (Table 5). Only the average lesion lengths of the SPFs of Gnome were significantly correlated between experiments. The effect of the SPFs of Gnome were not significant in Experiment 2.

Height data was collected on all the plants tested in Experiment 2 and on the plants tested in Run 2 of Experiment 1 (Table 6) to see if there was a correlation of plant heigth with lesion length.

Table 6. Summary of height data for the plants used in Experiments 1 (run 2) and Experiment 2.

	1/30/86 Experiment 1 run 2 only			5/22/86 Experiment 2		
	Average height	Standard deviation	Range	Average height	Standard deviation	Range
	-- cm --		-- cm --	-- cm --		-- cm --
Corsoy	52.2	13.7	33 - 83	64.8	7.6	51 - 79
Gnome	65.8	12.4	38 - 91	61.8	5.3	48 - 68
PI 297514	40.0	6.1	28 - 56	55.2	8.6	38 - 68
PI 359314	53.9	12.1	36 - 81	63.1	6.8	51 - 76
Weber 84	45.3	8.6	30 - 83	62.6	5.8	53 - 73

There was considerable variation in plant height which was probably due to variation within the growth environment of the plants.

From Experiment 1, run 2, data was collected on plant height and on the pot in which the plant was grown. An analysis of variance showed that the pot in which a plant was grown had a very significant ($P < .001$) effect on plant height indicating that conditions among pots varied enough to cause significant variation in plant height. The effect was significant for all cultivars except Weber 84.

In Experiment 1, Run 2, the correlation of height and lesion length was significant for the stems of Corsoy, PI 297514, and Weber 84 (Table 7). A significant negative correlation was observed for the stems of PI 297514 in Experiment 2. The range of heights was less in Experiment 2 than in Experiment 1 and there may not have been a sufficient range of heights to develop a good correlation.

Table 7. r values and probabilities for the correlation of plant heigth and lesion length from Experiments 1 (run 2) and 2.

	Experiment 1 (Run 2 only)		Experiment 2	
	r		r	
All genotypes	.533	***	- .152	
Corsoy	.596	***	- .040	
Gnome	.328		.345	
PI 297514	.875	**	- .458	*
PI 358314	.415		- .001	
Weber 84	.461	*	- .151	

*, **, *** denote significance at the .05, 101 and .001 alpha levels respectively.

DISCUSSION

A soybean cultivar is selected from a single plant at some point in a breeding program and the progeny are bulked thereafter. The earlier the generation in which the single plant is selected, the greater the heterozygosity of the selected plant. This will increase the heterogeneity of the future bulked generations. A population (future cultivar) would tend to have more heterogeneity among the individuals for a trait that is quantitative in nature and for which there has not been selection pressure. In dry beans there is evidence that resistance to S. sclerotiorum is a quantitative trait (5, 6, 7). At present there have not been any soybean cultivars developed using reaction to Sclerotinia stem rot as a major criterion of selection. Thus heterogeneity within a cultivar for reaction to Sclerotinia stem rot seemed a probable cause of the

unexplained variability in lesion lengths among the stems of a cultivar as determined by the WI assay.

The results indicated that genetic heterogeneity was not a major cause of the residual variation in the WI assay. The significant differences among the average lesion lengths of the SPFs in Experiment 1 were not repeated in Experiment 2 and the overall correlation of the average lesion lengths of the SPF's in the two experiments was not significant. It is possible that the WI assay was not sensitive enough to detect such heterogeneity. Only the most sensitive analyses performed found significant differences among SPFs. It is also possible that no variation existed among the 27 SPFs per cultivar that were tested and that a larger sample would uncover some residual heterogeneity. If heterogeneity does exists, it did not contribute significantly to the overall error term. When the SPF sum of square was returned to the error term it only accounted for 19.9 % and 26.8 % of the residual variability in Experiments 1 and 2 respectively. These contributions were in line with the contribution of the degrees of freedom associated with SPFs.

In some ancillary studies, all the stems of a single cultivar were derived from a single SPF. Considerable residual variability remained despite the genetic homogeneity of this material. In one of these tests the lesions on the stems of Corsoy ranged from 3 to 6.6 cm. PI297514 developed lesions ranging from 5.4 to 8.2 cm.

This suggests that environmental effects and methodological inconsistencies were the source of the residual variation. Variation in the microclimate of the inoculation sites within a box seemed unlikely due to relatively small box size and the fact that the boxes were well sealed. There also should be little variation in the inoculum. All the plugs of inoculum were drawn from approximately the same distance from the starting point of the culture assuring that the age of the mycelium was similar for all plugs. One plate of inoculum was used per replication so that variation among the plates was confounded with replications. Variation due to the inoculation technique was also minimized. All stems were inoculated immediately after they were cut to the final length and plugs of inoculum rarely fell off. The experiments were checked about five hours after initiation and any plugs which fell off were repositioned or replaced with fresh plugs. With the WI technique, all lesions appeared to initiate at approximately the same time and overall variation increased with time (Sneller *et al.* unpublished). This indicated that the inoculation process is uniform and that the factors causing the residual variability affect lesion growth, not lesion initiation.

By elimination of the other possible sources of variation, the growth environment of the plants appeared to be the most likely source of the residual variation. While efforts were made to maintain a uniform growing environment

among the plants, it became more variable as more plants were needed for an experiment. This variability was reflected in the height data taken on the plants used in this study. The height variation was not correlated with lesion length. Variation in the microclimate in the greenhouse may influence reaction to S. sclerotiorum independently of plant height.

Residual variation in the WI method was not reduced in this study. Progeny testing remains necessary to assure an accurate rating of a genotype. This will require testing a soybean line in a late generation in a breeding program to assure a high degree of genetic homogeneity among the progeny.

