GARZONIO, DENNIS MICHAEL

POD AND STEM BLIGHT OF SOYBEANS: THE RELATIVE IMPORTANCE OF SEED-BORNE AND SOIL-BORNE INOCULUM

Iowa State University PH.D. 1981

Pod and stem blight of soybeans: The relative importance of seed-borne and soil-borne inoculum

by

Dennis Michael Garzonio

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Plant Pathology, Seed and Weed Sciences
Major: Plant Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1981

TABLE OF CONTENTS

INTRODUCTION

Pod and stem blight, caused by fungi in the *Phomopsis-Diaporthe* complex, is responsible for serious losses in soybean seed quality in all soybean seed production areas of the world. Soybean plants become infected by the pathogen during the growing season, and, under certain weather conditions, severe seed infection may occur. Previous workers have suggested that infested soybean crop residues are a major source of *Phomopsis* inoculum (Athow and Caldwell, 1954; Gerdemann, 1954; Hildebrand, 1956; Lehman, 1923; Kmetz et al., 1979). Evidence for this is based on the fact that the disease has been shown to be more severe in fields which have been in a continuous soybean rotation than in those with corn in the rotational history (Kmetz, 1975). There is little experimental evidence, however, to directly link soil- or crop residue-borne inoculum to plant infection. There is likewise very little information on the importance of seed-borne inoculum with respect to pathogen transmission. In laboratory germination tests, *Phomopsis* has been shown to grow on germinated seedlings (Kulik and Schoen, 1981; Wallen and Cuddy, 1960), but it is not known whether the pathogen can be transmitted to emerged seedlings under field conditions. *Phomopsis* infested nonviable seeds also may contribute significant sources of inoculum when planted in the field.

The major objectives of this study are to demonstrate transmission of *Phomopsis* from seed- and soil-borne sources of inoculum, to determine the relative importance of seed- and soil-borne inoculum with respect to development of the disease in the field, and to develop methods to quantify soil-borne inoculum.

LITERATURE REVIEW

General Disease Aspects

Causal organism

Pod and stem blight of soybeans (*Glycine max* (L.) Merr.) was first reported in North Carolina in 1920 and referred to as *Phoma* blight (Wolf and Lehman, 1920). The disease was renamed pod and stem blight in 1922, and Lehman (1922) assigned *Phomopsis sojae* as the causal agent. Lehman (1923) then described a species of *Diaporthe* as the causal agent and named it *Diaporthe sojae* Lehman. Perithecia were observed only in culture until Wolf and Lehman (1926) reported them on overwintered stems. Wehmeyer (1933), in his monograph of the genus *Diaporthe*, reassigned *D. sojae* and *D. batatatis* Harter and Field, the causal organism of dry rot of sweet potato (Harter and Field, 1912), to varietal status under the type species *D. phaseolorum* (Cke. and Ell.) Sacc., the causal organism of pod blight of lima bean (Harter, 1917). Petrak and Sydow (1936) recorded *Phomopsis glycines* Petrak on soybean in Japan in 1936. Welch and Gilman (1948) reported both heterothallic development, characterized by scattered single perithecia and production of pycnidia on soybean stems, and homothallic development, characterized by caespitose clusters of perithecia and the lack of pycnidial production. The heterothallic form was recognized by these authors as *D. phaseolorum* var. *sojae* (Lehm.) Wehm. (Dps) and the homothallic form as a strain of *D. phaseolorum* var. *batatatis* (Harter and Field) Wehm. (Dpb). The latter variety aggressively attacked soybean stems, resulting in stem girdling and death. Stem girdling had previously been thought to be one of the symptoms of pod and stem blight. Crall (1950) suggested the use

of the term stem canker to differentiate the symptoms caused by Dpb from those caused by Dps. Athow and Caldwell (1954) then suggested that the stem canker organism was morphologically different from Dpb and proposed another variety of *D. phaseolorum* with the trinomial *D. phaseolorum* var. *caulivora* Athow and Caldwell (Dpc). The validity of the separation of Dps and Dpc has since been questioned (Threiner et al., 1959; Whitehead, 1966). More recently, Kmetz et al. (1974, 1975, 1978) suggested that, in addition to Dps and Dpc, an undescribed *Phomopsis* sp. was associated with pod and stem blight symptoms. These three fungi can be distinguished by culture characteristics (Kmetz et al., 1975). *Phomopsis* sp., however, produces only pycnidia in culture and no perithecia (Kmetz et al., 1975). Production of only *Phomopsis*-type pycnidia in culture, by isolates obtained from soybean plants with pod and stem blight symptoms, has been reported by several other workers (Athow and Caldwell, 1954; Gerdemann, 1954; Hildebrand, 1954; Lehman, 1923; Luttrell, 1947; Nicholson et al., 1972). Kmetz et al. (1978) suggest that pod and seed infection may be independent of stem infection and that the seed decay phase of the disease be named Phomopsis seed decay. They justify this name because *Phomopsis* sp. is the more prevalent of the three seed-borne organisms and both Dps and Dpc have *Phomopsis* asexual stages (Kmetz et al., 1978).

For purposes of this dissertation, *Phomopsis* sp. will be used to refer to that component of the complex and *Phomopsis* will be used to refer collectively to *Phomopsis* sp. and Dps.

Host range and distribution

Phomopsis has been associated with seed of pigeon pea (Ellis et al., 1978a) and mung bean (Nath et al., 1970), dead stem, pod, or leaf tissue of cowpea (Shanor and Taylor, 1944), soybean (Lehman, 1923), snap beans, lima bean, peanut, lupine, lespedeza, *Strophostyles helvola* (L.) Ell., okra, onion, and garlic (Luttrell, 1947), stem canker of *Abutilon theophrasti* Medic. (Hepperly et al., 1980), birdsfoot-trefoil (Whitehead, 1966), and soybean (Dunleavy, 1957), and fruit rot of tomato and pepper (Luttrell, 1947). In addition, it also has been isolated from healthy green soybean tissue prior to plant maturity (Kmetz et al., 1975).

Since the first report of the pathogen on soybeans in North Carolina (Wolf and Lehman, 1920), *Phomopsis* has been reported from all of the major soybean growing regions in the United States (Andrews, 1950; Bretz, 1944; Chamberlain and Gray, 1974; Crall, 1950, Fenne, 1949; Gardner, 1928; King, 1948; Larsh, 1944a, 1944b; Lehman, 1923; Nicholson et al., 1972; Pady, 1944; Petty, 1943; Peterson and Strelecki, 1965; Walker, 1944; Weimer, 1947) and throughout the world in Argentina (Atlas de Gotuzz, 1970), Brazil (Do Amaral, 1951), Cameroon (Bernaux, 1979), Canada (Koch and Hildebrand, 1944), China (Liu, 1948), Colombia (Patino, 1967), Guyana (Martyn, 1933), Hungary (Ersek, 1978), India (Nath et al., 1970), Italy (Mannerucci and Gambogi, 1978), Japan (Kurata, 1960), Korea (Sasaki, 1929), Malawi (Siddiqi, 1971), Puerto Rico (Ellis et al., 1978a), Rumania (Hulea et al., 1973), Senegal (Girard, 1979), Tanzania (Anonymous, 1976), and the USSR (Shoshiashvili, 1940).

