STUDIES ON THE REACTION OF SOYBEAN TO *SCLEROTINIA SCLEROTIORUM*, CAUSAL AGENT OF STEM ROT OF SOYBEAN

I. Further development of a laboratory assay for use in screening for resistance to stem rot, and field assessment of commercial cultivars

II. Screening of soybean introductions for stem rot resistance

by

Laura Beth Kao

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ABSTRACT

STUDIES ON THE REACTION OF SOYBEAN TO *SCLEROTINIA SCLEROTIORUM*, CAUSAL AGENT OF STEM ROT OF SOYBEAN

I. FURTHER DEVELOPMENT OF A LABORATORY ASSAY FOR USE IN SCREENING FOR RESISTANCE TO STEM ROT, AND FIELD ASSESSMENT OF COMMERCIAL CULTIVARS

II. SCREENING OF SOYBEAN INTRODUCTIONS FOR STEMROT RESISTANCE

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Improvements were made upon a laboratory assay for detecting resistance in soybean to *Sclerotinia sclerotiorum*. Stems of six-wk-old greenhouse-grown plants were excised, and the leavesand top portions of the plants were removed. Stems were inoculated with colonized disks of 2% millet agar. Resistance was assessed by measuring lesion lengths after seven days of incubation on moist vermiculite. A higher incidence of infection and greater uniformity in lesion lengths were obtained by reducing the temperature of incubation from 25$\pm$3 C to 21$\pm$1 C, and by inoculating cut stem apices instead of the axils of the first trifoliate leaves. This improved assay was used to search for resistance in over 800 soybean introductions, among which several were superior to Corsoy, a known partially resistant cultivar. Field assessment of commercial soybean cultivars for resistance to stem rot was also done during three consecutive years. Wide differences in susceptibilityof cultivars were seen, with some reproducibility.

To my partner in life, Cheng, and to my parents

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Introduction

Stem rot or white mold of soybeans (Glycine max (L.) Merr.) is caused by the discomycetous fungus, Sclerotinia sclerotiorum (Lib.) de Bary. This disease develops after bloom and causes spreading lesions on the above-ground parts of the plant accompanied by profuse growth of the fungus (72). The stem of the plant may become girdled, resulting in wilting and death of the foliage. Potential for yield loss in susceptible soybean cultivars is considerable (19,32). Due to the persistence of sclerotia, the fungal resting structures, and to the wide host range of the pathogen, control is difficult and frequently unsuccessful if environmental conditions are favorable for disease development. Differences in resistance and susceptiblity among soybean cultivars have been noted (33,45). Recent interest in the development of new cultivars incorporating resistance to S. sclerotiorum has led to the development of assays to detect resistance (16,21,44,45).

The organism

Sclerotinia sclerotiorum is an inoperculate, discomycetous fungus that produces stromata (i.e., the sclerotia) free from host tissue (43). The sclerotia are hard, irregularly shaped bodies consisting of a white medulla covered by a dark, melanized rind. The sclerotia produce

stipitate apothecia which in turn produce ellipsoidal, hyaline ascospores. The name of S. sclerotiorum was changed to Whetzelinia sclerotiorum in 1972, but the previous name was restored by Linda Kohn in 1979 (43).

Life cycle

Summaries of the life cycle of Sclerotinia sclerotiorum have been provided by Grau (31), Purdy (66), and Sinclair (72). In brief, the fungus forms sclerotia on or in the infected plant. The sclerotia serve as the resistant, overwintering structures and can germinate carpogenically to form apothecia when environmental conditions are favorable. Ascospores produced in the apothecia are forcibly ejected upward into the air stream, and are dispersed on air currents. There is no known functional conidial or repeating stage. Hyphae derived from the ascospores colonize nonliving or senescent plant parts or wounded plant tissue; from thence infection is established in healthy tissue. Sclerotia form on or in the diseased tissue, thus completing the life cycle. Alternatively, in the presence of a food source, sclerotia can germinate and infect plants directly.

Symptoms

The predominant features of stem rot infection are the presence on infected plants of the causal fungus as fluffy, white mycelium and later the black, hard-rinded sclerotia. Symptoms are most often observed on the main stem 15 to 40 cm above the soil line (33). Lesions originate at leaf

axils and advance up and down the stem from the node. Multiple stem infections can occur (72). Infection first appears as grayish, water-soaked lesions which become tan, then bleached white (31). The stem can be girdled, inhibiting movement of water and nutrients to the upper foliage (72). The foliage of girdled plants wilts and assumes a grayish-green cast. This is often the first indication of stem rot infection in the field. Tissue between leaf veins is discolored and dries up while veinal tissue remains green. As the disease progresses, the leaves become tan, curl and shrivel, but remain attached to the stems. Fluffy fungal mycelium covers the diseased area during periods of high relative humidity (31). Large sclerotia that completely occupy the normal pith region of stems are produced (64). Pod development and pod fill above the stem lesions are greatly reduced (33,72). Occasionally sclerotia are found in pods (72). Stem infections are rarely observed at the soil line (72), and roots do not develop symptoms (30,60). S. sclerotiorum is capable of attacking seedlings, but this occurs infrequently (66).

Pathogenesis

S. sclerotiorum can initiate infection from either germinating ascospores or from mycelium derived from sclerotia or ascospores (46). In stem rot of soybeans, ascospores serve as primary inoculum. Ascospores infect mature or senescent flowers, and after colonizing these tissues, the mycelium invades the main stem. The process is similar to

infection in beans. Much histological work on the infection process has been conducted on bean plants, and this information has been extrapolated to the infection process in soybeans.

Ascospore germination and subsequent formation of an appressorial mass *in vitro* were studied microscopically by Purdy (65). A germ tube emerges from the ascospore and upon contact with a surface, dichotomously branches to form a hand-like structure with the terminal cells becoming enlarged at the tips. This appressorial mass is darker in color than the hyaline hyphae from which it arises. Eventually, an infection peg appears in the circular area of each terminal cell in contact with the plant.

Abawi *et al.* (3) studied the time course of the infection process in bean. Ascospores germinated within 6 hours of being atomized onto bean blossoms. Within 24 hours, extensive germ tube branching and appressorium formation occurred. The appressoria then formed infection pegs which penetrated the epidermal layer of the bean blossom and formed vesicles therein. The vesicles gave rise to infection hyphae which ramified through the petals. At 48-72 hours after inoculation, the flower parts were thoroughly colonized and had aerial hyphae growing from them. Hyphal strands extruding from the blossoms produced appressoria when in contact with other tissues, facilitating spread to other tissues. Lumsden (46) emphasized the intercellular nature of growth of the infection hyphae that arise from the

vesicle. In infected stems, an organized, fan-shaped infection front of hyphae growing beneath the cuticle develops and girdles the stem. Then ramifying hyphae, which are of a smaller diameter than the infection hyphae, invade the dead or dying tissue both inter- and intra-cellularly. These hyphae emerge through stomates or breaks in the cuticle, giving the cottony appearance to infected tissue.

Various cell-wall degrading enzymes and the production of oxalic acid are associated with the process of pathogenesis (46,78). The most important of the enzymes are endo-polygalacturonase, pectinase, and pectin methylesterase. These and other enzymes aid infection by digesting host tissues, thus providing nutrients for the pathogen and reducing mechanical resistance of the plant tissues to fungal growth.

After tissue is thoroughly colonized, sclerotial initials form. Microscopically, these appear as clumps of short, barrel-shaped cells. Mature sclerotia form 3-7 days after infection.

Host range and distribution

Purdy (66) published information on the host range of S. sclerotiorum which included unpublished material from P.B. Adams. Adam's survey of the literature revealed that S. sclerotiorum infects members of 64 plant families, 225 genera, and 361 species. At least one more species has since been added to this oft-cited list (9). Given this wide host range which includes many economically important

plants, it is not surprising that many crops grown in rotation with soybeans or in the same area as soybeans are susceptible hosts. Among these are cabbage (16,21,58), green bean (16), lettuce (16,31), peanut (16,21), sunflower (16,21,30,31,37), rapeseed (28), safflower (37,55), mungbean (37), field peas (14,41), potatoes (31), celery (41), carrots (41), dry edible beans (30,31,42), Brussels sprouts (58), cauliflower (58), kale (58), turnip (58), Jerusalem artichoke (37) and sugarbeet (30). Some weeds associated with these crops are also hosts (2,9,26). Distribution of the fungus is worldwide, although it does not occur in every locality of every country (66). Stem rot of soybeans occurs in Brazil, Canada, Hungary, India, Nepal, South Africa, and the United States (72).

Economic significance

S. sclerotiorum can cause severe but sporadic losses to crops (1,9,18,26,30,31,42,50,53,55,57,59,61,66,79,80). Purdy (66) cites a table compiled by P.B. Adams from which Purdy estimates that crop production losses in the United States caused by S. sclerotiorum are in the millions of dollars. S. sclerotiorum is reported to cause significant economic losses in snapbean production in New York state, with 90% of plants being infected each year by early August (57). Nordin (61) lists S. sclerotiorum as the major cause of disease in spring-sown oil-seed rape and turnip rape in Sweden where it may cause yield losses up to 50%. Head rot of safflower caused by S. sclerotiorum is considered to be

one of four principal diseases of safflower in Montana (55). However, almost all researchers who study diseases caused by S. sclerotiorum acknowledge the sporadic nature of infection (26,31,33,42,51,53,59,61,62,72,78). For example, Grau and Radke (32) conducted field tests of soybean cultivars for three consecutive years in a naturally infested field. Although a disease severity index of 34 on a scale of 0 to 100 occurred the first year, no disease was found in the same field during the second year. During the third year, the disease severity index was 39. Not only can disease vary from year to year, it can also vary from site to site within the same year. Coyne et al. (24) obtained varied and occasionally widely different infection ratings of the same dry bean cultivars planted in the same year in two different white mold nurseries. Phrases such as "potentially destructive" (31), "the scattered nature of the disease" (59), or "frequently causes serious, but unpredictable, yield losses" (78) are added as qualifiers to descriptions of Sclerotinia-incited diseases.

Because of the sporadic nature of the disease, stem rot is not considered an extremely important disease of soybean. The American Phytopathological Society's Compendium of Soybean Diseases says, "The disease is considered of minor importance in the United States except for local outbreaks during prolonged wet periods, when plants may be killed before maturity" (72). To paraphrase L.H. Purdy, who was asked to report on the impact of Sclerotinia diseases at a

1979 symposium on *Sclerotinia*: if it were more important, more work would have been done on it (66). However, there are reports that incidence of stem rot of soybeans is increasing (15,19, 30-33). Several factors contribute to this phenomenon. The practice of narrow row spacing creates a humid, comparatively stable environment conducive to disease development (32). Irrigation has a similar effect because carpogenic germination of sclerotia requires water, as does germination of ascospores (1,2,32,34,70). Finally, soybean production is expanding into land formerly cropped to susceptible hosts and/or soybeans are being grown in rotation with susceptible hosts such as dry edible beans, green beans, cabbage, peanut, sunflowers and sugarbeets (19,21,30-33).

When conditions are favorable for *Sclerotinia* infection, yield losses can be significant. In field tests done with various cultivars, irrigation schemes, and row widths, Grau and Radke (32) demonstrated that *S. sclerotiorum* reduces yield of susceptible soybean cultivars and that disease severity and yield are inversely correlated. By omitting some irrigations during the growing season, disease severity could be reduced, resulting in 10 and 22% yield increases in 1979 and 1981, respectively, over plots given more irrigation. Chun *et al.* (19) also found an inverse correlation between disease incidence and yield. In field experiments conducted using 16 commercial soybean cultivars of varying susceptibility to stem rot, they found that for

every 10% increase in disease incidence, yield was reduced by 235 kg/ha or 7.8%. Although Grau and Heimann (31) suggest that yield is significantly reduced when 20% or more of the soybean plants are killed by Sclerotinia stem rot, the findings of Chun et al. (19) would indicate that even lower amounts of disease are capable of reducing yields.

In dry beans also, an inverse correlation between white mold reactions and yields of various cultivars was shown in the field (24,42). Kerr et al. (42) studied the relation of the disease to yield and yield components in dry edible bean. A survey of irrigated bean fields in western Nebraska over 4 years revealed that 30% of fields were infected with white mold resulting in an average loss of yield of 13%. Seed yield, weight per 100 seeds, number of seeds per five plants and number of pods per 5 plants were all decreased in diseased plants as compared to healthy plants. A higher proportion of smaller seeds was harvested from diseased plants than from healthy plants. Reduced seed number per plant was the major component of yield loss followed by reduced seed weight.

Besides the direct loss of yield due to the activity of the pathogen, there are other sources of profit loss to the soybean grower. Presence of sclerotia in a shipment of soybeans intended for human consumption can result in the rejection of the whole shipment at a foreign port of entry (9,72). Cultural practices adopted to prevent stem rot can also reduce yields. For example, elimination of irrigations

can reduce yield while reducing stem rot severity (32). Also, planting lower-yielding but more resistant cultivars can compromise yield (42).

Epidemiology

The vast majority of time during the life cycle of S. sclerotiorum is spent as sclerotia in or on the soil (8). Sclerotia can survive for 3 to 5 years or longer (7,8,63, 70). Some of the factors which reduce longevity in soil are flooding and desiccation, burial in soil, pesticides, soil fumigants, and the activity of soil inhabitants, both macro- and microscopic (7,8,10,41,50-54,62,82).

Laboratory studies on the effect of availability of water at various points in the life cycle of S. sclerotiorum revealed that water potentials from -1 to -64 bars did not hamper production of sclerotia, but sclerotia could not germinate to produce apothecia at water potentials of -6 bars or lower (34). There are contradictory reports as to whether sclerotia which have been desiccated can later produce apothecia (34,63). Alternate wetting and drying of sclerotia is detrimental to survival (32). Flooding has been shown to kill sclerotia in 23 to 45 days in various soil types regardless of the depth at which the sclerotia were buried (53).

Various workers have studied the effects of burial on survival of sclerotia in conjunction with cultivation methods (7,50-52). Buried sclerotia are degraded more quickly than those on the soil surface (50,51). Sclerotia

reach the soil surface from the plant on or in which they were formed by gravity, wind, or harvesting (70) and would be subject to burial by tillage. Only sclerotia in the top 2 or 3 cm of soil are functional; below this depth, the stipes of the apothecia have greatly reduced chances of reaching the soil surface (2,51). However, sclerotia recovered from soil depths greater than 3 cm were able to germinate carpogenically when exposed to proper conditions in the laboratory (70). This would indicate that buried sclerotia remain capable of producing apothecia should they be returned to the soil surface through further tillage. Since sclerotia can survive for several years in the soil, the possibility of this phenomenon occurring is not remote. Further, Phillips (63) showed that the longer sclerotia remain in soil, the shorter the length of time needed for carpogenic germination to occur. Thus, Merriman's advice (50) to practice deep plowing to reduce Sclerotinia-incited disease was modified later (51) to caution that if deep plowing is used as a control, further plowing should be avoided that might return viable sclerotia to the soil surface.