Studies are presently under way to investigate the possible causes of variation in the growth environment affecting resistance to S. sclerotiorum. By controlling the source of the variation it is hoped that the sensitivity of the test can be further improved. This would allow testing with fewer plants per line and increase the number of lines that can be screened at one time.

LIST OF REFERENCES

1. Blanchette, B.L., and D.L. Auld. 1978. Screening field peas for resistance to white mold. Crop Sci. 18:977-979.

2. Chamberlain, D.W. 1951. Sclerotinia stem rot of soybeans. Plant Dis. Rep. 35:490-491.

3. Cline, M.N. and B.J. Jacobson. 1983. Methods of evaluating soybean cultivars for resistance to Sclerotinia sclerotiorum. Plant Dis. 67:784-786.

4. Coyne, D.P., J.R. Steadman and H.F. Schwartz. 1977. Genetic variation, inheritance and breeding strategy for resistance to Sclerotinia sclerotiorum in beans (Phaseolus vulgaris L.). Rep. Bean Improv. Comm. 20:19-21.

5. Dickson, M.H., J.E. Hunter, M.A. Boettger and J.A. Cigna. 1982. Selection for resistance in Phaseolus vulgaris L. to white mold caused by Sclerotinia sclerotiorum (Lib.) De Bary. J. Amer. Soc. Hort. Sci. 107:231-234.

6. Fuller, P.A., D.P. Coyne and J.R. Steadman. 1984. Inheritance of resistance to white mold disease in a diallel cross of dry beans. Crop Sci. 24:929-933.

7. Grau, C.R. and H.L. Bissonette. 1974. Whetzelinia stem rot of soybeans in Minnesota. Plant Dis. Rep. 58:693-695.

8. Grau, C.R. and V.L. Radke. 1984. Effects of cultivars and cultural practices on Sclerotinia stem rot of soybean. Plant Dis. 68:56-58.

9. Hine, R.B. and J.E. Wheeler. 1970. The occureance of some previously unreported diseases in Arizona. Plant Dis. Rep. 54:179-180.

10. Huang, H.C. and D.G. Dorrell. 1978. Screening sunflower seedlings for resistance to toxic metabolites produced by Sclerotinia sclerotiorum. Can. J. Plant Sci. 58:1107-1110.

11. Hunter, J.E., M.H. Dickson amd A. Cigna. 1981. Limited -term inoculation: A method to screen bean plants for partial resistance to white mold. Plant Dis. 65:414-417.

12. Hunter, J.E., M.H. Dickson and M.A. Boettger. 1982. Evaluation of plant introductions of Phaseolus species for resistance to white mold. Plant Dis. 66:320-322.

13. Lockwood, J.L. and T.G. Isleib. 1983. Resistance of soybeans to white mold. Annual Report to the Soybean Promotion Committee of Michigan. 1983-84.

14. Lockwood, J.L. and T.G. Isleib. 1985. Resistance of soybeans to white mold. Annual Report to the Soybean Promotion Committee of Michigan. 1985.

15. Noyes, R.D. and J.G. Hancock. 1981. Role of oxalic acid in the Sclerotinia wilt of sunflower. Physiol. Plant Path. 18:123-132.

16. Phipps, P.M. and D.M. Porter. 1982. Sclerotinia blight of soybeans caused by Sclerotinia minor and Sclerotinia sclerotiorum. Plant Dis. 66:163-165.

17. Putt, E.D. 1958. Notes on differences in susceptibility to Sclerotinia wilt in sunflowers. Can. J. Plant Sci. 38:380-381.

18. Weiss, F. 1946. Check list revision. Plant Dis. 30:130-137.

19. Yorinori, J.T. and M. Homechin. 198 . Sclerotinia stem rot of soybeans, it's importance and research in Brazil. p. 582-588. in World soybean conference III proceedings. ed. Richard Shibles. Westview Press. Boulder.

IMPLICATIONS OF THESIS FINDINGS TO A BREEDING PROGRAM FOR RESISTANCE TO *SCLEROTINIA SCLEROTIORUM*

The WI method appeared to be a reliable indicator of field resistance to *S. sclerotiorum*. Several genotypes were identified which exhibited resistance to the fungus. This indicates that it would be possible to breed for resistance to the disease by transferring the resistance genes to adapted genotypes. Fortunately one of the most resistant cultivars was 'Corsoy' which is already adapted to the primary soybean producing regions of Michigan. Two plant introductions were also identified as being resistant. It would be interesting to investigate the genetic control of resistance in these sources to see if they could be used to further increase the resistance of Corsoy.

All the lesions resulting from the WI technique would be lethal to a plant. Complete resistance was not expressed and the ratings were relative. A resistant and susceptible check must be included in each experiment. The resistance as measured by the WI method, appeared to relate to the ability to limit infections to non-lethal dimensions. All three resistant cultivars still developed some lethal lesions, though significantly fewer than the more susceptible cultivar, Gnome. Thus resistance appeared to be incomplete and was ultimately expressed as a lower probability of a lethal lesion. The expression of resistance was also influenced by the environment as shown by variation between experiments.

There remained variation within a genotype for reaction to S. sclerotiorum. Genetic heterogeneity within a cultivar for resistance to the fungus was ruled out as a cause of the variability as were possible sources associated with the inoculation procedures. The variation appeared to be associated with some factor influencing lesion growth after lesion initiation. The most likely source of the variation appeared to be the growth environment of the plants. Environmental variation within an experiment lowers the heritability of the trait regardless of the genetic control of resistance. Selection efficiency will therefore be low unless the cause of this variation can be managed.

The variation necessitates the testing of several stems to obtain a reliable rating. The testing of progeny in a breeding program will require testing lines in later generations (F_4 or later) when a line produces relatively homogeneous progeny.