Symptoms and economic importance

Pod and stem blight of soybeans is characterized by the production of linear rows of pycnidia on soybean stems and random pycnidia production on pods, petioles, and, infrequently, on leaves. These signs of the pathogen, however, are only observed on senescent tissues. Severe seed infection is characterized by shriveled seeds either partially or totally covered by white mycelium. Such seeds are unlikely to germinate. Lightly infected seeds are often normal in size and appearance, showing no signs of infection. Many of these may be able to germinate, but may have reduced seed vigor (American Phytopathological Society, 1975; Athow, 1973; Kulik and Schoen, 1981). *Phomopsis* infection of seeds also has been related to loss in flour and oil quality (Hepperly and Sinclair, 1978b).

In general, the disease is more severe in the warm, humid areas of the Southern U. S. than in more northern states (Jacobsen, 1979; Koehler, 1944; Turnipseed, 1979; Walla, 1977; Whitney, 1978). However, the effect of pod and stem blight on yield is not well defined. Fungicides sprayed on the growing crop in Southern states do increase yields significantly (Backman et al., 1979; Horn et al., 1975; Ross, 1975). However, they control a spectrum of diseases including pod and stem blight. The disease can have an important effect on seed quality by reducing viability (Athow and Caldwell, 1954; Ellis et al., 1974b; Nicholson et al., 1972; Wallen and Cuddy, 1960; Wallen and Seaman, 1963). Decreased seed viability may then result in poor stand establishment (Ellis et al., 1975; Ellis et al., 1979; Kulik and Schoen, 1981; Schmitthenner and Kmetz, 1979; Wallen and Seaman, 1963) and subsequent yield reduction (Schmitthenner and Kmetz, 1979; Wallen and Seaman, 1963). However, because of the ability of soybean plants to

compensate for missing plants in the field, the influence of final stand on yield is variable and may depend on such factors as weather, cultivar, soil fertility, and weed pressure (Anonymous, 1981; Caviness, 1966; Hildebrand and Koch, 1947; Probst, 1945). Premature ripening of soybean plants, due to *Phomopsis* infection, also has been reported (Athow and Caldwell, 1954; Kmetz et al., 1979).

Current control practices recommend the foliar application of benzimidazole fungicides to control pod and stem blight (Ellis et al., 1974a; Hepperly et al., 1978; Ross, 1975). Fungicides are often applied, however, when disease severity does not warrant their application, and increased seed quality may not result (Huber, 1979; Jacobsen, 1979; McGee, 1979; McGee and Brandt, 1979). Consequently, significant amounts of time, money, and chemicals are utilized trying to control the disease unnecessarily.

Epidemiology

Although *Phomopsis* may overwinter in infected or infested soybean seed (Wallen and Seaman, 1963), the importance of seed as a source of inoculum is not clearly understood. McGee et al. (1980) found no relationship between infection level of planted seed and that on harvested seed in a field experiment in Iowa in 1978. Infected seed not killed by mild winters in Brazil has been reported to produce infected volunteer soybean plants, and the possibility exists that they may serve as an inoculum source (Fett, 1978). The importance of soybean crop residue as a source of *Phomopsis* and Dpc inoculum and as a means of overwintering for the pathogens has been suggested by several workers (Athow and Caldwell, 1954; Gerdemann, 1954; Hildebrand, 1956; Lehman, 1923; Kmetz et al., 1979). Primary inoculum of

both *Phomopsis* sp. and Dps, as pycnidia bearing alpha spores, and both Dps and Dpc, as perithecia bearing ascospores has been found on overwintered soybean stems (Kmetz et al., 1979). In the spring, alpha spores can be seen oozing in a gelatinous matrix from mature *Phomopsis* sp. and Dps pycnidia on the residues. These spores may be disseminated by rain splash. Later in the growing season, pycnidia of *Phomopsis* sp. develop on fallen cotyledons and petioles. These may contribute secondary inoculum, but this has never been proven (Kmetz et al., 1979). The contribution of primary or secondary inoculum of Dps and Dpc in the form of ascospores also is not clearly understood (Kmetz et al., 1979; Schmitthenner and Kmetz, 1979).

Plants can be infected by *Phomopsis* as early as the seedling stage of growth (Kmetz et al., 1978). However, although green tissues may be infected, they do not show signs of the pathogen, as expressed by pycnidial production, until plant senescence (Hill et al., 1981; Kmetz et al., 1979). Significant levels of seed infection do not occur before physiological maturity (Kmetz et al., 1978). Severe seed infection may then take place, however, if prolonged periods of wet weather occur. *Phomopsis* sp. infection of seed is more prevalent on the lower part of the plant, while Dps and Dpc infection is more prevalent in seed from upper parts (Kmetz et al., 1979). *Phomopsis* mycelium in infected seed is found primarily in the hourglass cell layer of the seed coat, less so in the parenchyma, and least in the palisade layer. Mycelial mats also may be found between the seed coat and cotyledons (Ilyas et al., 1975). Infection of seed does not necessarily result in death of the developing seed before harvest. Increases in seed germination during storage, resulting from reduced pathogen viability,

indicate that seed is not always killed at the time of infection, but possibly during the germination process (Wallen and Seaman, 1963).

Seed infection by *Phomopsis* has been associated with conditions of high relative humidity, temperature, and excessive rainfall (Balles, 1980; Kmetz et al., 1979; Lehman, 1923; Ross, 1975; Shortt et al., 1981; Spilker, 1977). Under field conditions in Kentucky, Balles (1980) reported that rainfall and relative humidity during the seed-filling period were more important environmental factors than temperature in determining the severity of seed infection. Plants infected with soybean mosaic virus may be predisposed to greater *Phomopsis* seed infection (Hepperly et al., 1979). Interactions between seed-borne fungi also have been detected. Roy and Abney (1977) demonstrated reduced pod and seed infection by *Phomopsis* when soybean plants were artificially inoculated with *Cercospora kikuchii* in the field. McGee et al. (1980) reported a significant negative correlation between *C. kikuchii* and *Phomopsis* seed infection.