The most important detriment to sclerotial survival in the soil is the activity of soil inhabitants. Various organisms have been shown to reduce sclerotial populations of S. sclerotiorum either by preventing their formation or by parasitizing and destroying sclerotia (8,10,41,50,53,54, 82). Twenty of 60 strains of bacteria isolated from the

soil degraded and/or prevented germination of sclerotia *in vitro* (82). Several fungi, *Trichoderma* spp., *Coniothyrium minitans*, and *Sporidesmium sclerotivorum*, are noteworthy sclerotial mycoparasites (8). Larvae of the insect, *Bradysia* sp., form holes in the rind through which other organisms, such as *Trichoderma*, can invade (10).

Weakening of the rind of the sclerotium, whether through the activity of other microbes or incomplete development, can result in decreased sclerotial survivability. Several fungal contaminants, including *Rhizopus*, *Fusarium*, *Mucor*, *Trichoderma* and *Alternaria* frequently grew from field-collected sclerotia (37,50). Scanning electron microscopy has revealed that the rind cells of sclerotia produced under natural conditions were collapsed and perforated compared to sclerotia produced in pure culture in the laboratory (50). These breaks in the rind can serve as portals of entry for further colonization by parasitic microorganisms. Incomplete melanization of the rind was also found to be correlated with fungal contamination during formation of sclerotia (37). Light brown, incompletely melanized sclerotia were prone to myceliogenic germination when placed on moist sand and exhibited no dormancy. Conversely, intact, fully-melanized sclerotia remained dormant until exogenous nutrients were provided or until the rind was damaged.

Interestingly, while studying the efficacy of mycoparasites on sclerotia of *S. sclerotiorum*, Mueller *et al.* (54) discovered that the presence of mycoparasites frequently

increased apothecial production. Such a phenomenon would be an evolutionarily advantageous trait since expedient propagation when threatened with death would increase the chances of an organism's genes being carried to a new generation.

Whether a sclerotium is formed on the surface of the plant or inside the pith can affect its survival and germination in the soil, presumably because of the difference in the exposure to microbiota. Merriman (51) collected sclerotia from the pith or from the outside of bean stems and buried them in the soil directly, or after first inserting them into lengths of healthy bean straw, the ends of which were plugged with cotton. The sclerotia which had been formed on exposed plant surfaces were degraded more rapidly than those formed in the pith. Sclerotia which were placed directly in the soil were degraded more rapidly than those inserted in bean straws; however apothecium production was increased in buried sclerotia. These findings also relate to the observation of Phillips (63) that sclerotia formed on sunflower root surfaces "invariably" begin germinating carpogenically sooner than those formed in stem cavities when placed in moist conditions in the laboratory.

Populations of sclerotia in natural soils ranged from zero to less than 10 sclerotia per kilogram of soil (8). Neither the number of sclerotia in the soil (62,70) nor the number of apothecia present (12,17,52,62,81) was correlated with disease incidence. Despite this, a minimum population is evidently necessary to incite a moderately severe epidemic (70).

Sclerotium population density can be increased in the absence of a susceptible economically important host by formation of secondary sclerotia (7,8,70), or by production of sclerotia on weed hosts (8,9,26). Adams (7) buried sclerotia in the soil and followed their population changes for three years. At some of his sampling times, he found more sclerotia present than he had originally buried, which he attributed to production of secondary sclerotia. Inoculum density was constant in winter and spring, but declined in the summer and autumn. The decline was attributed to the detrimental effects of drying and remoistening of soil. Sclerotia under fallow conditions survived as well as those cropped to hosts. Despite heavy disease development on susceptible hosts, inoculum density did not significantly increase. Schwartz and Steadman (70) followed the population of sclerotia in the soil of a field planted first to a susceptible crop and then to a succession of resistant hosts for three years. The population density decreased from the original 6.2 sclerotia/kg of soil to 1 sclerotium/kg of soil at the end of this four-year span.

Sclerotia need a period of conditioning before they can germinate to form apothecia. In reviewing the literature pertaining to this subject, Phillips (63) found that the minimum resting period needed by sclerotia of S. sclerotiorum before carpogenic germination was variously reported to be from 7 to 141 days. His own research indicated that 90 days were required before unconditioned sclerotia began

to germinate. By these results, sclerotia should be able to germinate in the same season in which they are formed. However, another group found that only 10-20% of sclerotia collected from the field in the fall germinated in the laboratory, whereas 70% of those collected in the spring germinated (70). Protocols for producing ascospores in the laboratory call for incubating sclerotia at 4 C for several weeks, thus simulating the requisite conditioning period (56).

Cool temperatures and ample moisture are conducive to carpogenic sclerotial germination and the development of epidemics caused by S. sclerotiorum (2,34,56,61,63,72). Continuous moisture for approximately 10 days is required for apothecial development (2). In dry climates, irrigation provides this moisture (2,70). The most conducive environment for apothecial production in a dry edible bean field in Nebraska was the irrigation furrow, especially where the plant canopy shaded the soil surface (70). In heavy-canopied crops or with narrow row-spacing, dew can provide moisture at the soil surface (28). In years with heavy rainfall, increased development of apothecia with a concomitant increase in disease incidence has been noted (61).

The temperature range suitable for apothecial production was 10-25 C, with an optimum of about 10 C (1). Growth of the fungus in vitro and development of lesions occurs in a similar range with an optimum of 20 ± 5 C; 30 C is too hot for fungal growth or lesion development (1,18,60,64,69,71,

80). Continuous exposure to 35 C for three weeks reduced the survival of sclerotia (7); however, it would be unlikely that such conditions would occur in the field. The natural range of temperatures encountered in temperate climates should not be prohibitive to carpogenic germination and disease development. Air temperatures 10 cm above the soil in a bean field were between 10 and 30 C during the period when white mold was first observed until it approached maximum severity (10 August to 31 August 1976) (80).

Ascospores need free moisture at the plant/fungus interface and a source of nutrients for infection to occur on intact plants (1,3,34,48,65). However, once they have landed on a plant, ascospores can await the development of proper conditions for germination and infection (1,34). Ascospores withstood continuous exposure to 0% relative humidity for 21 days in the laboratory, and survived under field conditions for up to 12 days when atomized onto bean leaves (34). Flower blossoms have been documented to provide the nutrients for infection in beans (3) and soybeans (21,31). Senescent plant parts, ragweed flower parts, or clumps of ragweed pollen have been shown to provide nutrients for ascosporic infection in cabbage (26,59). An exception to the requirement for a food base for ascosporic infection was found in bean by Abawi (5). Pre-flowering bean plants with genetically-induced necrosis or plants infected by another pathogen were susceptible to ascosporic infection through these lesions.

There are contradictory reports as to whether size of sclerotia is correlated with the number of apothecia produced (27,54). An average of two apothecia are formed per sclerotium (70), and sclerotia are capable of repeated germination to form new apothecia (27). The potential for production of ascospores by a single sclerotium was calculated to be as high as 2.3×10^8 (70). Apothecial production lasted for 16 and 18 weeks at two sites in Victoria, Canada (51). Apothecia can release ascospores for 2 to 17 days, with an average of 9 days (70).

Mycelium derived from sclerotia has low competitive saprophytic ability (1,59). Sclerotia placed 1 cm (1) or over 2 cm (59) away from a plant were unable to form mycelium that would traverse the distance to the plant and cause infection. Mycelial production apparently requires an exogenous source of nutrients (1,2,37,62,65). If a dead leaf bridges the distance between the sclerotium and the plant, infection can be accomplished (2,62,65). The primary function of sclerotia, therefore, lies in the production of apothecia.

Spread of S. sclerotiorum can occur by various means. Sclerotia-containing soil adhering to seedling transplants, to equipment or to animals can be a means by which the fungus is transported (8). Irrigation water can spread plant debris which contains sclerotia through a field or to another field. Debris containing 8 sclerotia per 10 g dry weight of plant material was collected from the water sur-

face of irrigation furrows in bean fields (70). Sclerotia can survive passage through the digestive tract of some animals, and consequently can be spread by movement of animals which have eaten food contaminated by S. sclerotiorum (8). Infection by ascospores adhering to pollen carried by honeybees has been shown to occur experimentally (76), but its prevalence as a means of dispersal under natural conditions has not been studied. There are reports of the seeds of bean and other hosts being internally and externally infected with mycelium of S. sclerotiorum (58,74). Sclerotia mixed in with seed lots can spread the fungus as well (72).

Air-borne ascospores are the primary means of dispersal of S. sclerotiorum (1,2,15,26,31,59,62,66,80,81). Ascospores are important in local infections (2,12,59) and in spreading disease from field to field (1,2,62,81). Williams (8) trapped ascospores 30-150 cm above the soil surface and 150 m from a source. Patterson and Grogan (62) found no apothecia in their disease study plots, but did find them in adjacent fields and orchards. Thus, they attributed disease in their plots to ascospores produced elsewhere. There is even the possibility that ascospores are carried by wind from sites of production extremely remote from infected fields. Spores identified as Sclerotinia sp. were among those trapped during airplane flights at altitudes of 500-18,000 feet (49).

After initial infection by ascospores, secondary spread

may occur through plant-to-plant contact (79). This process is considered to have only a minor role in epidemics, however (2). There is no functional conidial stage in Sclerotinia (43), thus no secondary spread occurs by this means.

Disease Control

Control measures utilized against S. sclerotiorum include tillage practices (32,36,51), weed control (26), decreased irrigation (32,52), flooding (53), and fungicides (31,69). Other potential means of control include biological control (10,41,54,77), fumigation (12), disease forecasting (28,61), and planting resistant cultivars (31) or cultivars with an open plant architecture (9,13,23,24,29,33,71). Deep plowing was advocated since it was more effective in burying sclerotia than harrowing (50,51). Planting in wide (76 cm) rows was shown to be less conducive to disease development than narrower rows (32). Weed control eliminates weed hosts which can harbor the pathogen and also provide nutrients for ascosporic infection of crop plants (9,26). Steadman (75) pointed out that Sclerotinia-incited diseases are not controlled consistently or economically. After reviewing control options, he concluded that "resistance and microclimate modification appear to be the most useful control measures for field crops."

Although differences in resistance among cultivars of soybean and other plants have been noted, (6,11,14,16,17,20-22,25,33,35,38-40,44,45,47,55,67,68,78,79), the resistance

mechanisms are not precisely known. Plant architecture has been investigated as a possible factor in disease escape (11,13,23,24,29,32,33,57,71,), as has row spacing (32,36). The microenvironment in dense-foliaged cultivars or close row spacing of beans or soybeans is more conducive to disease development than open-canopied cultivars or wide row spacings, respectively. Two groups (13,57) found that the microenvironment between rows was less subject to wide variations in temperature and humidity than the environment above the canopy once the plants had formed a dense leaf canopy. Blad et al. further found that the canopy air temperature was 7-8 C lower in heavily-irrigated plots than in plots irrigated less than half as much (13). Grau and Radke (32) hypothesized that the more stable environment beneath the canopy might keep sclerotia from wetting and drying fluctuations, which can be lethal. Their studies indicated that disease severity increased as row width decreased. In peanut, cultivars with an upright growth habit and with small leaves were less susceptible to Sclerotinia minor infection than cultivars with dense, spreading-type canopies (22). In beans, whether the growth habit was determinate or indeterminate was not the sole determinant of disease reaction; rather, the distribution of the leaf area, especially near the ground was more important (71).

Physiological resistance also exists (11,22,24,39,78). Growth of the fungus through a tolerant white bean cultivar was slower than through more susceptible cultivars (78).

Early maturation has been associated with disease avoidance (24,29), although other reports (11,33) claim that there is no relationship between resistance and maturity group.

A reddish brown, restricted lesion frequently has been noted in association with plant resistance to S. sclerotiorum (16,21,30,64,71,73). The fungus is localized within a 10-40-mm-diameter area around the site of inoculation; color of the plant tissue in this area ranged from light brown to reddish-brown (71,73).

The relation of resistance to S. sclerotiorum and flower color has been investigated. Grau et al (33) observed in field trials that soybean cultivars with white flowers were relatively susceptible, while some cultivars with purple flowers were less so. However, they doubted that resistance was expressed in the flowers themselves. Although Grau et al. do not appear to have been aware of it, two groups had previously shown that blossoms of beans became colonized when inoculated in the laboratory, irrespective of flower color or genotype (71), or resistance of the plant to white mold (40). Additional evidence which contradicts Grau's proposal was the finding of Cline and Jacobsen (21) that two white-flowering soybean cultivars were moderately resistant in inoculations made in the greenhouse. Boland and Hall (16) also obtained results which failed to correlate resistance with flower color.

Assays for resistance to Sclerotinia scerotiorum

A range of reactions to S. sclerotiorum has been noted in soybean (16,17,20,21,33,44,45), bean (6,11,25,35,39,40, 78,79), safflower (55), sunflower (38,67), peanut (22), field peas (14), alfalfa (68), and lettuce (47). Exploitation of these differences could lead to development of more resistant cultivars of these crops. In temperate climates, detecting resistance in the field is usually limited to one trial per year. Furthermore, field trials are subject to the seeming capriciousness of the fungus and environment (24,32). Therefore, the development of assays utilizing greenhouse- or growth chamber-grown plants has been explored. The advantages of such assays are: 1) ability to produce test material year-round and to thus test more varieties and plant lines, and 2) ability to standardize factors such as the amount of and method of application of inoculum and environmental conditions. A disadvantage is that conditions inherent to field experiments such as row-spacing and development of a closed plant canopy that might affect disease development cannot be reproduced in the greenhouse, growth chamber, or laboratory.

Assays using hosts other than soybean

Three-hundred and eighty-eight plant introductions (PI's) of field pea were screened for resistance to white mold using a mist-chamber assay (14). An oat kernel infested with S. sclerotiorum was placed against the base of

a 10-day-old pea plant which had been conditioned in a mist chamber for 24 hours previously. Inoculated plants were returned to the mist chamber for 3 days, after which they were rated on a visual scale based on lesion length. Thirty-eight PI's that had the shortest lesions were assayed a second time. The range of disease severities was higher in the second test, but the relative ranking of the lines was the same. In two other experiments, disease ratings of 10 varieties which were included in both experiments were significantly correlated ($r=0.84$). Thus differences in disease reaction were identified, and results of the assay were reproducible.

Various methods of inoculation were examined by Madjid *et al.* (47) during the development of an assay to detect the reaction of lettuce to *S. sclerotiorum*. Lettuce seedlings, 14-31 days old, were inoculated 1) by placing agar blocks colonized by mycelium 1 cm from the base of the plants, 2) with a mycelium-agar suspension sprayed on the plants or 3) with sclerotia which were incorporated into the potting mix. The percentage of survivors was determined 15 days later. Of these inoculation methods, the agar block technique was most satisfactory because the mycelial spray method required undefined "optimal environmental conditions", which were not always available and the sclerotial inoculum failed to infect. Significant differences in survival percentage were detected, but all cultivars tested showed a low level of resistance.

Oxalic acid was applied to alfalfa plants in an attempt to develop an assay for detecting resistance to S. trifoliorum (68). Like S. sclerotiorum, S. trifoliorum produces oxalic acid during pathogenesis. When the oxalic acid method was compared with the application of kernels colonized with S. trifoliorum to the surface of a tray of seedlings, no significant relationship between results of the two assays was shown. The authors concluded that the oxalic acid procedure was unsatisfactory.

Tu (78) investigated differences of reaction of different cultivars of white bean to oxalic acid. He placed the petioles of excised bean leaves in vials containing different concentrations of oxalic acid. After different periods of time, the percentage of leaf area with symptoms was determined. Symptoms increased with concentration of oxalic acid and with duration of time the leaves were exposed to the oxalic acid solution. Although the apparent rate of diffusion of oxalic acid in leaf tissue paralleled the growth of S. sclerotiorum in bean, he did not advocate an oxalic acid assay for detection of resistance since the intent of his paper was rather to prove that oxalic acid had a role in pathogenesis.

Huang and Dorrell (38) devised an assay for screening sunflower seedlings which has similarities to Tu's assay. The roots of 3-week-old seedlings were washed and the intact seedlings were placed in a vial containing autoclaved culture filtrates of S. sclerotiorum. Ratings based on degree

of wilting were made after 24 hours. Autoclaved culture filtrates which had been lyophylized and reconstituted worked as well as those which were freshly prepared. They detected a rather narrow range of reactions, from moderately resistant to highly susceptible, in the 16 cultivars tested. They recommended that this test be used as an adjunct to field testing.

Ascospores often have been used as inoculum in resistance assays. Tu (78) inoculated 10-day-old white beans by having apothecia discharge ascospores directly onto misted seedlings in a plastic container. After inoculation, pots were covered with a clear plastic bag and kept in a growth room. Plants were monitored for enlargement of lesions on leaves at 2-day intervals. After 8 days, two known susceptible cultivars were totally collapsed while a tolerant variety showed brown flecks that rarely reached 2 mm in diameter. If the tolerant variety did begin to rot, the rate of disease progress was approximately one-third that of the susceptible cultivars. Tu tested only three cultivars and did not repeat his results. His results seem contradictory to those of others (3,21,48,69), who found that pre-bloom plants could not be infected unless wounds were created, and that ascospores would not infect unless a food source was present. Although not quantified, the inoculum concentration used was very high; perhaps the ascospores which did not infect were used as a nutrient source by those that did.

Two laboratories employed assays utilizing excised leaves in moist chambers (1,6,69). Inoculum was placed on the center of leaves and the lesion diameters were later recorded. Saito (69) utilized ascospores or mycelium growing on agar as inoculum, and found that agar inoculum was preferable since ascospores could not invade directly into host tissue unless a wound was made or nutrients were added to the spore suspension. Abawi (6) used agar inoculum and found that all cultivars and breeding lines tested by this method were highly susceptible.

An assay utilizing plant tissue culture methods to test bean germplasm is being developed at North Dakota State University (35). Relative growth of bean calli on tissue culture medium containing culture filtrates of different isolates of S. sclerotiorum is taken as an index of susceptibility or resistance.

In an investigation of inheritance of resistance in bean to white mold, Abawi et al. (4) developed an assay designed to simulate the natural infection process. Plants were grown in a greenhouse to the blossom stage, then were inoculated by spraying 2 ml of a suspension containing 2000 ascospores/ml onto their blossoms. Immediately afterwards, the plants were put in a mist chamber where they were incubated for 1 week before being rated on a scale based on number and severity of lesions. Although they claimed that "the ascospore inoculation procedure...appears reliable and effective in detecting high levels of resistance to white

mold", subsequent work has cast doubt on this claim.

Hunter, one of the authors of the above-mentioned paper, and other researchers (40) attempted to repeat this work. Lines that had been reported to be resistant previously were susceptible in the repeated tests. The fact that contact between blossoms and healthy green tissue was left to chance in this method was considered to be a flaw. To remedy this, they sprayed detached blossoms with ascospores, and then placed them in the axils of the leaves. This method produced more uniform disease incidence and severity. A subsequent experiment showed that pieces of celery petiole colonized with S. sclerotiorum were as effective as ascospore-sprayed blossoms in inciting disease. Either 95% or higher relative humidity in a growth chamber or a mist chamber was required for disease development.

An experiment was conducted to determine if earlier removal of colonized celery petiole inoculum would reduce disease severity and thereby help differentiate levels of resistance (40). Inoculum was removed from 2-3 week old plants 12, 24, 36, 48, and 72 hours after inoculation. Optimal length of inoculum exposure was reported to be 24-48 hours, even though another experiment had indicated that if inoculum was left on for more than 24 hours, separation between susceptible and partially resistant cultivars was difficult.

The same laboratory employed a modification of the

limited term inoculation method to screen Phaseolus PI's for resistance to white mold (39), then to evaluate progenies of crosses made to increase resistance (25). The change in protocol was as follows: after plants were inoculated with colonized celery pieces and incubated in a mist chamber for 48 hours, they were rated as having collapsed or upright stems. Plants with upright stems were grown on a greenhouse bench for 5 more days after which time the number of surviving plants was recorded. Of the 449 PI's screened, 29 had 50% or more survivors. Cultivars and breeding lines which had a low incidence of white mold in the field were highly susceptible when inoculated by this method. All lines could be killed if the inoculum was left on the stem in a humid chamber for longer than 48 hours. Breeding to build up minor genes for resistance was carried out, resulting in a 17% increase in survivorship to the limited term inoculation procedure by the F_4 generation (25).

Assays utilizing soybean

Soybean cultivars have been tested for reaction to S. sclerotiorum in the field by groups located in Wisconsin (31.33), Ontario (17), and Michigan (45). Results from these tests often determined the selection of cultivars used in development of laboratory assays (16,19,21,45,73).

Cline and Jacobsen (21) in essence repeated the work of Hunter et al. (40) using soybean instead of bean. Ascospores were sprayed onto blooming plants which were

incubated in a mist chamber, but no differences in stem rot reaction could be detected, since all cultivars except two had nearly 100% plant death. Detached flowers sprayed with ascospores and placed in the leaf axils resulted in higher disease incidence than spraying whole flowering plants with ascospores. When ascospores were sprayed on the whole plant, more disease was obtained when plants were incubated in a mist chamber than in polyethylene bags placed in a growth chamber; however, variability was greater in the mist chamber. Plants inoculated with colonized carrot pieces, then placed in polyethylene bags had no significant differences in disease severity among 16 cultivars; disease severity was high in all. Finally, pieces of colonized celery were applied to the second or third node of a 4-week-old soybean by wrapping moistened cotton around the stem and inoculum, the plant was bagged and placed in a growth chamber. After 24 hours, the inoculum was removed, the plants were wetted, rebagged, and placed back in the growth chamber for a week. Statistically significant differences in the resulting disease severities were detected, but the experiment was not repeated.

In order to identify stem rot-resistant cultivars adapted to Ontario, Boland and Hall (16) employed the limited term inoculation procedure as described by Hunter et al (40) with two modifications. Colonized green bean was used instead of celery as inoculum. Inoculated plants were incubated in the mist chamber for 50 to 144 hours (mean of

101 hours), before the inoculum was removed and the plants were placed in a growthroom. This is considerably longer than the 24 hours advocated by Hunter *et al.* (40). The cultivar Evans was included in all experiments as a susceptible control. To compare the different experiments, disease ratings (0-5) were expressed as percentages of the rating for Evans in each test. Ten of the 43 cultivars tested had significantly lower ratings than Evans in one trial; the average of three trials resulted in only four cultivars having significantly lower ratings than Evans. They encountered considerable variability in their assay. For example, Corsoy 79, the least susceptible cultivar tested had a percentage disease rating of 6, 62, and 50 in three trials.

The assay of Chun and Lockwood (20) utilized plants grown in a greenhouse for 3-4 weeks without fertilizer. The plants were excised at the soil level, the lateral leaves were removed, and stems were placed on 500 cc of silica sand moistened with 200 ml of distilled water in a tray lined with plastic film. The trays were tilted at 30 degrees from the horizontal to keep the sand moist but not saturated. The stems were inoculated with 5-mm-diameter disks of *S. sclerotiorum* grown on 2% millet seed agar which had been coated with 0.3% water agar to aid in adhesion of the inoculum to the stem. Inoculum was placed on the axil formed by the stem and petiole of the first trifoliate leaf. Trays were covered with plastic wrap and incubated on a

laboratory bench for 6 days. Lesion lengths were measured from the point of inoculation to the end of the water-soaked lesion. Further tests showed that tilting the trays was not required and that moist vermiculite could be used instead of moist sand as the substratum (David Chun, personal communication). Results of this assay showed significant differences among cultivars, but the incidence of infection was very variable.

Attempts to improve the assay of Chun and Lockwood were made by Sneller (73), who tried two modifications, both involving inoculations made on an internode of the excised soybean stem. In one, the inoculation site was rubbed with a chloroform-soaked cotton swab and allowed to dry before inoculation. In the other, nail polish was applied to the inoculation site, allowed to dry, then peeled off. Both inoculation techniques resulted in a high number of stems which escaped infection with resulting increase in variability. High variability was also noted when the inoculum was placed on intact tissue. Sneller tried removing inoculum after various periods of time, as in the limited term inoculation technique of Hunter *et al.*(40), but found no significant relationship between cultivar reaction and duration of inoculation. The concentration of millet seed in the agar on which *S. sclerotiorum* was grown was reduced to 0.3% from the usual 2.0% in an attempt to better discriminate between reactions of susceptible and resistant varieties. However, inoculation with the less concentrated millet

seed agar resulted in many more stems escaping infection. The lesions that developed were no different in length than lesions produced by inoculation with 2.0% millet seed agar. Multiple inoculations were made along the full lengths of stems of 5- to 10-week old soybean plants, at approximately 6 cm intervals. The older the tissue, (i.e., the lower on the stem), the shorter the lesions that developed from inoculations made to sites treated with nail polish which had been stripped off after drying.

This thesis describes studies done to improve the laboratory assay designed by Chun and Lockwood (20). In addition, soybean plant introductions were screened for resistance to stem rot using an improved assay in an attempt to identify resistant germplasm that could be used in breeding. Field trials of commercial soybean varieties grown in a S. sclerotiorum-infested plot were also conducted and disease development in the field was observed.

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PART I. FURTHER DEVELOPMENT OF A LABORATORY ASSAY FOR USE IN SCREENING FOR RESISTANCE, AND FIELD ASSESSMENT OF COMMERCIAL CULTIVARS

Introduction

Sclerotinia stem rot or white mold caused by Sclerotinia sclerotiorum (Lib.) deBary is a sporadically occurring but potentially destructive disease of soybean. In Michigan, soybeans are a major crop, grown frequently in rotation with corn and other graminaceous crops (13). Currently advocated cultivational practices such as planting in narrowly-spaced rows help to create beneath the plant canopy a cool, moist environment which is conducive to the development of the disease (11). The pathogen has a wide host range, many members of which are economically important (21). Stem rot can be prevalent when soybeans are planted on land formerly cropped to susceptible hosts such as dry beans.

Differences in susceptibility of soybeans to stem rot have been demonstrated in the field (5,6,12) and in the greenhouse (4,8,16,17). Evaluation of varietal resistance in the field is extremely laborious and permits only one cycle of evaluations during a growing season. Moreover, disease may be variable (5,11), such that selection of single resistant plants in a breeding program would be impossible. Consequently, interest in developing assays to detect resistance to stem rot using greenhouse- or growth chamber-grown plants has arisen (4,7,8,16,17,22). This thesis describes studies done to improve the laboratory assay designed by Chun and Lockwood (7). In addition, field trials of commercial soybean varieties were conducted in a field infested with S. sclerotiorum.

MATERIALS AND METHODS

The pathogen

Sclerotinia sclerotiorum isolate G from soybean was obtained from W. L. Casale of our department. In 1986, an isolate was obtained from soybeans growing in a field plot at the Michigan State University Plant Pathology farm and was designated isolate L. The two isolates were maintained on millet seed agar (per L: 20 g millet seed ground in a Wiley mill to pass through a sieve with 0.85 mm openings [20-mesh], 20 g agar) in 9-cm-diameter petri dishes, each containing 20 ml agar. The agar plates were inoculated in the center and cultures were grown at 22 ± 2 C for 4-6 (usually 5) days. The inoculum consisted of 5-mm-diameter disks cut with a cork borer from two concentric circles 1-1.5 cm from the edge of the culture. Two experiments were conducted to compare the two isolates.

In some experiments, ascospores were used as inoculum. To produce ascospores, sclerotia were either rinsed in 17 C running tap water for 4 wk or were placed in moist vermiculite at 4 C for 6 wk before being placed on moist sand or vermiculite and incubated at 22±2 C by a north-facing window. Ascospores were collected onto Millipore membrane filters (2 um pore size) with a vacuum apparatus as described previously (23) and were stored at 22±2 C either in petri dishes for short periods of time (1-6 wk) or in vials over silica gel crystals for longer periods.

Soybean plants

Seeds were germinated in moist vermiculite for two days. Uniform seedlings were transplanted into 11-cm-diameter X 14-cm-high plastic pots of 946 cc (32 oz.) capacity, containing a potting mix consisting of a steamed mixture of sandy loam soil, sphagnum peat, and sand (5:3:2, v:v:v) with 3-7 (usually 3) plants per pot. Later, seeds were planted directly into the potting mix. Plants were grown for 3-7 wk in a greenhouse at various times of the year, without fertilizer unless stated otherwise. The temperatures in the greenhouse ranged from 20-35 C, and day lengths were extended to 12 hr in the winter time by the use of fluorescent lamps which were later replaced with high-intensity sodium lamps. Plants were cut off at soil level, put into polyethylene bags, and brought into the laboratory where leaves and growing tips were excised prior to inoculation. The cultivars Corsoy and Evans were used routinely in the development of the method. Corsoy was known to be partially resistant to stem rot in the field (11,12) whereas Evans was regarded as susceptible (12). Later, Weber 84, which was highly susceptible in the field experiments, was substituted for Evans.

Inoculation procedure

Excised stems were laid on 1000 cc vermiculite (2 cm depth) in plastic trays of 26 X 18 X 6 cm dimensions. The trays were first lined with a single layer of plastic film

(Borden Sealwrap, Borden Chemical Co., North Andover, MA), three layers of which also were used to cover the trays to retain moisture. The vermiculite was moistened with 500 ml distilled water, and 7 to 16 stems were arranged parallel to each other in the tray. Stems were inoculated by applying a disk of inoculum at various sites on the stems. In most experiments, inoculum was applied either to the axil of the first trifoliolate leaf or on the cut apex of the stem. In some experiments, inoculation was performed on sites on an internode which had been treated with either chloroform or clear nail polish which had dried then been stripped off. To aid in adhesion, inoculum disks were dipped in 0.3% water agar before being applied to stems. Trays with inoculated stems were incubated on a laboratory bench for 5-10 days at ca. 25±3 C, or in later experiments at 21±1 C.