Agronomic Aspects

In general, seed infection is greater in early, rather than in late, maturing varieties (Balles, 1980; Kmetz, 1975; Shortt et al., 1981; Wilcox et al., 1974). A suggested explanation is that early varieties mature when conditions conducive to seed infection are more likely to be present than for later maturing varieties (Balles, 1980; Kmetz, 1975). This is further suggested by the fact that early planting often results in increased seed infection (Balles, 1980; Dhingra and Sediyama, 1979; Kilpatrick and Hartig, 1955; Kmetz, 1975) and an overall lowering of seed quality (Green et al., 1964; Smith et al., 1961) compared to late planting of the same varieties.

Weed density appears also to influence seed quality. Dhingra and da Silva (1978) reported increased seed infection by *Phomopsis* with increased weed pressure as did Anderson and McWhorter (1976) who reported on decreased seed quality. Chagas and Dhingra (1979), however, found no correlation between weed density and seed infection. It is probable that high weed populations provide a microclimate of high humidity favorable for fungal development (Dhingra and da Silva, 1978).

The effect of soil potash level on *Phomopsis* seed infection is also controversial. Crittenden and Svec (1974), Mascarenhas et al. (1976), and Jeffers and Schmitthenner (1976) reported decreases in numbers of visible moldy seed with increased potassium fertilizer level, but Svec et al. (1976) reported no benefit in adding potassium fertilizer. In the latter study (Svec et al., 1976), however, potassium level was much higher in the soil prior to fertilizer application than in the study by Crittenden and Svec (1974). These findings (Crittenden and Svec, 1974) are in agreement with those of Schmitthenner and Jeffers (Ohio Agric. Res. and Dev. Center, Wooster, personal communication), who found that potassium application was beneficial in reducing the numbers of visible moldy seed and *Phomopsis* sp. infection, as determined by agar plating, only in soils in which the potash level was so low that plants showed foliar symptoms of potassium deficiency.

Crop rotation and residue management also can influence the severity of disease. Under field conditions in Ohio, Kmetz (1975) reported that *Phomopsis* sp. infection of seed was less when soybeans followed corn than when soybeans followed soybeans. However, rotational practice had no effect on Dps or Dpc seed infection except when harvest was delayed two

months. Then, Dps infection was higher in seed when soybeans followed corn than when soybeans followed soybeans. In addition, *Phomopsis* sp. was isolated more frequently from soybean crop residue recovered from unplowed than plowed soil when soybeans followed soybeans. Incidence of Dps or Dpc when soybeans followed soybeans was greater in fall- than spring-plowed or unplowed soil.

Wilcox and Abney (1971) reported increased *Phomopsis* infection of soybean seed when pods on partially broken lateral branches were in contact with the soil surface. Moderate lodging, however, did not result in increased seed infection.

Increased seed infection also has been associated with delayed harvest situations and has been attributed to wet conditions favoring pathogen development during the delay (Dhingra and Sediyama, 1979; Ellis et al., 1976; Kmetz et al., 1974; Wilcox et al., 1974).

Disease Control

Fungicides

At present, four fungicides, Benlate 50W (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate), Mertect 340F (2-(4'-thiazolyl)-benzimidazole), Bravo 500 (tetrachloroisophthalonitrile), and Topsin-M thiophanate-methyl (dimethyl((1,2-phenylene)bis(iminocarbonothioyl))bis(carbamate)) are registered for use on soybeans in the United States by the Environmental Protection Agency (Ellis et al., 1974a, Hepperly et al., 1978; Ross, 1975). Two applications are made at the R3-R4 growth stage and 14-21 days later, respectively. In addition to pod and stem blight, other diseases (anthracnose, Septoria brown spot, Cercospora frogeye leafspot, purple seed stain)

are included in the control recommendation. These diseases usually are severe enough in the Southern U.S. for fungicide application to result in increased yield and seed quality (Backman et al., 1979; Horn et al., 1975; Ross, 1975; Turnipseed, 1979).

Because the major effect of disease in the Northern U.S. is to reduce seed quality, a special registration for Benlate 50W has been issued to the states of Ohio and Kentucky for soybeans grown only for seed. It allows for one application of fungicide at one to two pounds per acre at the R5 growth stage instead of one-half to one pound per acre being applied at each of two applications as described previously. As significant levels of seed infection do not occur before R7 (Kmetz et al., 1978) and the fungicide is taken up by the seed after foliar application (Ellis and Sinclair, 1975), one application of fungicide just before seed infection takes place (R5-R6) should ensure optimum concentration of fungicide in the seed at the time when protection is most needed (McGee and Brandt, 1979). McGee and Brandt (1979) suggested that the success of either application method depends on the application of fungicide before seed infection occurs, as Benlate 50W did not eradicate *Phomopsis* in infected seed. Under delayed harvest situations, foliar application of Benlate 50W has been shown to be effective in protecting soybean seed from *Phomopsis* infection (Ellis and Sinclair, 1976; Ellis et al., 1976).

Various schemes have been proposed (Brandon, 1979; Bretches, 1979; Hepperly et al., 1978) to predict the need for fungicide application based on the existence of various factors assumed to favor disease development. These schemes are based on limited knowledge of the disease epidemiology, and, except for the scheme proposed by Bretches (1979), which reportedly

reduces the number of fungicide applications by 30% as compared to an across-the-board recommendation (Turnipseed, 1979), there appears to be no reports of their validation. A new scheme is currently being developed (McGee, 1980), which utilizes pod-borne inoculum before physiological maturity to indicate the risk of severe seed infection developing by harvest maturity to provide a basis for determining the need for fungicide application.

Treating of infected soybean seed prior to planting with captan (cis-N-((trichloromethyl)thio)-4-cyclohexene-1,2-dicarboximide), thiram (bis(dimethylthio-carbamoyl)disulfide), or benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) has increased field emergence (Ellis et al., 1975, 1978b; Hammond and Backman, 1978; Hepperly and Sinclair, 1978a; Schmitthenner and Kmetz, 1979) and improved yield (Schmitthenner and Kmetz, 1979). However, effects on yield have tended to be inconsistent (Wall, Dept. of Plant Pathology, Seed and Weed Sciences, Iowa State University, Ames, personal communication). Captan and Thiram were effective only against *Phomopsis* infection in the seed coat, but because the systemic nature of benomyl allows the compound to penetrate the seed coat, it also was effective in eradicating infection in embryo tissue (Ellis et al., 1975).

Cultural practices

Certain practices also are recommended for pod and stem blight control. These include: avoidance of early planting, avoidance of delayed harvest, use of late maturing varieties, maintenance of adequate weed control, maintenance of adequate soil potash, fall plowing of residue, and crop rotation. Heavily infected seed stock such as breeders seed or foundation seed

may be preserved by allowing for the reduction in viability of *Phomopsis* through prolonged seed storage (Wallen and Seaman, 1963). This practice, however, would not be practical in salvaging commercial seed sold to growers, as seed vigor deteriorates over time due to physiological changes during seed aging.

MATERIALS AND METHODS

Disease Patterns in the Field in Relation to Inoculum Source

Detection of *Phomopsis* on seeds

Phomopsis infection of seed lots to be planted in field experiments (Table 1) was determined using the Iowa State University soybean seed health test (McGee et al., 1980). In this test, seeds were surface sterilized in 0.5% sodium hypochlorite for 30 seconds, rinsed in sterile distilled water for five seconds, and then placed on autoclaved blotters in crispers (25.0 x 15.0 x 4.0 cm). Each blotter was moistened with 75 ml of sterile distilled water containing 0.5 mg/ml of 2,6 dichloro-6-nitroaniline (Botran 75W), added to suppress the growth of contaminant *Rhizopus* spp. Two crispers, each containing 50 seeds, were prepared for each seed lot. These were incubated at 25 C for seven days in the dark and the number of seeds from which *Phomopsis* developed was then counted.

For seeds harvested from field plots, *Phomopsis* infection was estimated by surface sterilizing seeds in 1.3% sodium hypochlorite for one minute, rinsing in sterile distilled water for 30 seconds, and then incubating them on potato dextrose agar plates adjusted to pH 4.5 with lactic acid (APDA). After incubation for 5-7 days at 28 C in the dark, the number of seeds from which *Phomopsis* developed were counted. No attempt was made to differentiate *Phomopsis* sp. from Dps. Dpc, the causal agent of stem canker occasionally was isolated from seed in these studies, but it was not counted. Dpc was distinguished from *Phomopsis* by the cultural characteristics of appressed growth and lack of pigmentation compared to

Table 1. Description of seed lots used in 1979 and 1980 field experiments

Growing season	Number	Variety	Maturity group	Seed-borne *Phomopsis* (%)	Germination (%)
1979	1	Amsoy 71	2+	2.0[a]	98.5[b]
	2	Amsoy 71	2+	10.0	84.5
	3	Wells	2	0.0	95.0
	4	Wells	2	6.0	82.0
	5	Beeson	2+	1.0	85.0
	6	Beeson	2+	8.0	79.0
1980	7	Wells	2	0.0	96.0
	8	Wells	2	4.0	90.0
	9	Wells	2	26.0	82.5
	10	Wells	2	37.0	49.0
	11	Wells	2	46.0	30.0
	12	Wells	2	77.0	27.0

[a]2 replicates/50 seeds, determined by blotter test.

[b]4 replicates/50 seeds, determined by paper towel germination.

the flocculose, aerial growth and the presence of orange or yellow pigmentation of *Phomopsis* isolates.

Seed germination test

For seed lots planted in the field, germination was tested by germinating four replicates of 50 seeds each of each seed lot in paper towels for seven days at 25 C in the dark (Association of Official Seed Analysts Rules Committee, 1978).

Detection of *Phomopsis* infection of plant parts

At various growth stages classed according to the system of Fehr et al. (1971), plants were sampled randomly from within each experimental unit (plot) in the field and plant parts were tested for *Phomopsis* infection by surface sterilizing and plating 3.2 cm sections of stems, hypocotyls, or whole pods on APDA as previously described for seeds. If not plated on the same day of sampling, they were stored at 10 C for no more than five days before evaluation.

Stem infection by *Phomopsis* also was determined on green plants by spraying them in the field with a 24 mg/ml solution of the isopropylamine salt of N-(phosphonomethyl) glycine (Roundup 41% a.i.). Two weeks later, plants were sampled and percent stem infection was determined by counting the number of stems with pycnidia of *Phomopsis* using a dissection microscope. For plants at the stage of harvest maturity, stem infection was determined by counting the number of stems with pycnidia as described above. Care was taken to distinguish *Phomopsis* pycnidia from acervuli of *Colletotrichum* spp., which also were readily found. The extent of stem colonization by *Phomopsis* was estimated, only in 1980, by dividing each stem equally into three sections, and then evaluating each section for stem area colonized using a rating scale, where 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75% and 4 = 76-100%.

Progression of *Phomopsis* infection on plant parts

In 1979, two seed lots of each of the three varieties Amsoy 71, Wells, and Beeson were selected with a range of seed-borne *Phomopsis* (Table 1, no. 1-6). On May 17, 1979, each seed lot was planted in three fields

(locations) in which the previous rotational practice had been continuous soybeans, corn-soybean rotation, or continuous corn (Table 2). These were assumed to represent different levels of soil- or crop residue-borne inoculum of *Phomopsis*. The fields were located within 11.3 Km of each other. There were a total of 96 experimental units (plots) per location arranged in a four block split-split plot design, with sampling time being the whole plot, variety the sub plot, and infection level the sub-sub plot. Individual plots at each location consisted of four 4.8 m rows, 0.76 m apart, with 100 seeds per row. During the growing season, each field was machine cultivated twice and hand weeded twice.

Table 2. Summary of fields used in 1979 and 1980 field experiments

Location	Rotational practice	Years in rotation	Growing season	Fertilizer[a]	Weed pressure
Agronomy Farm	Continuous soybeans	10	1979	none	slight
			1980	none	slight
Curtiss Farm	Corn-soybean	5	1979	0/67/101	moderate
			1980	0/67/101	slight
Woodruff Farm	Continuous corn	10	1979	0/67/101	severe
			1980	0/67/101	slight

[a]Kg/ha of N, P, and K.

Seedling emergence was counted two weeks after planting in one of the two middle rows of each four row plot used for the V4-V5 sampling. Progression of disease development was determined by measuring *Phomopsis* infection at four sampling times or growth stages during the growing season

by plating, a hypocotyl and stem section at V4-V5 (June 17), a lower, middle, and upper flower or pod and stem section at R2 (July 25), a lower, middle, and upper pod and seeds within them at R7 (September 20) and R8 (October 9), respectively, from each of 10 plants randomly selected from one of the two middle rows of each four row plot at each location. At each sampling time, a total of 240 plants were evaluated at each location. At R8, stem infection based on visible pycnidia also was determined on the 10 stems from which pods had been obtained for plating. At V4-V5 and R2, stem infection also was measured by spraying the other middle row not used for plating with Roundup and 10 plants chosen at random were evaluated for the presence of *Phomopsis*.