In an experiment to determine whether inoculations made on cut stem apices of otherwise intact plants would yield results similar to the excised stem assay, intact plants in pots (1/pot) were brought to the laboratory where the top of the plant was excised 30 cm from the soil level leaving 3 or 4 expanded trifoliate leaves on the plant. Colonized agar inoculum was placed on the cut stem surface. A stake was inserted in the soil and a polyethylene bag was placed over the plant and stake and bound around the lip of the pot with tape. In another experiment, the same design was used except that inoculation consisted of placing soybean flowers of unknown genotype which had been sprayed with an ascospore

suspension in the axil of the second trifoliate leaf.

Lesion lengths were measured as the distance from the site of inoculation to the farthest extent of the lesion as determined by scraping the edge of a ruler down the base of the lesion until it was obstructed by healthy tissue. Where more than one stem was used per treatment in a replication, mean lesion lengths in each replication were calculated as the sum of individual lesion lengths divided by the total number of inoculated plants, whether infected or not.

Experimental design

Various designs were employed. In general there were 5-16 stems per treatment, arranged in 3-14 replications, with 1 to 3 (usually 1) trays serving as a replication. Significant differences between means were determined by Tukey's Honestly Significant Difference test following analysis of variance. Experiments were repeated one or more times unless otherwise stated.

Field experiments

During three consecutive years, 1985-1987, 16 soybean cultivars from maturity groups 0-III were evaluated for reaction to Sclerotinia stem rot in a field on the Michigan State University farm. Planting and maintenance of the field were performed, respectively, by Dr. Thomas Isleib and Clifford Zehr. The field had been artificially infested in 1982 by broadcasting screenings containing sclerotia of S. sclerotiorum, which were obtained from elevators used to

store dry edible beans. The field was planted to soybeans in 1983, and in 1984, a test similar to the field tests of 1985-1987 was performed with 20 soybean cultivars.

In each of the three years, 1985-1987, four 4-m (14 ft.) rows of each of 16 cultivars were planted 50 cm (20 in.) apart in each of five replications in a balanced 4 X 4 lattice design, using a different randomization each year. Beginning at flowering, ca. 4 cm water was applied weekly by overhead irrigation, unless otherwise stated. In September, disease was determined in the center two rows of each plot as follows: 0-no disease, 1-infected plant, remaining alive, and 2-infected plant, dead. Since analyses of data showed no differences in rankings of cultivars by disease indices or incidence, the data are expressed as disease indices.

In 1985, planting was done on 20 May, and disease ratings were made on 9 and 10 September.

In 1986, due to late spring rains, planting was delayed until 18 June. The summer was a wet one, with rainfall occurring approximately weekly. In addition to the 16 commercial varieties, 10 soybean plant introductions (PI's) were planted in the infested field in a randomized complete block design with two replications. Disease ratings were made on 23 and 24 September.

Another plot removed from the infested field was planted on 18 June 1986 to the 16 commercial varieties and 10 PI's using 20-foot rows of each. This latter field received no irrigation. It was weeded manually in mid-July.

Plants grown in the uninfested field were brought into the laboratory periodically and assayed for stem rot resistance by the excised stem method.

Visual inspections for presence of apothecia and disease development were made throughout the summer of 1986 at approximately weekly intervals. Plates of exposed Sclerotinia-selective medium (3) were placed for 3 and 4 hours during the afternoons of 8 and 18 August at six locations in the infested field, 50 cm above the ground, facing west. Both days were clear with moderate westerly winds. The plates were brought into the laboratory, wrapped with Parafilm (American Can Company, Greenwich, CT) and inspected for development of S. sclerotiorum.

In 1987, planting was done on 15 May. Irrigation was initiated soon after emergence of the seedlings. Since no apothecia were found by visual inspection the previous year, sclerotia were placed at 18 sites in the field at marked locations so that apothecial development could be monitored at these known locations as well as in the rest of the field during the biweekly field monitoring. On 6 June, sclerotia from three sources were placed in styrofoam cups which had the bottoms removed and were buried at different depths in the infested field. The three sources of sclerotia were as follows: 1) sclerotia which had overwintered in soybean debris in the infested field were collected on 20 April 1987 and stored in the laboratory at 22±2 C, 2) sclerotia were grown aseptically on green beans in the laboratory and did

not receive a cold treatment, 3) sclerotia were collected from millet seed agar cultures of various ages which had not received a cold treatment. Disease ratings were made on 3 and 4 September. The styrofoam cups containing the soil and sclerotia were gathered and brought back to the laboratory where the sclerotia were recovered and observed for physical damage.

Upon observation in the field that some stems were considerably thicker than others, an unrepeated experiment was conducted comparing 3-field-grown plants with thin (2-4-mm-diameter) stems and thick stems (6-10-mm-diameter).

Data were analyzed by analysis of variance and differences between means were distinguished by Tukey's Honestly Significant Difference or Fisher's Protected Least Significant Difference tests.

RESULTS

Isolate of fungus

Isolates G and L induced lesion lengths which were not significantly different (Table 1). Thus, after November 1986, isolate L was used routinely. Since there were ongoing attempts to correlate the laboratory results with field results, it was considered advantageous that the isolate used in the laboratory tests be one obtained from the infested field.

Table 1. Effect of two isolates of Sclerotinia sclerotiorum used as inoculum on lesion development in the excised-stem laboratory assay[a].

Cultivar	Mean lesion length (cm)[b] Isolate G	Isolate L
Corsoy	7.5 a	6.4 a
Weber 84	7.8 a	6.8 a
Hodgson 78	10.4 b	10.3 b

[a]Plants were grown in the greenhouse for 6 wk. The stems were arranged in a randomized complete block design with three replications and two stems of each treatment per replication. Plants were fertilized with 0.6 g 20-20-20 NPK 2 and 4 wk after planting.

[b]Lesion lengths followed by the same number were not significantly different by Tukey's Honestly Significant Difference test ($P=0.05$). There was no interaction between cultivar and isolate of fungus by analysis of variance.

Ascospores as inoculum

An unrepeated experiment was designed to compare two types of inocula: mycelium-colonized agar and ascospores. Either a 5-mm-diameter plug of colonized millet seed agar or a 10 ul drop of water containing a suspension of 5500-6000 ascospores was applied to the cut end of 7-wk-old stems of four varieties of soybean (Table 2). The resulting lesion lengthsfrom the two inoculation techniques werestrongly correlated ($r=0.97$), and lesions originating from the two kinds of inocula were comparable. However, in a later experiment 100 ul drops containing 300 ascospores each in a 0.1 M sucrose solution applied to cut ends or internodes largely failed to infect. The lack of infection in the

latter experiment was probably because the larger drops tended to roll off the inoculation sites and because the inoculum density used was lower. The first experiment indicated that the lesions originating from ascospores are comparable to those incited by colonized agar. Because of the ease of producing and handling mycelium-colonized agar, and the precedent set by Chun and Lockwood (7), agar inoculum was used routinely.

Table 2. Comparison of mycelium-colonized agar and ascospores in a water suspension as inocula in the excised stem assay[a]

	Average lesion length (cm)[b]	
Cultivar	Agar	Ascospores
Corsoy	6.3 ab	4.2 a
Weber 84	11.2 abcd	7.2 abc
Hodgson 78	15.2 cd	12.7 bcd
Gnome	16.3 d	15.0 cd

[a]Plants were arranged in a completely randomized design with 6 observations per treatment. Plants were fertilized three times with 0.6 g 20-20-20 NPK 2, 4, and 6 wk after planting.

[b]Lesion lengths followed by the same letter do not differ by Tukey's Honestly Significant Difference test ($P=0.05$). There is no interaction between form of inoculum and variety of soybean by analysis of variance.

Handling of plants

Intervals of 1 or 4 hr between excision and inoculation of stems, or keeping excised stems on ice or at ambient temperature, did not influence subsequent disease development. Therefore, cut stems were kept at ambient temperature

and were inoculated within 2 hr of cutting. In an unrepeated experiment, stems of six soybean varieties were excised at the base, placed in polyethylene bags and put in the coldroom at 4 C for 5 days prior to being assayed. Resulting lesion lengths were similar to those encountered when there was no time delay before inoculation. Despite this evidence that refrigerating or keeping plants on ice before assaying caused no difference in the resulting assay, plants were routinely assayed within 2 hr after excision in the greenhouse.

Stems usually were incubated under the ambient light of the laboratory. In some instances, trays were covered with aluminum foil for 3 days to prevent upturning of the stem tips, which sometimes dislodged inoculum disks, then were incubated in the ambient light of the laboratory.

Age of plants

Lesion development was compared in stems of Corsoy and Weber 84 plants 3, 4, 5, 6 and 7 wk old. These ages corresponded, respectively, to growth stages V2, V3, V4, V5-V6 and V7-R1 (9). Plants were fertilized by applying 0.6 g 20-20-20 (NPK) to the soil in each pot once weekly after the plants reached 2 wk old. Stems were inoculated at the cut apices and were incubated at 21±1 C. In Corsoy, lesion lengths tended to decrease in older plants, whereas in Weber 84, only the lesions in 7-wk-old plants were shorter than those in younger plants (Figure 1). Differences between the

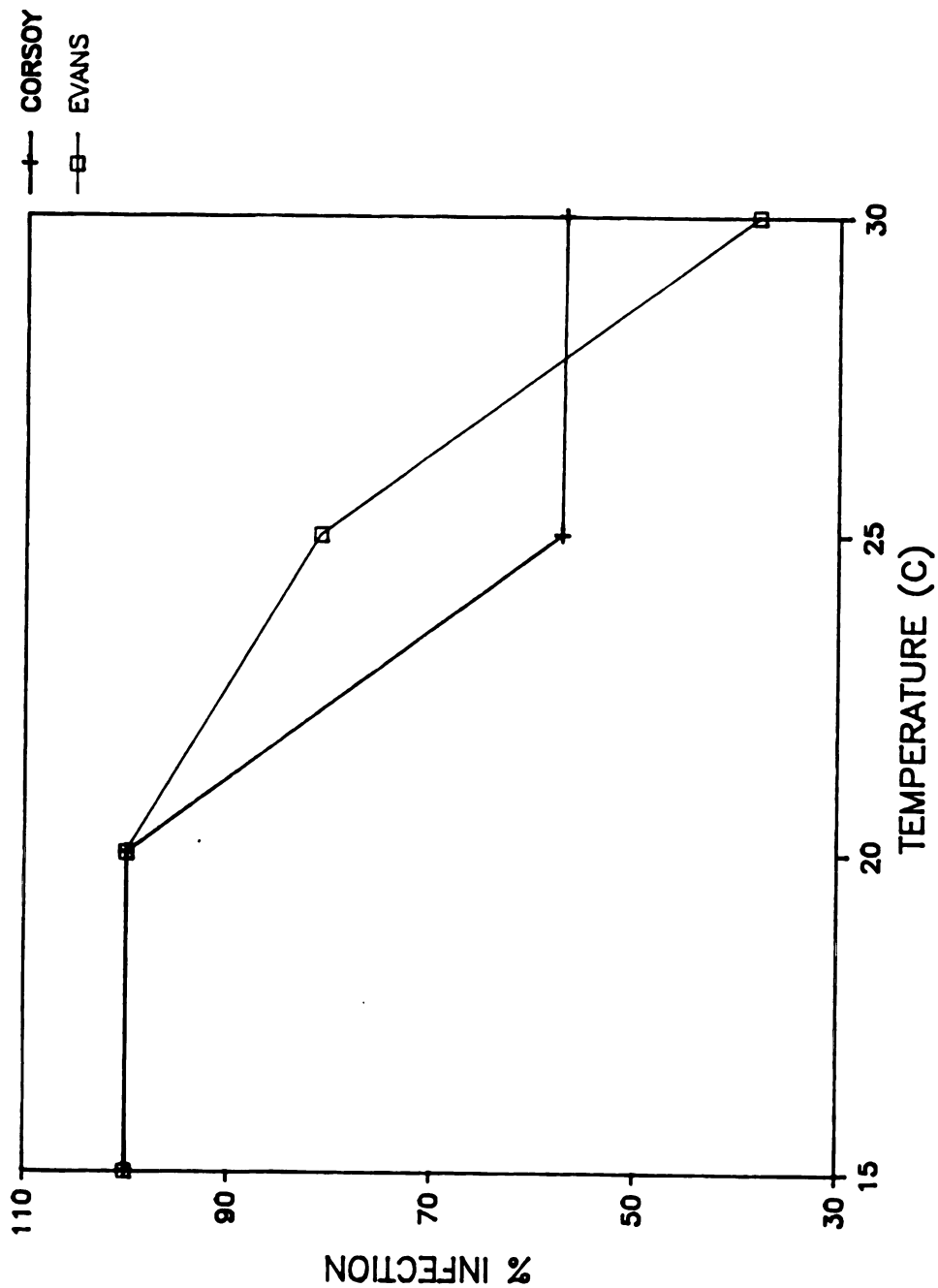

Figure 1. Effect of age of soybean cultivars Corsoy and Wever 84 on the development of Sclerotinia stem rot on excised stems in the laboratory.

two cultivars were greatest in plants 5, 6 or 7 wk old (P = 0.05) (Table 3).

Table 3. Effect of age of soybean cultivars Corsoy and Weber 84 on the development of Sclerotinia stem rot on excised stems in the laboratory[a]

Plantage (wk)	Lesionlength (cm)[b]	
	Corsoy	Weber 84
3	9.3 ab	10.3 a
4	6.7 bcd	8.3 abc
5	5.8 cd	10.3 a
6	4.7 de	9.9 a
7	2.4 e	6.2 bcd

[a] Stems (2 per treatment per cultivar in each of 7 replications) were inoculated at excised apices with mycelial inoculum disks.

[b] Means followed by the same letter did not differ by Tukey's Honestly Significant Difference test (P=0.05). There was no significant interaction between age of plant and cultivar of soybean.

Supplementary fertilizer

During some of the assays, plants grown for longer than 4 wk showed symptoms of nitrogen deficiency. Several experiments indicated that the use of fertilizer to correct the deficiency resulted in reduced mean lesion lengths. For example, 0.6 g fertilizer (20-20-20, NPK) was applied to the soil surface of pots in which four cultivars were growing weekly, after the second wk. Stems from 5-wk-old plants were inoculated at cut tips and incubated at 21±1C. Mean lesion length for stems of fertilized plants was 3.8 cm and

that for unfertilized plants was 7.6 cm. These differences were statistically significant ($P = 0.05$).

Site of inoculation

The axil of the first trifoliolate leaf and the cut stem apex were compared as inoculation sites, using stems from 4- and 5-wk-old plants of Corsoy and Evans, and an inoculation temperature of 21±1 C. Inoculation of cut terminals gave longer lesions and more infected stems than did inoculation at the leaf axils of plants at either age (Table 4).