In 1980, the experiment was repeated using six seed lots of the variety Wells with a greater range of seed-borne *Phomopsis* than that in 1979 (Table 1, no. 7-12). There were a total of 96 experimental units per location arranged in a four block split plot design, with sampling time being the whole plot, and infection level the sub plot. The seed lots were planted at all three locations on May 21, 1981, in the same continuous soybean and corn fields used in 1979. The corn-soybean rotation field used in 1980 also was located at the Curtiss Farm, had been in rotation for five years, and was approximately 0.2 Km from the rotation field used in 1979 (Table 2). Seedling emergence was evaluated two weeks after planting and sampling occurred at V4-V5 (June 18), R3 (July 30), R7 (September 14), and R8 (October 6), using the same procedures as in 1979. A quantitative measurement of stem colonization by *Phomopsis* also was obtained at R8 on

plants which were used to estimate plant infection based on visible pycnidia on stems.

To establish possible relationships between temperature and rainfall on progression of plant infection by *Phomopsis* throughout both the 1979 and 1980 growing seasons, daily average temperature and precipitation were obtained from meterological records of the Iowa State University Agronomy Farm.

Potassium level in relation to rotational practice

Because of the potential for variability in *Phomopsis* seed infection in relation to potassium levels in the various fields, potassium measurements were determined in the soil and leaf tissues for each location. Soil potash was measured June 11, 1980, in plots used in the 1979 and 1980 field experiments by collecting and bulking eight, 2.0 x 15.2 cm deep core camples from each location and having these analyzed by the Iowa State University Soil Testing Laboratory. Potassium concentration in leaves was measured only in 1980 at R3 for seed lots seven and 12 (Table 1) by removing one lower and one upper leaf from each of 10 plants used for plating per plot, bulking the samples across blocks for a total of 40 lower and 40 upper leaves per treatment, drying the leaves at 65 C for 24 hours, and then grinding the leaves in a Stein mill for one minute. The ground leaf samples were sent to the Research-Extension Analytical Lab, Ohio Agricultural Research and Development Center, Wooster, Ohio, for analysis.

Relationship between lodging and pod infection

The influence of lodging on pod infection at R8 was determined by evaluating lodging per plot both in 1979 and 1980. Lodging was based on the average erectness of the main stem of all plants at maturity, where: 1 = all plants erect, 2 = slight lodging, 3 = plants lodged at a 45 degree angle, 4 = severe lodging, and 5 = all plants horizontal.

Phomopsis Transmission from Seed-borne Inoculum

Preparation of artificially inoculated diseased seed

Artificially inoculated nonviable infested seed was produced by adding 40 ml of a spore suspension of *Phomopsis* containing approximately 8 x 10^7 conidia/ml to 100 g of Wells soybean seed which had previously been mixed with 50 ml of distilled water and then autoclaved in a 500 ml Erlenmeyer flask. The seed was incubated for seven days at 28 C in the dark, then removed and dried on trays under a continuous flow of sterile air in a bio-hazard hood. These were then stored in cloth bags before use. Viable infected seed was produced by spraying Wells seed with the same spore suspension of *Phomopsis*, incubating the seed for two days at 28 C and 70% RH, and then removing, drying, and storing the seed as described above.

Field and greenhouse experiments

The *Phomopsis*-free seed lot of the variety Wells, referred to in Table 1 as number seven, was divided into five lots. Two lots were sprayed with a spore suspension of *Phomopsis* to produce viable infected seed as described above. These simulated infected seed lots in which the majority of the seeds were viable. One of these lots was then treated with the

fungicide Captan 30DD (0.6 ml fungicide in 1.8 ml water/454 g seed). The third seed lot consisted of a 1:1 mixture of *Phomopsis*-free seed with artificially inoculated nonviable infested seed prepared as previously described. This treatment simulated a seed lot containing a high percentage of nonviable infested seed. A fourth seed lot was prepared in the same manner as the third lot, using autoclaved noninfested seed and the fifth seed lot was the *Phomopsis*-free seed. Each treatment was replicated four times and planted in 1980 in a randomized complete block design at the continuous soybean and corn fields, respectively. Plots consisted of a single 4.8 m row in which 100 seeds were planted for a total of 20 experimental units per location. Repeated measurements of plant infection were made at 5, 12, 19, and 26 days after emergence by plating a hypocotyl section from each of 10 plants per plot on APDA as previously described. For the 5 and 12 day measurements, both cotyledons on each seedling also were plated.

The relative importance of *Phomopsis* infested nonviable seed and infested crop residue as sources of inoculum also were compared in a greenhouse experiment. Four liters of an autoclaved greenhouse soil mix (2 soil:1 sand:1 peat moss), steamed for four hours at 100 C, was added to each of nine trays (40.0 x 27.9 x 8.4 cm). Prior to planting, soybean stems covered with *Phomopsis* pycnidia, previously collected from the Bruner Farm on October 17, 1978, and stored on the roof of the Seed Science Center until March 6, 1980, were ground in a Stein mill for 30 seconds. A group of three trays each had 0.0, 2.5, or 7.5 g/l of ground stems incorporated into the top 2.5 l of soil mix in each tray, respectively. Twenty *Phomopsis*-free seeds of the variety Wells (Table 1, no. 3) were then

planted 3.2 cm deep into each of the nine trays. In addition, 0, 2, or 4 artificially inoculated nonviable infested seeds, prepared as previously described, were planted 3.2 cm deep at a distance of 2.5 cm from each *Phomopsis*-free seed in each of a group of three trays containing 0.0, 2.5, and 7.5 g/l of incorporated ground stems, respectively. Each treatment was replicated twice in a randomized complete block design for a total of 18 experimental units. After incubation in the greenhouse for three weeks at approximately 20 C, plant infection by *Phomopsis* was determined by plating both cotyledons and a hypocotyl section from each of 10 randomly selected plants from each tray on APDA as previously described.

Quantification of Soil-borne Inoculum

A series of experiments was carried out to develop a technique for bio-assaying *Phomopsis* inoculum in soils. In the first experiment, four trays (40.0 x 27.9 x 8.4 cm) were each filled with four liters of an autoclaved greenhouse soil mix as previously described. Soybean stems covered with *Phomopsis* pycnidia, previously collected from the Agronomy Farm on October 6, 1980, and stored in the Seed Science Center until January 21, 1981, were ground as described above, and 100 g of infested ground stems were mixed into the soil mix of two of the trays. A spore suspension of *Phomopsis* containing 1.3×10^7 conidia/ml was poured into 3.2 cm deep furrows, 5.1 cm apart, in the two remaining trays. Sixty *Phomopsis*-free seeds of the variety Wells (Table 1, no. 7) were then planted into each of the four trays. After incubation in the greenhouse for nine days at approximately 20 C, 25 seedlings from each of one tray containing residue and one tray containing the spore suspension were evaluated for *Phomopsis*

infection by plating cotyledons and whole roots, hypocotyls, and stems on APDA as previously described. The remaining seedlings in each of the two trays were sprayed to runoff with a 24 mg/ml solution of the isopropylamine salt of N-(phosphonomethyl)glycine (Roundup 41% a.i.), incubated an additional seven days, and then evaluated by plating. Twenty-three days after planting, 25 seedlings in each of the two remaining trays were evaluated by plating whole roots, hypocotyls, and stems on APDA and the remaining seedlings sprayed with Roundup and evaluated as above.

In a second experiment, soil and residue from each of the fields used in the 1980 field experiments were evaluated for the presence of *Phomopsis* during the growing season on June 18, July 30, and September 14. Using a 0.1 m^2 quadrat, two liters of soil-residue mixture was collected from the upper two cm depth at one sampling site in each of the four blocks at each location. Each two liter soil-residue mixture then was divided into a soil-only and residue-only fraction by first crushing the mixture with a steel pipe to break-up soil aggregates and then passing it through 1.27 and 0.32 cm screens to trap residue in the screens. Residue which failed to screen out when passed through the screens, was removed by passing the remaining soil-residue mixture over a gravity separation table repeatedly three times. No attempt was made to separate soybean from corn residue collected at each location. The residue fraction then was ground for 30 seconds in a Stein mill and mixed with two liters of a 1:1 mixture of nonsteamed sand and peat moss and both fractions were each then placed into trays (40.0 x 27.9 x 8.4 cm) containing three liters of the sand-peat moss mixture. Twenty *Phomopsis*-free seeds of the variety Wells (Table 1,

no. 7) were then planted 3.2 cm deep into each tray. The trays were each watered with 500 ml of water and incubated at 25 C in continuous light in sealed germination carts. After four days, the trays were sprayed with 500 ml of water from an overhead sprinkler to facilitate inoculum dispersal. Seedling infection was then measured after an additional seven day incubation period by plating on APDA a hypocotyl section and both cotyledons each from 10 randomly selected seedlings per tray.

To improve the sensitivity of the bio-assay, a test was developed using only crop residue. The procedure involved placing 50 *Phomopsis*-free seeds of the variety Wells (Table 1, no. 7) each into three crispers (27.0 x 19.0 x 9.5 cm) containing a cellulose pad (Kimpak, made by Kimberly-Clark Corporation, Neenah, Wisconsin) moistened with 150 ml of water and 100 g of ground soybean crop residue, prepared as previously described. These seeds were then covered with an additional 100 g of ground residue. After incubation for 14 days at 25 C in continuous light in sealed germination carts, a whole hypocotyl and both cotyledons from each of 40 randomly selected seedlings in each crisper were assayed for *Phomopsis* infection by plating on APDA as previously described. This test then was used in March, 1981, to bio-assay for *Phomopsis* inoculum on the soybean crop residue in the three fields with different rotational histories used in the 1980 field experiments. This experiment had the objectives of measuring the inoculum in the residues and the amount of surface residue in the experimental sites. Surface residue was collected at each location in 0.5 m^2 quadrats from as many sampling sites as was needed to provide the 600 g of residue necessary to perform the test and assayed as described above.

Estimates also were made of the amount of surface residue in the experimental sites and reported as grams of residue per m^2 surface area.

Statistical Analysis

Data were analyzed using the statistical analysis system (SAS) available through the Computer Center at Iowa State University. Individual field experiments were analyzed across rotational practice fields in a combined analysis of variance (ANOVA). ANOVA tables containing model statements, degrees of freedom, mean squares, and significance levels, for all experiments in which they were calculated, are found in the Appendix. When main effects were found significant at either the .05 or .01 significance level, LSD's (.05) were calculated and included in the tables found in the Results and Appendix sections.

RESULTS

Disease Patterns in the Field in Relation to Inoculum Source

Effect of seed-borne inoculum on field emergence and yield

In 1979, field emergence ranged from an average low of 38.5% at the corn-soybean rotation (CSR) field to a high of 74.7% at the continuous corn (CC) field (Table 3, Appendix Table 1). Generally, there appeared to be a minor influence of seed-borne inoculum on emergence, with significant negative correlations between the two parameters being obtained only at the continuous soybean (CS) field (Table 4). In 1980, field emergence ranged from an average low of 50.9% at the CC field to a high of 73.2% at the CS field, with a marked reduction in emergence as seed-borne inoculum increased (Table 5, Appendix Table 2). Significant negative correlations were obtained at all three locations between seed-borne inoculum and field emergence (Table 6).

Progression of *Phomopsis* infection of plant parts

The severity of *Phomopsis* infection of soybean plant parts was directly related to rotational practice with, in general, most severe infection occurring in the CS field, less in the CSR field, and least in the CC field (Table 7, Appendix Tables 1, 2, 7, 8, 10, 12, 14, 15). This effect was consistent for all plant parts measured, at each growth stage, and in both years of the study. In general, there was a trend toward increased severity of infection as the growing season progressed, with the exception

Table 3. Field emergence of soybean seed as affected by variety, seed-borne inoculum, and rotational practice in 1979

Variety	Seed-borne inoculum (%)	Rotational practice[a]		
		CS	CSR	CC
Amsoy 71	2.0	73.5%[b]	34.8	79.5
	10.0	51.8	26.3	61.0
Wells	0.0	75.0	33.0	73.5
	6.0	75.5	41.3	81.5
Beeson	1.0	71.0	44.8	75.5
	8.0	75.5	51.0	77.0
LSD (P=0.05)		7.7	NS	8.0
Mean		70.4	38.5	74.7

[a]CS = continuous soybean, CSR = corn-soybean rotation, CC = continuous corn.

[b]Four replicates/100 seeds.

Table 4. Correlations between seed-borne *Phomopsis* and field emergence in 1979 as affected by rotational practice

Rotational practice[a]		
CS	CSR	CC
-.512*	-.020	-.359

[a]CS = continuous soybean, CSR = corn-soybean rotation, CC = continuous corn.

*Significant at the 0.05 level of probability.

Table 5. Field emergence of soybean seed as affected by seed-borne inoculum and rotational practice in 1980

Rotational practice	Seed-borne inoculum (%)	Field emergence (%)
Continuous soybean	0.0	85.3[a]
	4.0	85.0
	26.0	78.5
	37.0	74.8
	46.0	62.5
	77.0	53.0
	LSD (P=0.05)	7.1
	Mean	73.2
Corn-soybean	0.0	84.8
	4.0	84.3
	26.0	81.5
	37.0	64.5
	46.0	54.3
	77.0	49.3
	LSD (P=0.05)	9.5
	Mean	69.8
Continuous corn	0.0	68.3
	4.0	64.0
	26.0	59.5
	37.0	41.5
	46.0	37.5
	77.0	34.5
	LSD (P=0.05)	13.0
	Mean	50.9

[a]Four replicates/100 seeds.