Table 4. Effect of inoculation site on development of lesions in the laboratory assay[a]

	Corsoy		Evans	
Inoculation site	Lesion length, cm	% infection	Lesion length, cm	% infection
Leaf axil	3.8	58	3.7	67
Cut apex	9.6	100	12.6	100

[a]Stems were arranged in a completely randomized design with 12 stems per treatment.

Based on the field observation that leaves and petioles of some cultivars were colonized by S. sclerotiorum, but that the fungus often failed to invade stem of the plant, and that a reddish-brown resistant reaction was often seen where the colonized petiole met the main stem, experiments were conducted in which 4-5 cm of petiole of the first

trifoliate leaf was left on the excised stem and agar inoculum was place at the end of the petiole. Inoculation on cut apices was also included as a treatment. The lesion length resulting from inoculation of a petiole tip was a reflection of petiole length rather than of resistance of the main stem to invasion (Table 5). Thus there was no advantage to detecting differences between cultivars by this technique over inoculation of the cut stem apices.

Table 5 . Comparison of inoculation of 4-cm-long petioles attached to excised stems vs. cut apices of stems[a]

	Lesion length (cm)	
Cultivar	Inoculated on cut apex	Inoculated on cut end of petiole
Corsoy	9.0 a	4.2 b
Weber 84	10.9 a	4.9 b
Beeson 80	10.0 a	4.3 b

[a]Stems were arranged in a randomized complete block design with five replications, with three plants of each treatment per replication. There is no interaction between variety of soybean and inoculation site.

Application of inoculum to treated internodes

In an attempt to reproduce the findings of Sneller (22), the internodes between the first and second trifoliate leaf of soybean stems were inoculated after having been either swabbed with chloroform or treated with nail polish which was allowed to dry, then removed.

Table 6 gives the results of an experiment representative of five experiments done to test the

different inoculation techniques. Six cultivars were employed. Corsoy and Hodgson 78 are considered to be resistant varieties; Ozzie is susceptible in laboratory assays, but escapes disease in the field; Elgin is moderately susceptible; Gnome and Weber 84 are highly susceptible. The average lesion lengths were generally longest and incidence of infection was 100% when inoculations were made on cut stem apices. With the nail polish treatment, incidence of infection also approached 100%; lesions were usually slightly shorter than with cut end inoculation. Inoculation on chloroform-treated sites resulted in highly variable infection incidence which was reflected in the average lesion lengths, as well.

Table 6. Comparison of inoculation on cut apices, chloroform-treated internodes or nail polish-treated internodes on development of lesions and percentage infection of stems in the excised stem assay[a]

	Lesion length (cm)				Percentage infection			
Cultivar	cut[b]	chlor.[c]	nail pol.[d]		cut[b]	chlor.[c]	nail pol.[d]	
Corsoy	9.8	3.5	8.1		100	66.7	100.0	
Ozzie	9.9	2.2	3.8		100	50.0	66.7	
Elgin	9.5	4.1	6.7		100	83.3	100.0	
Hodgson 78	13.0	4.5	7.4		100	100.0	100.0	
Gnome	11.3	5.3	8.5		100	83.3	100.0	
Weber 84	10.3	4.5	6.4		100	40.0	100.0	

[a]There was no interaction between cultivar and inoculation method by analysis of variance.

[b]Inoculation on cut stem apices.

[c]Inoculation on chloroform-treated internodes.

[d]Inoculation on internode treated with nail polish which was allowed to dry, then was peeled off.

In one test,a treatment in which the internodes were rubbed with a dry cotton swab was included as a control lest breakage of trichomes due to rubbing was complicating the chloroform-treatment inoculation technique (data not shown). Incidence of infection with this treatment was extremely low (25%) and lesions tended to be short (4 out of 6 were less than 1 cm long). The results obtained with this treatment were similar to those obtained when inoculations were made on an untreated internode. Since the results indicated that any mechanical damage resulting from applying chloroform with a cotton swab did not result in increased disease, the dry cotton swab treatment was left out of subsequent chloroform-treatment experiments.

In another test, inoculum was placed on a shallow cut made with a razor blade on an internode. Resulting average lesion lengths for Corsoy, Ozzie, Elgin and Hodgson 78 were 2.1, 6.9, 14.5, and 5.7 cm. The lengths of the lesions were similar to those initiated at sites of nail polish application and removal.

Temperature of incubation

To assess the effect of incubation temperature on disease development, stems of cultivars Corsoy and Evans were incubated at 15, 20, 25 and 30 C in the dark and on the laboratory bench at 25±3 C in ambient laboratory light. Plants were 4 wk old and were inoculated at cut ends. Stems incubated at 15 and 20 C had longer mean lesion lengths and a greater proportion of infected stems than those incubated

at 25 and 30 C (Figures 2 and 3, Table 7). Stems in trays incubated on the laboratory bench had the least disease of all.

In an experiment in which the lesion length was measured every 24 h, it was noted that once infection was established on a stem, lesion development progressed at a similar rate for the cultivar regardless of temperature (data not shown). Since lesions appeared earliest at 20 C, this accounted for the longer lesions at that temperature.

In a second experiment, inoculated stems were incubated at temperatures of 20, 22, 24 and 27 C (Table 8). Percentages of plants infected and lesion lengths again were greatest at 20 and 22 C. Henceforth, stems for all experiments were incubated on a laboratory bench at 21±1 C.

Flower color

Isolines of Harosoy were assayed by two methods to determine whether there was a relationship between the ability of S. sclerotiorum to invade plants which bore flowers of different color. The lines tested and the results of the two tests are shown in Table 9. In the first experiment, inoculum was placed on the cut apices of the stems. In the second experiment, the inoculation sites were internodes between the first and second trifoliate leaves which were rubbed with a chloroform-soaked cotton swab and allowed to dry before inoculum was applied. Differences in

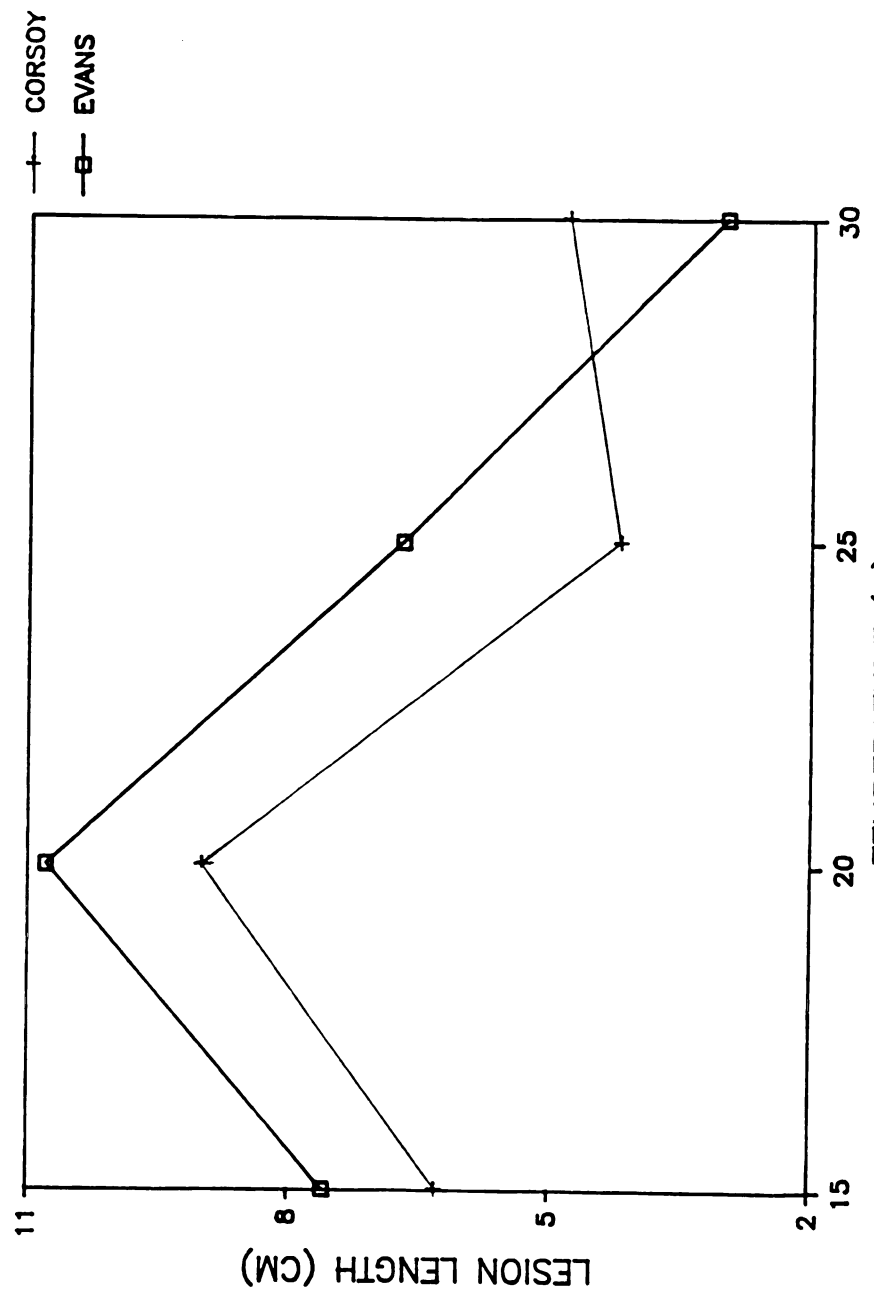

Figure 2. Effect of temperature on lesion length development of Sclerotinia stem rot in excised stems of soybean cultivars Corsoy and Evans

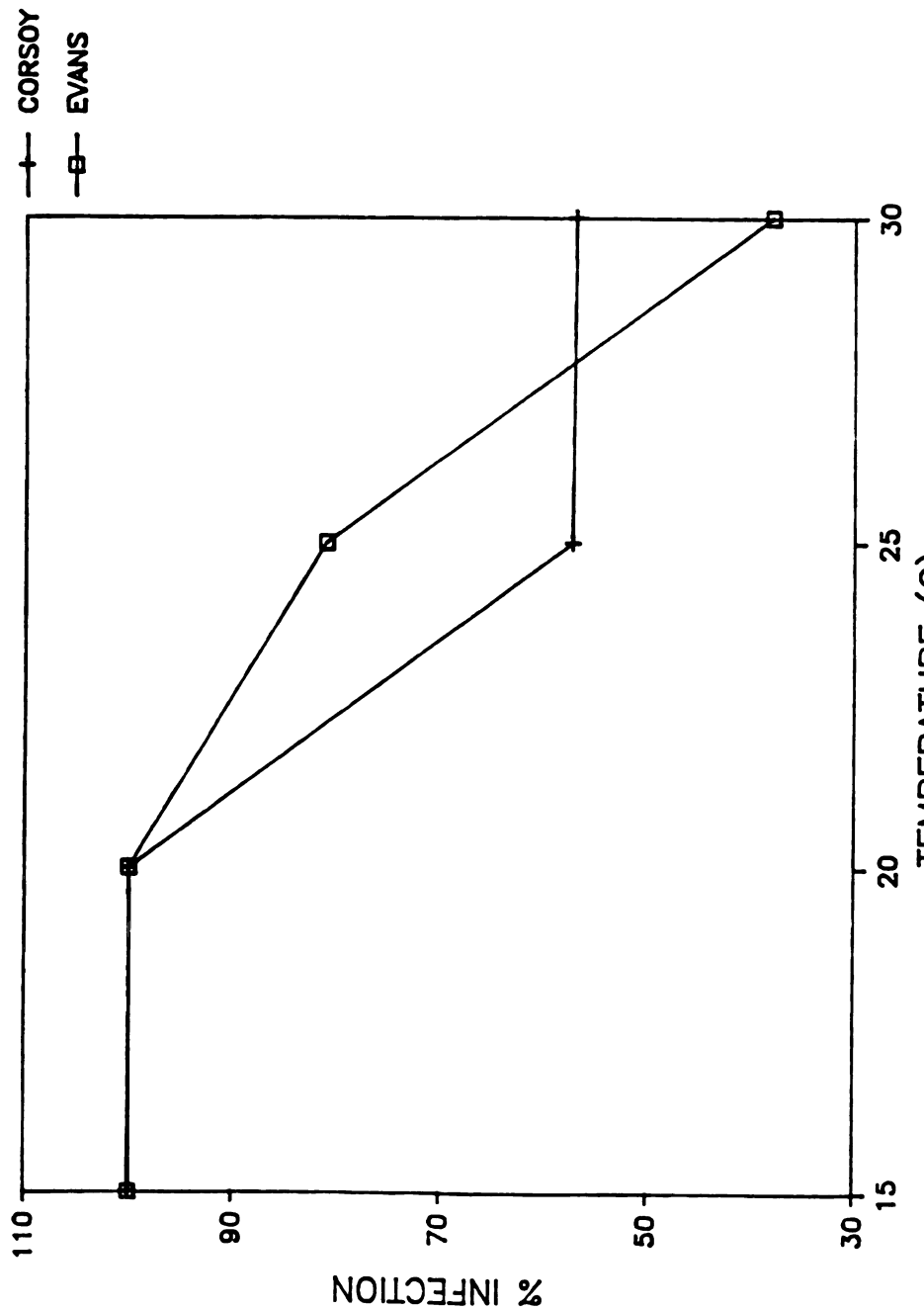

Figure 3. Effect of temperature on percent infection of excised stems of soybean cultivars Corsoy and Evans in the laboratory assay for detecting resistance to Sclerotinia sclerotiorum.

Table 7. Effect of temperature on development of Sclerotinia stem rot in excised stems of soybean cultivars Corsoy and Evans[a]

Temperature (C)	Lesion length (cm)[b]		Infected plants (%)[b]	
	Corsoy	Evans	Corsoy	Evans
15	6.3 ab	7.6 ab	100 a	100 a
20	9.0 a	10.8 a	100 a	100 a
25	4.2 bc	6.7 bc	57.3 b	81.0 a
30	4.8 bc	3.0 cd	57.0 b	38.0 b
Room [c]	1.1 c	1.8 d	14.3 c	23.7 b

[a]Stems were arranged with 7 stems per treatment in each of 3 replications.

[b]Means in a column followed by the same letter did not differ by Tukey's Honestly Significant Difference test ($P=0.05$). There was no significant interaction between variety and temperature of incubation.

[c]25±3 C.

Table 8. Effect of temperature on development of Sclerotinia stem rot in excised stem of soybean cultivars Corsoy and Evans[a]

Temperature (C)	Lesion length (cm)		Infected plants (%)	
	Corsoy	Evans	Corsoy	Evans
20	2.7 bcd	6.5 a	42 bc	72 ab
22	4.4 abc	6.0 ab	89 a	89 a
24	0.9 d	3.0 cd	11 cd	31 cd
27	0.3 d	0.0 d	6 cd	0 d

[a]There were 3 stems per treatment in each of 6 replications. There was no significant interaction between variety and temperature of incubation.

lesion lengths were not detected in either experiment (Table 9). Variability was considerably higher using the chloroform-treatment inoculation technique. Flower color was not related to stem reaction.