Table 6. Correlations between seed-borne *Phomopsis* and field emergence in 1980 as affected by rotational practice

Rotational practice[a]		
CS	CSR	CC
-.918**	-.876**	-.809**

[a]CS=continuous soybean, CSR=corn-soybean rotation, CC=continuous corn.

**Significant at the 0.01 level of probability.

Table 7. Soybean stem, pod, and seed infection by *Phomopsis* in 1979 and 1980 as affected by rotational practice and plant growth stage

Rotational practice	Stem infection (%)[a] 1979 V4-V5	1979 R2	1979 R8[b]	1980 V4-V5	1980 R3	1980 R8[b]	1980 R8[c]
Continuous soybean	83.3[d]	97.0	90.4	67.5	26.3	98.3	1.7
Corn-soybean	10.8	80.8	96.7	35.0	10.8	73.1	1.0
Continuous corn	0.4	3.3	73.3	5.0	1.7	72.6	0.6
LSD (P=0.05)	8.5	16.1	8.1	10.2	8.4	19.1	0.4

	Pod infection (%)[a] 1979 R2	1979 R7	1979 R8	1980 R3	1980 R7	1980 R8
Continuous soybean	9.5[e]	86.0	80.7	0.8	38.3	47.0
Corn-soybean	7.8	96.7	94.7	0.1	31.4	23.2
Continuous corn	0.8	60.4	56.2	0.0	5.6	4.6
LSD (P=0.05)	5.0	10.4	8.	0.4	5.2	5.6

	Seed infection (%)[a] 1979 R7	1979 R8	1980 R7	1980 R8
Continuous soybean	11.2[f]	7.4	7.3	8.8
Corn-soybean	17.2	7.7	4.3	3.1
Continuous corn	0.8	0.7	0.1	0.3
LSD (P=0.05)	6.1	5.1	2.6	4.8

[a]Potato dextrose agar plating.

[b]Stems with visible *Phomopsis* pycnidia.

[c]Stem rating index, where 0=no visible pycnidia, 1=1-25% stem area colonized, 2=26-50%, 3=51-75%, 4=76-100%.

[d]Four replicates/60 plants.

[e]Four replicates/180 pods.

[f]Four replicates/approximately 450 seeds.

of stem infection in 1980, when a decline occurred between the V4-V5 and R3 growth stages. This, however, could have resulted from exceptionally warm, dry weather in July of that year, which might have eradicated infection. Average daily temperatures and rainfall between the two growth stages was 23.8 C and 8.3 cm, respectively, in contrast to 1979, when temperature averaged 21.8 C and 17.9 cm of rainfall fell during the same period (Table 8). Generally, disease development was less in 1980 than in 1979, and it is probable that rainfall was partially responsible for this, as only two-thirds of the amount of moisture fell in 1980 as in 1979 (Table 8). Measurements of stem infection by the Roundup technique gave essentially the same results as the plating technique (Appendix Tables 1, 2, 7, 8). Seed-borne inoculum in planted seed was not related to subsequent plant infection either at the V4-V5 stage of growth (Table 9, Appendix Tables 1, 2) or at later growth stages (Appendix Tables 1, 2, 7, 8, 10, 12, 14, 15) in any of the rotations.

Plant infection followed a distinct pattern in relation to position on the plant in both years of the study, whereby infection levels on the lower parts of the plant on stems, pods, or seeds were greater than those at the middle or upper parts of the plant (Tables 10, 11, Appendix Tables 3, 4). Seed-borne inoculum was not related to severity of infection of the harvested seed at any of the positions on the plant in any of the fields (Table 11, Appendix Tables 3, 4), nor was it related to pod or stem infection (Appendix Tables 3, 4, 9, 11, 13).

Table 8. Summary of temperature and rainfall for the 1979 and 1980 growing seasons[a]

Year	Growth stage	Days	Average daily temperature	Rainfall	Days with rainfall
1979	Planting-V4/V5	31	19.6 C	14.2 cm	12
	V4/V5-R2	38	21.8	17.9	12
	R2-R7	56	21.1	18.0	20
	R7-R8	20	14.8	4.1	3
		145	19.3	54.2	47
1980	Planting-V4/V5	28	20.7	10.7	12
	V4/V5-R3	42	23.8	8.3	15
	R3-F7	46	23.1	17.1	14
	R7-R8	22	15.3	0.3	4
		138	20.7	36.4	45

[a]Data obtained from meteorological records of the Iowa State University Agronomy Farm.

Table 9. Soybean stem infection by *Phomopsis* at the V4-V5 growth stage in 1979 and 1980 as affected by rotational practice, variety, and seed-borne inoculum

	1979			1980		
Rotational practice	Variety	Seed-borne inoculum (%)	Stem infection (%)[a,b]	Variety	Seed-borne inoculum (%)	Stem infection (%)
Continuous soybean	Amsoy 71	2.0	85.0[c]	Wells	0.0	72.5
		10.0	77.5		4.0	70.0
	Wells	0.0	82.5		26.0	60.0
		7.0	87.5		37.0	62.5
	Beeson	1.0	87.5		46.0	67.5
		8.0	80.0		77.0	72.5
Corn-soybean	Amsoy 71	2.0	10.0	Wells	0.0	37.5
		10.0	22.5		4.0	27.5
	Wells	0.0	17.5		26.0	22.5
		6.0	10.0		37.0	27.5
	Beeson	1.0	2.5		46.0	42.5
		8.0	2.5		77.0	52.5
Continuous corn	Amsoy 71	2.0	2.5	Wells	0.0	0.0
		10.0	0.0		4.0	2.5
	Wells	0.0	0.0		26.0	5.0
		6.0	0.0		37.0	5.0
	Beeson	1.0	0.0		46.0	7.5
		8.0	0.0		77.0	10.0

[a]Potato dextrose agar plating.

[b]No significant difference (P=0.05) in stem infection in 1979 or 1980 within rotational practice regardless of seed-borne inoculum.

[c]Four replicates/10 plants.

Table 10. Soybean stem and pod infection by *Phomopsis* at the R8 growth stage in 1979 and 1980 as affected by rotational practice and plant height

Rotational practice	Stem infection[a,b]							
	1979				1980			
	Lower	Middle	Upper	LSD (P=0.05)	Lower	Middle	Upper	LSD (P=0.05)
Continuous soybean	--	--	--	--	2.4[c]	1.7	1.1	0.2
Corn-soybean	--	--	--	--	1.3	1.0	0.6	0.1
Continuous corn	--	--	--	--	0.9	0.7	0.3	0.1
	Pod infection (%)[d]							
	1979				1980			
	Lower	Middle	Upper	LSD (P=0.05)	Lower	Middle	Upper	LSD (P=0.05)
Continuous soybean	100.0[e]	89.2	52.9	7.2	88.8	30.0	22.1	7.1
Corn-soybean	99.6	95.3	88.9	4.4	50.8	12.9	5.8	6.2
Continuous corn	82.0	49.0	37.5	8.2	7.5	2.9	3.3	3.7

[a]Stem rating index, where 0 = no visible pycnidia, 1 = 1-25% stem area colonized, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.