Table 9. Harosoy isolines used to determine effect of flower color on resistance of soybean to Sclerotinia sclerotiorum

M.S.U. Acc. #[a]	Illinois Acc. #[b]	Mutant gene	Phenotype	Test 1[c]	Test 2[d]
I83096	Harosoy-L_2	none	Purple	8.4	6.7
I83184	L62-0906	w_1	White	8.2	5.0
I83186	L72-1138	w_4	Near white	7.7	7.9
I83185	L72-1078	W_3w_4	Purple throat	9.8	6.3
I83187	T235	wm	Magenta	9.9	4.0
I83188	L63-1612	$wmRps_1$	Magenta	9.2	5.1
I83097	L61-5047	Rps_1rxp	Purple	8.8	4.3

[a]Michigan State University Accession Number

[b]Illinois Accession number

[c]Stems of 5-wk-old plants were arranged in a randomized complete block design with 5 replications, with 3 stems of each isoline per replication. Plants were fertilized with 1.5 g 12-12-12 NPK 2 and 4 wk after planting.

[d]Stems of 6-wk-old plants were arranged in a randomized complete block design with 14 replications, one plant of each isoline per replication.

Assay with potted plants

An experiment was done to determine whether inoculations made on cut stem apices of otherwise intact plants would yield results similar to the excised stem assay and perhaps be more closely related to results of field

testing. Potted plants which had their tops excised 30 cm above soil level were inoculated on the cut apex, then were covered with a polyethylene bag. Lesion lengths were significantly correlated (r=0.59, P=0.05) with the 1985 field disease indices but not with the 1984 field data (6) (r=.27) (Table 10). A variant of this experiment in which soybean flowers of unknown genotype were sprayed with ascospores and placed in the axils of the second trifoliate leaves gave no lesions.

Table 10. Mean lesion lengths resulting from cutapex inoculation of potted soybean plants covered with a polyethylene bag and incubated on a laboratory bench for 8 days[a]

Cultivar	Average lesion length (cm)[b]	Cultivar	Average lesion length (cm)[b]
Hardin	5.7 c	Century	9.5 abc
Corsoy 79	6.3 bc	Hobbit	10.2 abc
Corsoy	6.3 bc	Beeson 80	10.6 abc
Ozzie	7.4 bc	Gnome	10.7 abc
Wells II	8.5 abc	Pella	11.0 abc
Hodgson 78	8.6 abc	Weber 84	11.2 ab
Sprite	9.1 abc	Nebsoy	13.3 a
Evans	9.3 abc		

[a]Plants were arranged in a completely randomized design with 4 replications. Plants were fertilized with 1.5 g 12-12-12 NPK 2 and 4 wk after planting.

[b]Lesion lengths followed by the same letter are not significantly different by Tukey's Honestly Significant Difference test (P=0.05).

Commercial cultivars

At various stages in the development of the laboratory method, commercial cultivars of soybean were assayed for their reaction to stem rot. Statistically significant

differences among cultivars usually occurred, but cultivar rankings were not always consistent from one experiment to another (Table 11). In spite of variability among experiments, Corsoy frequently was one of the most resistant of the various cultivars in the laboratory assay.

Commercial cultivars were evaluated for stem rot reaction in an infested field in 1985, 1986, and 1987. The most susceptible cultivars were Gnome, Weber 84 and Hobbit, with 40.4-52.4% of plants infected. Nebsoy, Sprite, Century, Wells II, Elgin and Beeson 80 were intermediate with 18.0-29.3% of plants diseased. Evans, Corsoy, Corsoy 79, Hardin, Pella, Hodgson 78, and Ozzie had the least disease, with 0-12.0 % disease incidence.

In 1986, disease development in the infested field was poor, with disease incidence ranging from 0.0% to 0.5% in individual plots. No apothecia were observed throughout the growing season. No ascospores were trapped on the selective medium. Symptoms were not observed until 4 September. Close monitoring of three plots every 2 or 3 days after that date until 15 September revealed extremely little plant-to-plant spread.

In 1987, no apothecia were observed growing from the sclerotia which were buried in styrofoam cups throughout the field or elsewhere. Upon examination of the sclerotia in the laboratory, no visible damage to them was seen. Disease incidence was again low, but was higher than in 1986. Average disease indices among the varieties ranged from 0 to

Table 11. Reactions of soybean cultivars to Sclerotinia stem rot in laboratory assays and in field tests in 1984[a] and 1985.

Cultivar	Maturity group	Lesion lengths in laboratory assays(cm) Test 1[bc]	Test 2[cd]	Disease incidence (%) in field experiments 1984[ace]	1985[ce]
Gnome	II	7.6	9.4	39.5	52.4
Weber 84	I	8.4	7.5	31.1	48.5
Sprite	III	1.8	8.4	-	28.8
Century	II	7.5	7.3	19.1	28.1
Elgin	II	1.5	8.5	15.5	21.2
Vickery	II	-	-	13.8	-
Amsoy 71	II	-	-	13.7	-
Amcor	II	-	-	13.4	-
Corsoy	II	6.8	3.3	-	7.9
Corsoy 79	II	5.8	3.0	13.3	5.3
Lakota	I		-	13.3	-
Beeson 80	II	1.9	8.7	12.6	18.0
Harcor	II	-	-	12.4	-
Wells II	II	7.9	7.0	11.9	23.1
Nebsoy	II	9.5	10.8	10.0	29.3
Hodgson 78	I	1.9	7.9	6.8	2.8
Pella	III	4.8	6.7	6.7	3.8
Simpson	0	-	-	6.1	-
Hardin	I	5.9	2.7	5.7	7.1
Hobbit	III	5.9	9.5	4.1	40.4
Dawson	0	-	-	2.8	-
Evans	0	7.0	5.2	2.2	12.0
Ozzie	0	5.3	6.7	_	0.0
LSD		3.0	2.1	12.4	13.6

[a]Data of Chun et al. (6).

[b]Data are based on 3.5-wk-old excised stems (9/cultivar) inoculated at leaf axils and incubated at 25 C.

[c]Correlation coefficients: test 1 X test 2 = 0.08, 1984 X 1985 = 0.72, test 1 X 1984 = 0.25, test 1 X 1985 = 0.31, test 2 X 1984 = 0.28, test 2 X 1985 = 0.61.

[d]Data are based on 5.5-wk-old excised stems (9/cultivar) inoculated at cut terminals and incubated at 20 C.

[e]Percentage of diseased plants in two, 5-m rows in each of three replications (1984), or two, 4-m rows in each of 5 replications (1985). LSD values are Fisher's Protected LSD (P=0.05).

11.7% and the variability was extremely high (coefficient of variation = 191.3%). However, the ranking of the 16 cultivars was similar to the ranking of 1985, (r=0.69), especially at the extremes. Ozzie and Hodgson 78 had the least disease in both of these years and Gnome had the most disease.

There was no apparent relationship of maturity groups I, II, or III with disease reaction. In 1985, these maturity groups were represented among the cultivars with greatest and least disease.

Thirteen of the cultivars tested in 1985 had been tested the previous year in the same plot. The disease reactions in the two tests were significantly correlated (r=0.72, $\underline{P}$=0.05). The greatest discrepancy occurred with Hobbit, which had 4.1% infected plants in 1984 and 40.4% in 1985. When Hobbit was excluded, the correlation coefficient for the remaining 12 cultivars was 0.89. The reason for the large discrepancy with this cultivar is unknown.

Attempts were made to verify the results obtained by the laboratory assay by comparing these data with disease incidences obtained with commercial cultivars in the field. Ten assays performed in the laboratory using greenhouse-grown plants of the 16 field-tested cultivars in 1985 had varying correlations with the 1984 and 1985 field results, ranging from r=-0.17 to r=0.63. Two examples of results from assays performed with greenhouse-grown plants are given in Table 11.

On 28 September, 1985, healthy green stems of 10 cultivars that had not yet senesced were brought from the field and assayed by stem inoculation in the laboratory. The results showed a significant (P=0.05) correlation with the field data (r=0.68). This result prompted the planting of the commercial varieties in a non-infested field to provide plant material for laboratory assays. Six times during the summer of 1986 and once in 1987, plants from the uninfested field plot were brought into the laboratory and assayed. Correlation coefficients ranging from -0.25 to 0.54 resulted from comparisons of the laboratory assays with disease ratings of the field experiments in 1984 and 1985 (Table 12). There also was a wide range of correlation coefficients among the laboratory tests, from -0.48 to 0.90. Not all varieties were tested at each sampling date, hence the varying number of comparisons upon which the correlation coefficients are based.

Thickness of stem

Field-grown plants with thick (6-10-mm-diameter) stems tended to have shorter lesions than did plants with thin (2-4-mm-diameter) stems. For example, when thick stems were assayed by inoculation on cut ends, the resulting average lesion length for Corsoy and Weber 84, respectively, were 2.0 and 10.3 cm; for thin stems, the corresponding values were 4.1 and 16.0 cm. Since lesion lengths of thin and thick stems tended to differ, in any given assay, stems of like diameter should be used when possible.

Table 12. Correlation coefficients between disease incidences of 16 commercial varieties tested in an infested field and lesion lengths obtained in laboratory assays of the same varieties grown in a non-infested field

Correlation coefficient between experiments at dates cited								
		Date of collection of plants and assay						
		1986						1987
	Field 1985	8-1	8-23	8-28	9-4	9-12	9-19	7-17[c]
Field 1984[a]	.72 (13)[b]	.07 (11)	.43 (6)	.04 (8)	.25 (7)	.25 (7)	.05 (12)	.01 (13)
Field 1985		-.25 (13)	.47 (8)	-.07 (9)	.31 (9)	.54 (9)	.13 (14)	.12 (16)
8-1-86			-.30 (8)	.27 (9)	.34 (9)	.16 (9)	.23 (13)	-.35 (15)
8-23-86				.90 (5)	.54 (6)	.40 (6)	-.09 (8)	.14 (10)
8-28-86					.52 (8)	-.15 (8)	-.48 (8)	.27 (10)
9-4-86						.72 (9)	.34 (9)	.04 (10)
9-12-86							.71 (9)	-.41 (10)
9-19-86								-.18 (16)

[a]Data for Field 84 are from Chun et al (6).

[b]Numbers in parentheses denote the number of varieties compared in calculating the correlation coefficient.

DISCUSSION

The method for determination of soybean reaction to S. sclerotiorum as described by Chun and Lockwood (7) was an improvement over field testing in that plants could be grown in the greenhouse and assayed throughout the year. Low reliability of disease development in the field was also avoided. The assay had the advantage of not requiring a mist chamber or a growth chamber with high (90-98%) relative humidity as some assays have (1,2,4). The assays of Boland and Hall (4) and Abawi et al. (1,2) require that plants be left in a mist chamber for 2-6 days and one week, respectively. In such assays, space in the mist chamber can limit the quantity of plants assayed.

The use of mycelium-colonized agar as inoculum has advantages of ease of production and uniformity that have also been noted by Madjid et al (18). These workers tried three forms of inocula: sclerotia, mycelium-colonized agar blocks, and a mycelial-agar suspension applied as a spray, in their development of a greenhouse assay for use in screening lettuce for resistance to S. sclerotiorum. Sclerotia failed to infect. There was no significant difference between the use of agar blocks or mycelial suspensions as inoculum; both were effective. However, the mycelial-agar suspension required undefined optimal environmental conditions which were not always available, thus making colonized agar blocks the preferable inoculum. Some other assays require laborious procedures for the production of inoculum (1,2,4). For example, Boland and Hall (4) have

a multi-step procedure in which cultures are transferred to three different media over a 16-day period with the end result being inoculum consisting of colonized green beans which are wrapped around soybean stems. Use of ascospores requires production of apothecia, a process that requires a conditioning period in a cold environment that has been variously reported to be from 1 to 20 wk long (20). A recently published protocol for inducing apothecia from sclerotia requires 15 wk and includes a cold treatment and exposure to near-ultraviolet light before sclerotia form fertile apothecia (19). Ascospore use also requires making a microscopic count of ascospores and adjusting the inoculum density.

Despite the innate advantages of a laboratory assay which uses agar inoculum and has no special equipment requirement, the Chun and Lockwood (7) assay was not completely satisfactory because of the number of stems escaping infection and because of variability due to asynchronous initiation of lesion development. These problems limited the ability to distinguish differences among treatments or cultivars. In my work, these disadvantages were overcome to some extent by standardizing such factors as age and stem-diameter of plants, but variability was reduced most by placing the inoculum on cut stem apices rather than at the leaf axils, and by reducing the incubation temperature after inoculation from 25 ± 3 C to 21 ± 1 C (Table 7). With these changes, infection was usually close to 100% and variability

in lesion lengths was also reduced. Results using plants of different ages suggested that differences in cultivar response may be greatest in plants at least 5 wk old.

A remaining problem is that of reproducibility of results. Work with single plant families indicated that variability of lesion lengths resulting from inoculation was not due to genetic heterogeneity within a cultivar (22). Lack of reproducibility may be due to varying environmental conditions in the greenhouse. Cline and Jacobsen (8) made the observation that disease severity resulting from their limited-term inoculation method was affected by the light intensity under which plants were grown, with etiolated tissue being more susceptible than non-etiolated tissue. Although no experiments were done to quantify the phenomenon in the current research, it was observed that when the fluorescent lamps were replaced by high-intensity sodium lamps, the plants were shorter, developed shorter lesions, and gave less variable results than previously. However, in the course of the year, conditions within the greenhouse were still quite variable: in the summer, the lamps are turned off and white-wash is applied to the exterior of the greenhouse to reduce the temperature and light intensity, while in the winter, the days are lengthened with artificial lighting and the average temperature is lower than during the summer. It is not yet known what growing conditions will produce plants whose reaction to stem rot most closely mimics that in the field.

In comparing the response of a number of cultivars in the field with stem lesions of plants grown in the greenhouse, correlation coefficients ranged from r=-0.53 to r=0.86. Correlations are not enhanced by such cultivars as Ozzie which had no disease in the field in 1985, yet is fully susceptible by the laboratory assay. Boland and Hall (5) encountered results similar to those reported here in comparing field trials and greenhouse evaluations. Correlation coefficients for cultivars common to both kinds of test ranged from 0.03 to 0.41 and were not significant. Discouragingly, my work shows that plants grown in the field also do not respond to the laboratory assay in a manner consistently reflective of their disease reaction in the field. It would intuitively seem that assays using stems from the field would be more highly correlated with field results than would stems from greenhouse-grown plants. The results of the original laboratory assay using field-grown material showed a significant correlation coefficient with field results (r=0.68, $\underline{P}$=0.05). However, subsequent assays never reproduced this phenomenon. In fact, the tests did not always correlate well with each other. There was a tendency for high correlations between assays done in successive weeks (Table 12) such as in the last four tests done in 1986, which gave correlation coefficients of r=0.90, 0.52, 0.72, and 0.71. However, correlation coefficients between tests which were performed after a greater time interval than one week were usually not significantly correlated.

Besides the work done at Michigan State University, two other groups have done field-testing of soybean cultivars in Sclerotinia-infested fields in recent years (5,11,12). Results in one year were never identical to those of another year. Sometimes a researcher has identified the tendency of a cultivar's reaction to the pathogen, such as "moderately susceptible" or "resistant", but later work has not confirmed such placement. For example, Grau et al.(10,12) found the cultivar Evans to be moderately susceptible in one field study. Based on this report, the original work done to improve the assay of Chun and Lockwood (7) included Evans as the susceptible control. Then, two years of results from the field at Michigan State indicated that Evans was among the more resistant cultivars, so it was replaced by Weber 84 in experiments done to study factors influencing experimental variability. Boland and Hall (4) also worked extensively with Evans, using it as the standard against which all other cultivars were judged in their growthroom studies. However, in their field trials in 1981, 1982, and 1984, Evans had 62.4, 23.1, and 7.5% diseased plants, respectively. Moreover, Evans was not consistent in disease ranking relative to other cultivars that they tested. Other cultivars that they tested in two or three field trials also gave contradictory results: some were among the most susceptible one year and among the most resistant another year. This phenomenon was also noted in the cultivar Hobbit in field tests done at Michigan State University (6, this thesis).

The problem of inconsistent disease reactions of cultivars in field tests is complicated by the sporadic nature of disease development from year to year. In tests done three years in a row in an infested field in Wisconsin, no disease developed during the second year (11). That study was concerned with the effects of irrigation on disease development, and its general finding was that the greater the amount of water, the greater the disease development. During the second year, the optimally irrigated plots received more natural precipitation and irrigation water than in the other two years, so "the absence of Sclerotinia stem rot...was not expected" (11). Similarly, the lack of disease development in the Michigan State plot during the wet summer of 1986 was surprising. Boland and Hall (5) had a general decrease in disease incidence in the three years for which they provide data. Maximum percentages of diseased plants were 82.0, 56.8, and 22.4% in 1981, 1982, and 1984, respectively. In studies at Michigan State (6,this thesis), the maximum disease indices for 1984-1987 were, respectively, 39.5, 52.4, 0.5, and 11.7%. These results and those of Boland and Hall (5) illustrate the dilemma of whether field data should even be considered valid if disease is below a certain level. The tendency has been to regard the data as insignificant in years in which disease is low. Thus, field testing is rife with unresolved problems, as has been shown with variable disease development in Wisconsin (11,12), Ontario (5), and Michigan State

University (6,this thesis).

There is the possibility that a microbiological or physical factor unfavorable to S. sclerotiorum develops in the test plots repeatedly cropped to soybean, but this would not explain a high incidence of disease development following a year in which there was none (11). The field- and laboratory-produced sclerotia that were placed in the infested field as part of the studies reported in this thesis did not appear visibly damaged when they were recovered in the fall. This would be evidence against the build-up of a microbiological component that was causing the demise of sclerotia.

Another problem with the field studies done to date is that different researchers test only cultivars grown in their region and they frequently do not use the same set of cultivars in their tests each year. This results in little overlap in the cultivars tested by different research groups. Even when common cultivars were tested, results were sometimes discrepant, as has already been illustrated in the case of Evans. However, in some instances, results with common cultivars have been similar. As an example, field tests from Michigan State (6,this thesis) agree with those of Grau et al. (12) in that Corsoy and Hodgson 78 were among the more resistant cultivars, whereas Gnome was highly susceptible. Ideally, the laboratory assay should be compared with the disease reactions of a set of cultivars with a wide range of reactions to the pathogen, based on

many years of field testing done at many locations. Currently, due to the sporadic nature of the disease and varying choice of cultivars tested by the different researchers, this body of knowledge is unavailable.

Another possible reason for the frequent lack of correlation between field and laboratory results is that an assay based on the rate of lesion expansion in the excised stems may by-pass other components of resistance that may be operating in the field. The inoculum in field conditions is presumed to be ascospores which infect the flowers; from this initial infection, the pathogen invades the soybean stem (8,10,12). Therefore, components of resistance may also reside in the flowers, or in the cuticle or wax layers on the stem.

The treatments of Sneller (22) using chloroform or nail polish treatment of stems were repeated in my work to examine additional components of resistance. Sneller mistakenly believed that treatment with chloroform removed the cuticle. Treatment with chloroform dissolves epicuticular waxes, but not the cuticle (M. Bukovac, R. Hammerschmidt, personal communications). Sneller also claimed that the nail polish treatment removed cuticular wax only. However, this treatment strips off the numerous trichomes on the stem, resulting in holes that could serve as portals of entry directly into the plant epidermal cells. (M. Bukovac, personal communication). In any case, both Sneller's and my work showed that treatment of stem internodes with chloroform or nail polish resulted in increased infection over

that on untreated internodes, which largely failed to develop any lesions upon inoculation. The nail polish technique yielded results similar to those obtained when the cut end was inoculated or when the epidermis and possibly some underlying cortex cells of the stem were removed with a shallow cut of a razor blade. However, variability was higher in both of the alternative inoculation techniques, and they were more labor-intensive as well. Thus, inoculation on the cut apices was found to be preferable to either of the alternative inoculation techniques.

Flower color was proposed as another possible factor in resistance of soybean plants to infection by S. sclerotiorum, based on the observation by Grau (12) that soybean cultivars with white flowers were relatively susceptible while some cultivars with purple flowers were less so. Two groups have reported exceptions to this proposal (4,8). Work reported here with Harosoy isolines indicates that the stem tissue of plants differing only by genes that regulate flower color does not vary significantly in its ability to support fungal colonization.

It is perhaps noteworthy that Corsoy frequently has been the most resistant of the cultivars tested in the laboratory as well as demonstrating relatively little disease in field tests reported in this thesis and in studies of others (11,12). Corsoy (8) and the closely related cultivar, Corsoy 79 (4), also were relatively resistant in growth chamber tests.

Whether a consistently high correlation between field and laboratory reactions of plants to stem rot can be achieved is not known. Boland and Hall (5) identified cultivar height, lodging severity, and maturity as factors which affected disease development in one or more of their three years of field trials. They rightly say, "the identification of cultivar characteristics that affect the responses of soybean cultivars to disease under field conditions increases the difficulty of identifying sources of field resistance to this pathogen in the growth room". This, of course, applies to laboratory assays as well. Despite this, a cultivar or introduction in which a lesion is severely limited after stem inoculation, would seem likely to be highly resistant to the disease.

PART II. SCREENING OF SOYBEAN INTRODUCTIONS FOR STEM ROT RESISTANCE

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary causes stem rot or white mold, a minor but potentially destructive disease of soybean (*Glycine max*) (L.) Merrill (10). Control of this pathogen through cultural practices such as planting in widely spaced rows (11) can help reduce disease in wet years when it occurs, but if environmental conditions are unsuitable for disease development, then the loss in yield due to wide row spacing would be for naught. Chemical control of diseases has not been found to be economically feasible for Michigan soybean growers (13). Control of diseases of low-value field crops such as corn and soybean is best done by planting resistant cultivars. Cultivars resistant to the major soybean disease in Michigan, Phytophthora root rot, are available and utilized (13). Breeding for resistance to stem rot of soybean has not historically been employed. However, in the early 1980's, interest in this pathogen increased because of increasing incidence of stem rot, due to planting in narrowly spaced rows and to soybeans being planted in fields formerly cropped to other susceptible crops (6). Researchers working with dry bean developed a growth chamber assay which they used to screen plant introductions of *Phaseolus* spp. in order to identify lines that could be used for breeding resistance to white mold of bean caused by *S. sclerotiorum* (14,15). This "limited-term inoculation procedure" was adapted for use in soybean by Cline and Jacobsen (8), and

was reported to be a "feasible" method for evaluating soybean cultivars for resistance to stem rot. However, this group has not pursued screening. Lockwood and Isleib (16,17) became interested in developing an assay for evaluating soybean reaction to stem rot using greenhouse-grown plants. A method using excised stems incubated on moist sand in trays in the laboratory was developed (7) and used to screen 50 plant introductions (17). Working independently, a group at Guelph, Ontario has further adapted the limited-term inoculation assay to evaluate 43 soybean cultivars for resistance to S. sclerotiorum (4).

The preceding portion of the thesis described further development of the laboratory assay described by Chun and Lockwood (6). In this section, screening of plant introductions (PI's) obtained from the United States Department of Agriculture (U.S.D.A.) Soybean Germplasm Collection located at Urbana, Illinois for resistance to stem rot is described. This work was funded by the Soybean Promotion Committee of Michigan and is still in progress.

Materials and methods

S. sclerotiorum isolate L was obtained from soybean on the Michigan State University Plant Pathology farm. The fungus was maintained on 2% millet seed agar as described previously (this thesis). Agar disks were used as inoculum. Disks (5-mm diameter) were cut with a cork borer from two or three concentric circles 1-2 cm from the edge of 4-6-day-old cultures.

Soybean seeds were planted in 11-cm-diameter X 14-cm-high plastic pots of 946 cc (32 oz.) capacity containing a potting mix consisting of a steamed mixture of sandy loam soil, sphagnum peat, and sand (5:3:2, v:v:v). Four plants of each PI were sown in each of four pots. One week after planting, plants were thinned to three per pot. Plants were grown for 6 wk; 1.5 g of 12-12-12 NPK fertilizer in a granular formulation was applied to the surface of the soil approximately 1, 3, and 5 wk after planting. During August through November 1987, the plants in pots on the perimeter of the greenhouse bench were attached to stakes to prevent their falling over and blocking the greenhouse aisles. Day length was extended to 12 hr with high-intensity sodium lamps or fluorescent lights during January through May 1987 and late-September to the present. During mid-May through late-September, artificial lights were turned off, and during this time, white-wash shading was applied to the exterior of the greenhouse to reduce the temperature and light intensity. The temperature in the greenhouse varied from 20 to 35 C.

Plants were cut off at the soil level, placed in polyethylene bags and transported to the laboratory where leaves and the tops of the plants were excised, providing stem sections consisting of the bottom 25-30 cm of the plant. Excised stems were laid parallel to the short axis of plastic trays of 54 X 26 X 6 cm dimensions on 2000 cc vermiculite moistened with 1 L distilled water (1.5 cm depth). Stems were inoculated by applying a disk of agar

inoculum to the cut surface at the stem apices. Disks were coated with 0.3% water agar to aid their adhesion. The trays were covered with three layers of plastic film (Borden Sealwrap, Borden Chemical Co., North Andover, MA), one layer of which was also used to line the tray before addition of vermiculite. The trays were incubated on a laboratory bench at 21±1 C for 6-9 (usually 7) days before rating. Resistance was determined by measuring the length of the lesion from the site of inoculation to its farthest extent as determined by scraping the edge of a ruler down the the lesion toward the stem base until it was obstructed by healthy tissue.

The experimental design consisted of a randomized complete block design. There were usually eight replications, with one plant of each PI per replication, but the 60 most recently assayed PI's had only four replications each. Data were analyzed by Tukey's Honestly Significant Difference test following analysis of variance. The cultivar Corsoy, which has been shown to be a resistant cultivar in both growthroom (8) and field (6,12) studies, was included in all experiments.

Results

Fifty-six experiments have been done to date. Seven hundred and ninety-eight PI's have been screened (Table 1). Average lesion lengths of the PI's were expressed as percentages of the average lesion length of Corsoy for ease of comparing among experiments (Figure 1). Six categories were

Table 1. Plant introductions (PI) screened for resistance to stem rot by the excised stem assay.

PI #	Maturity Group	PI #	Maturity Group	PI #	Maturity Group
19.986	IV	54.859	II	68.427	II
30.594	II	862	II	430	II
599	II	865	I	436	II
600	II	873	II	439	II
36.653	I	55.089-1	III	443	II
47.131	II	887	IV	446	II
54.583	III	56.563	IV	448	II
591	III	57.334	III	449	IV
592	III	58.955	IV	454	II
600	IV	59.849	IV	455	II
54.604	II	60.269-2	IV	68.457	II
606-1	IV	272	III	457-1	II
606-2	IV	279	II	461	II
607	II	296-1	II	461-1	II
608	II	296-2	III	465	II
608-1	II	970	IV	465-1	II
608-2	III	61.940	III	466	II
608-3	III	944	IV	470	III
608-4	IV	947	IV	474	II
608-5	III	62.199	IV	474-2	I
54.609	III	62.202	III	68.475	II
610-1	III	202-2	IV	475-1	II
610-4	IV	248	IV	479	III
613	III	483	III	479-1	III
614	IV	63.271	I	480	II
615	III	468	IV	481	II
615-1	III	945	IV	483	III
615-2	IV	64.698	IV	484-1	II
617	IV	747	IV	484-4	II
618	III	65.338	II	488	II
54.619	II	65.341	II	68.494	III
620	III	346	II	500	II
620-2	III	354	II	503	II
809	I	379	III	508	II
818	II	388	II	516	II
834	I	68.011	IV	521	II
853	I	398	III	521-1	III
854	I	410	II	522	II
855	00	421	II	523	III
857	I	423	III	526	II

Table 1 (cont'd.).

PI #	Maturity Group	PI #	Maturity Group	PI #	Maturity Group
68.528	III	68.670-1	II	68.761-3	III
530	II	670-2	II	762	II
530-2	III	671	II	763	II
533-1	III	676	II	765	II
533-2	III	679	III	768	IV
535	III	679-2	IV	770	I
543	II	680	II	778	II
551-2	II	680-2	II	788	II
551-3	I	683	II	795	II
554	I	685	II	806	III
68.555	II	68.687	II	68.815	II
560	III	692	IV	69.500	II
562	II	692-2	III	501	II
564	II	694	II	503	II
572	I	696	II	507	I
576	I	701	III	507-1	IV
585	II	704	II	512	II
586	I	706	II	515	III
587	II	708	II	532	II
598	II	709	II	533	I
68.599	III	68.710	III	69.991	II
600	II	712	II	992	II
604-1	III	713	II	993	III
604-2	I	715	II	995	III
609A	III	718	II	996	II
609B	II	722	0	70.001	III
610	I	725	II	009	II
621	III	728	II	013	IV
622	II	729	II	014	III
627	II	731	III	016	I
68.629	II	68.732	II	70.017	I
639	II	732-1	III	019	III
642	II	736	II	021	II
644	IV	741	II	70.023	III
648	III	746	I	70.027	I
655	II	748	II	036	II
658	II	748-1	III	076	III
661	II	756	III	077	II
663	II	759	III	078	II
666	II	761	II	080	III

Table 1 (cont'd.).

PI #	Maturity Group	PI #	Maturity Group	PI #	Maturity Group
70.084	II	70.471	III	78.243	I
087	I	473	III	79.583	III
089	II	473-1	I	586	II
70.091	II	476	II	587	III
188	III	478	II	593	II
189	III	485	I	596	II
192	III	490	IV	602	II
197	II	495	II	609	II
199	III	500	III	610	I
201	III	501	III	613	II
70.202	III	70.503	II	79.616	III
208	IV	507	II	617	I
212	III	515	III	620	III
70.213	III	516	II	627	III
224	II	519	III	628	III
228	II	520	I	645	III
229	IV	528	III	648	I
241	I	541	III	691	III
242	II	559	III	691-4	III
242-2	IV	561	II	692	III
70.242-4	0	70.566	III	79.693	III
243	IV	71.161	I	694	I
247	III	444	IV	695	II
70.251	II	463	IV	696	IV
253	III	506	IV	699	I
453	II	845	III	703	II
456	II	850	II	710	III
457	II	850-1	III	712	II
458	II	72.227	IV	726	III
459	II	232	III	727	I
70.460	II	72.328	II	79.732-3	IV
461	II	337	II	732-4	IV
462	III	341	II	737	II
463	II	342	II	739	0
466-3	IV	73.583	III	743	IV
466-4	III	585	II	745	II
467	IV	587	II	746	II
469	III	772	II	747	II
469-1	III	780	II	756	II
470	III	78.242	II	760	III

Table 1 (cont'd.).

PI #	Maturity Group	PI #	Maturity Group	PI #	Maturity Group
79.761	II	80.828-2	IV	81.764	IV
773	II	831	III	765	I
797	III	834-1	IV	766	III
825-1	IV	834-2	IV	767	II
835	III	837	IV	768	II
846	II	841	III	770	II
848	II	844-2	III	771	II
848-1	III	844-3	III	772	I
862-1	II	845-1	III	773	II
863	II	845-2	III	775	I
79.870-1	I	80.847-1	III	81.777	IV
870-2	III	847-2	IV	780	III
870-4	IV	81.023	IV	785	III
870-6	IV	027	III	971	II
872	III	029-1	IV	82.183	I
874	III	029N	II	184	II
874-1	III	030	IV	210	IV
885	II	030-1	III	218	IV
80.459	III	031-1	III	232	III
461	III	031-2	III	235	III
80.466-1	III	81.033	I	82.246	IV
466-2	IV	034-1	IV	246-1	III
469	II	034-2	IV	259	IV
470	III	035	II	263-1	IV
471	II	037-1	IV	263-2	II
471-1	III	037-2	III	263-3	II
473	IV	037-3	III	264	IV
479	IV	037-4	I	278	III
480	III	037-5	IV	291	IV
481	III	038	III	295	IV
80.485	II	81.040	I	82.296	IV
488	IV	041	III	302	III
488-1	II	041-1	III	307	IV
494	II	042-1	IV	308	III
498-1	IV	042-2	IV	312N	IV
536	II	044-1	III	315	IV
671	II	044-2	III	325	IV
822	III	667	III	326	IV
825	III	761	III	509	IV
828-1	IV	763	II	527	IV

Table 1 (cont'd.).

PI #	Maturity Group	PI #	Maturity Group	PI #	Maturity Group
82.532	II	84.964	I	87.065	II
534	IV	965	II	524	II
544	IV	992	II	531	I
554	IV	85.012	II	619-1	II
555	IV	014	II	628	II
558	IV	021	II	631	II
581	IV	340	II	88.288	I
83.853	IV	492	II	293	II
858	IV	508	II	293A	II
868	IV	625	II	294	II
83.881	IV	85.671	II	88.294-1	II
881A	IV	86.002	II	295	I
889	IV	021	I	295-1	II
891	IV	022	II	296	II
892	IV	023	II	298	II
893	IV	031	II	301	II
915	IV	038	II	303	II
923	IV	045	II	304	II
925	IV	046	II	307	II
945-3	I	050	II	308	II
84.580	II	86.069	II	88.309	II
609	II	089	II	311	II
637	II	102	II	313	II
665	II	112	II	351	II
666-1	II	113	II	352	II
668	I	115	II	355	II
668-1	II	122	II	356	II
673	II	133	I	357	II
673-1	II	137-1	II	358	II
674	I	410	I	442	II
84.681	II	86.411	I	88.443	I
683	II	416	I	455	II
683A	II	443	II	468	II
686	I	454	II	479	II
750	II	463	II	484	I
810	I	737	I	495	II
896	II	741	II	497	I
921	II	878	II	508	II
928	II	878-2	II	777	II
954	II	972-1	II	787	II

Table 1 (cont'd.).

PI #	Maturity Group	PI #	Maturity Group	PI #	Maturity Group
88.797	I	89.156	II	91.732-1	I
798	II	167	II	732-2	I
803	II	170	II	733	I
804	I	171	II	92.109	II
805-2	I	90.180	II	460	II
805-4	II	560	II	464	II
810	II	567	I	465	II
825	II	570	II	468	I
997	II	91.091	II	469	I
89.000	II	102	II	470	I
89.001	0	91.104	II	92.561	II
003-1	II	107	II	563	II
004	II	109	II	565	I
005-5	II	110	I	569	II
006-2	II	110-1	I	570	II
008	II	114	II	571	II
012	II	115	II	572	II
013	II	116	II	573	II
014	II	117	II	576	II
053	II	119	II	580	II
89.055	I	91.120	II	92.582	II
055-1	II	120-2	II	583	II
056-3	I	123	I	589	II
057	I	124	II	592	II
058	I	126	II	595	II
059	II	129	II	596	II
060	I	132-2	II	598	II
061-1	I	138	II	603	II
063	II	141	II	607	II
064	II	144	II	611	II
89.065	II	91.150	II	92.625	I
065-2	II	156	II	627	II
070	II	161	II	629	II
072	II	164	II	630	II
073	II	167	II	633	II
075	II	171	II	639	II
138	II	180	II	649	I
153	II	557	II	660	II
154	II	559	II	661	II
154-1	II	725-3	II	671	II

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Table 1 (cont'd.).

PI #	Maturity Group	PI #	Maturity Group	PI #	Maturity Group
92.677	II	132.205	0	153.233	0
681	II	206	I	234	00
683	II	207	0	235	0
684	II	214	00	236	I
687	II	215	I	237	0
694	II	217	00	240	0
696	II	135.589	II	247	I
698	II	590	II	249	0
705	II	142.491	I	250	I
706	I	151.249	00	252	00
92.717	II	152.361	0	153.255	I
719	II	573	00	261	0
733	II	153.203	00	263	I
734	II	208	00	264	II
748	II	209	0	265	0
93.217	II	210	00	270	0
560	II	211	00	273	0
565	II	212	00	274	I
96.152	I	213	0		
171	II	214	I		
96.188	II	153.215	I		
193	I	217	00		
195	II	218	00		
201	II	219	00		
97.605	II	221	00		
103.414	II	222	00		
131.531	I	225	00		
132.201	I	226	II		
203	00	229	I		
204	0	230	00		

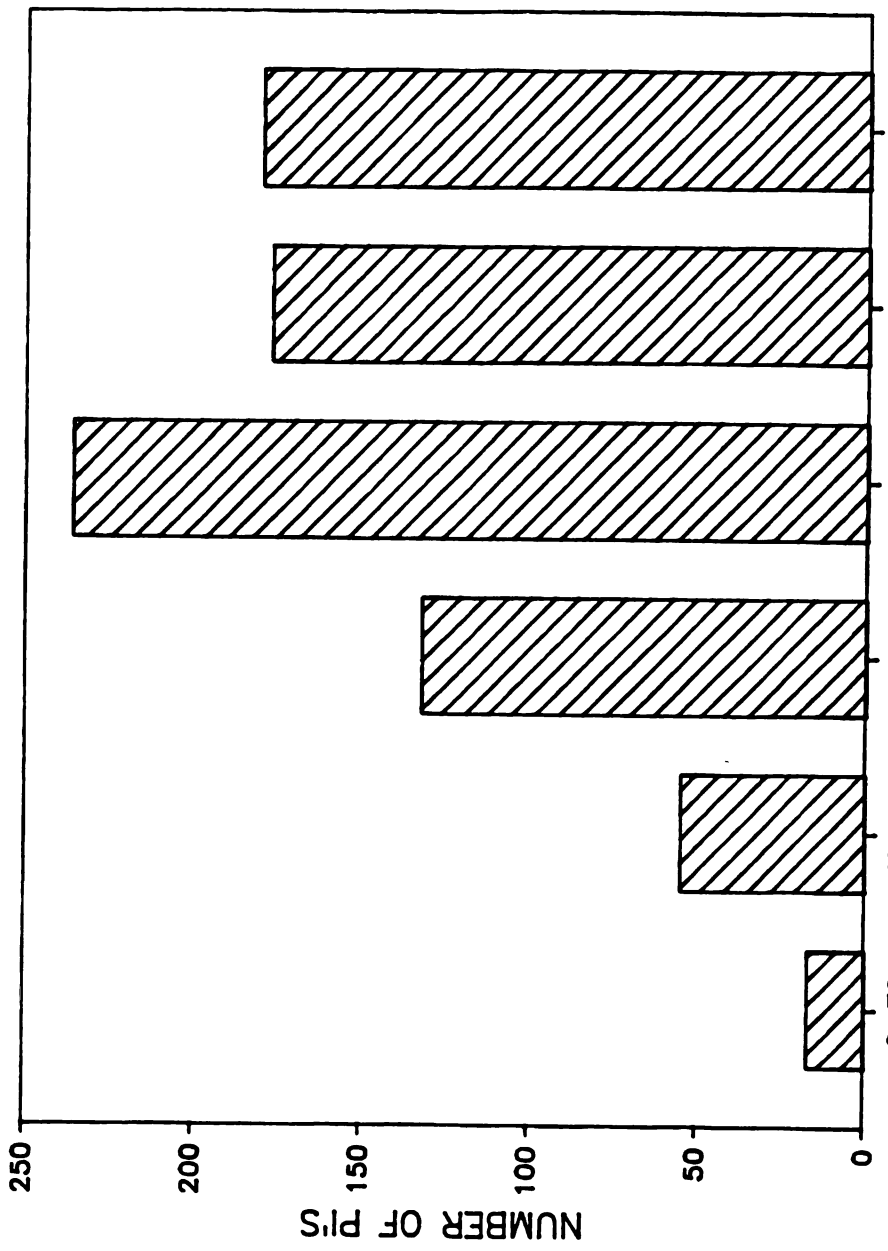

Figure 1. Frequency distribution of results of screening of soybean plant introductions (PI'S) for resistance to stem rot caused by S. sclerotiorum using the excised stem laboratory assay.

arbitrarily chosen: <70%, 70-90%, 90-110%, 110-130%, 130-160%, and >160% of the average lesion length of Corsoy. Seventeen cultivars had average lesion lengths less than 70% of those of Corsoy (Table 2). Of these, six were significantly different from Corsoy.

Table 2. Plant introductions with average lesion lengths 70% or less of the average lesion length of Corsoy

54.600	68.708 *	80.218
54.604	70.077	84.666 *
54.606-2	70.243 *	84.750 *
54.608-2	70.485	84.625
54.608-3	79.737	86.021
54.855 *	80.459 *	

* Denotes significantly different than Corsoy by Tukey's Honestly Significant Difference test (P=0.05).

The vast majority of PI's had longer average lesion lengths than Corsoy. Corsoy consistently had the lowest or one of the lowest average lesion lengths, although its average lesion length varied from 1.7 to 14.7 cm (average = 8.5) in different experiments. One hundred and fifty cultivars have been identified for further assaying either because they had short lesion lengths or because the experiment in which they were performed had a high coefficient of variability, making retesting desirable. About half of these have been retested with varied reproducibility of results. Table 3 shows representative results of retesting, with data expressed as average lesion lengths and percentages of the lesion length of Corsoy. In

many instances, resistance was confirmed in subsquent testing.

Table 3. Reaction of representative plant introductions to S. sclerotiorum in two laboratory tests

	Test 1		Test 2	
PI number	Ave. lesion length (cm)	% of Corsoy	Ave. lesion length (cm)	% of Corsoy
68.768	11.9	81	8.6	223
79.699	8.5	107	11.5	107
79.732-4	5.7	72	10.4	96
79.870-6	8.2	99	8.4	77
80.459	6.2	76	5.5	51
80.841	7.5	90	9.8	92
81.035	3.0	60	7.9	107
81.761	3.0	62	10.5	143
82.555	5.8	298	9.9	126

Discussion

Corsoy was chosen as a resistant control due to its resistance in tests done in the field (12) and laboratory tests (6,8,this thesis). There was variability in the average lesion length of Corsoy from experiment to experiment as can be seen in Table 3. For example, PI 68.768 had a lesion length of 11.9 cm in the first test, which corresponded to 81% of Corsoy's lesion length. In a second test, PI 68.768 had a shorter average lesion length, 8.6 cm, but was 223% of that of Corsoy which had an extremely short lesion. Conversely, PI 82.555 had a lesion length 298% of that of Corsoy in the first test, despite having a short average lesion length (5.8 cm). A second test resulted in longer lesion lengths for both Corsoy and

PI 82.555, so that an average lesion length of 9.9 cm for PI 82.555 was only 126% of that of Corsoy. These two examples are extreme; usually Corsoy was not so widely varying in its reaction in the assay. In fact, the author became more convinced of the validity of the laboratory assay due to Corsoy's repeatedly having one of the shortest lesion lengths in all but a very few tests. In the exceptions, Corsoy was intermediate among the PI's tested. PI's were liberally selected for repeated testing either because they gave reactions comparable to or better than Corsoy or because the validity of the experiment was in doubt due to Corsoy's giving an intermediate reaction.

There are over 7,000 soybean PI's in the U.S.D.A. Soybean Germplasm Collection. These accessions were obtained from all over the world, particularly from eastern Asia, where the soybean was first domesticated. The PI's represent a wide range of genotypes. Seeds are of widely varying sizes, shapes, and colors, and produce plants varying widely in morphology. Of the over 800 PI's subjected to the laboratory assay so far, several have been more prohibitive to the growth of S. sclerotiorum through the stem tissue than Corsoy, the resistant control. These PI's require further testing in the laboratory, and more practically, in the field. If the resistant reaction is reproducible, these PI's can be used as sources of resistance in a breeding program. Originally, the first 500 PI's were requested and tested in numerical order, so that PI's from maturity groups 00-IV were assayed. At the

suggestion of the president of the Soybean Promotion Committee of Michigan, the next 400 PI's included only maturity groups 00, 0, I, and II, which represent only those adapted to Michigan. If genes for resistance are identified by this assay, chances of transferring these genes to cultivars that can be grown in Michigan are enhanced.

During the screening to date, several PI's have been identified which limit the ability of S. sclerotiorum to grow through the stem tissue. Although further testing is needed, PI's identified as being resistant in the excised stem assay can be used in breeding for resistance to stem rot. Given the range of reactions shown thus far (Figure 1), it is likely that resistance is polygenic. To increase resistance, it should be possible to combine genes through crossing lines which show resistance.

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