[b]Stem rating index not evaluated in 1979.

[c]Four replicates/60 plants.

[d]Potato dextrose agar plating.

[e]Four replicates/60 pods.

Table 11. Soybean seed infection by *Phomopsis* at the R8 growth stage in 1979 and 1980 as affected by rotational practice, variety, seed-borne inoculum, and plant height

		1979				
Rotational practice	Variety	Seed-borne inoculum (%)	Lower	Middle	Upper	LSD (P=0.05)
Continuous soybean	Amsoy 71	2.0	25.0%[a,b]	3.0	3.0	
		10.0	8.0	0.0	1.0	
	Wells	0.0	36.0	4.0	3.0	
		6.0	34.0	11.0	2.0	
	Beeson	1.0	2.0	0.0	0.0	
		8.0	0.0	1.0	0.0	
	Mean		17.5	3.2	1.5	4.1
Corn-soybean	Amsoy 71	2.0	19.0	1.0	2.0	
		10.0	8.0	0.0	5.0	
	Wells	0.0	29.1	12.0	6.0	
		6.0	27.0	6.0	3.0	
	Beeson	1.0	11.0	4.0	1.0	
		8.0	4.0	1.0	0.0	
	Mean		16.4	4.0	2.8	4.2
Continuous corn	Amsoy 71	2.0	0.0	0.0	0.0	
		10.0	4.0	0.0	0.0	
	Wells	0.0	2.0	0.0	0.0	
		6.0	3.0	0.0	0.0	
	Beeson	1.0	2.0	0.0	0.0	
		8.0	0.0	0.0	1.0	
	Mean		1.8	0.0	0.2	1.0

[a]Potato dextrose agar plating.

[b]Four replicates/approximately 25 seeds.

Variety	Seed-borne inoculum (%)	1980 Lower	Middle	Upper	LSD (P=0.05)
Wells	0.0	11.0	2.0	1.0	
	4.0	21.0	2.0	1.0	
	26.0	23.0	1.0	2.0	
	37.0	24.0	2.0	0.0	
	46.0	34.0	13.0	1.0	
	77.0	19.6	2.0	0.0	
		22.0	3.7	0.8	6.2
Wells 4.0	0.0	7.0	0.0	0.0	
	4.0	9.0	0.0		
	26.0	8.0	2.0	1.0	
	37.0	2.0	0.0	0.0	
	46.0	17.0	1.0	0.0	
	77.0	7.0	1.0	0.0	
		8.3	0.3	0.2	3.1
Wells	0.0	0.0	0.0	0.0	
	4.0	1.0	0.0	0.0	
	26.0	0.0	0.0	0.0	
	37.0	0.0	1.0	0.0	
	46.0	2.0	0.0	0.0	
	77.0	1.0	0.0	0.0	
		0.7	0.2	0.0	0.6

Potassium level in relation to rotational practice

Potassium analysis revealed differences in soil potash between the fields used in 1979 and 1980 (Table 12). In general, plant available potassium was lowest at the CS field and highest at the CC field both in 1979 and 1980. All values were, however, above the deficiency range. Leaf potassium followed the same trend as soil potassium, with the lowest concentration of potassium in soybean leaves at the CS field and the highest at the CC field. Concentration in leaves always was greater in upper than lower leaves regardless of location. No signs of potassium deficiency were seen in any of the locations in either year.

Table 12. Potassium concentration in soil and leaves as affected by rotational practice

	Soil (Kg/ha)[a]		1980 leaves[b]		
Rotational practice	1979	1980	Lower	Upper	Mean
Continuous soybean	188 (M)[c]	165 (L-M)	0.44[d]	1.27	0.86
Corn-soybean	198 (M)	229 (H)	1.07	1.65	1.36
Continuous corn	250 (H)	321 (H)	1.10	1.82	1.46

[a]Measured June 11, 1980, 0-15 cm depth.

[b]Percent of oven dried weight.

[c]ISU test classification, where L-M=low-medium, M=medium, and H=high.

[d]Average of seed lots seven and 12 (see Table 1).

Relationship between lodging and pod infection

Marked differences in the amount of lodging occurred between the three rotational practice fields in 1979 (Table 13, Appendix Table 1). In the CC field with the greatest amount of lodging, pod infection was, however, lowest. In the CS field in which the least amount of lodging occurred, pod infection was less than at the CSR field, but greater than the CC field. Although lodging was not as severe in 1980 (Table 13, Appendix Table 2) as in 1979, again the CC field had the greatest amount of lodging and the CS field the least. In contrast to 1979, pod infection was greater at the CS field than at the CSR field in 1980.

Phomopsis Transmission from Seed-borne Inoculum

When seeds, treated in various ways to simulate seed-borne inoculum, were planted in a field with a CS rotation history, *Phomopsis* was readily recovered within five days of emergence from an average 7.0% and 12.0% of hypocotyls and cotyledons, respectively, in the various treatments and from over 50% of hypocotyls after 26 days (Figures 1, 2, Appendix Table 5). There were, however, no differences in the amount of infection or in the pattern of development of infection between inoculated and noninoculated treatments. When the seedlots were planted in a field with a CC rotation, overall seedling infection was greatly reduced, with an average hypocotyl infection level for all treatments of less than 5% after 26 days. Differences between seed treatments did occur, however. Significantly more hypocotyl and cotyledonary infection occured in seedlings grown from viable infected seed than in the other treatment. Fungicide treatment of the seeds markedly reduced the infection level in the former treatment,

Table 13. Soybean plant lodging and pod infection by *Phomopsis* at the R8 growth stage in 1979 and 1980 as affected by variety, seed-borne inoculum, and rotational practice

Variety	Seed-borne inoculum (%)	Rotational practice[a] Lodging[b] CS	CSR	CC	LSD (P=0.05)	Pod infection[c] CS	CSR	CC	LSD (P=0.05)
				1979					
Amsoy 71	2.0	2.0	2.8	4.0		80.8[d]	92.5	64.4	
	10.0	2.0	3.0	3.5		80.9	95.7	64.2	
Wells	0.0	1.5	2.0	3.0		83.3	96.7	46.7	
	0.6	1.0	1.8	3.0		82.5	95.8	40.0	
Beeson	1.0	1.8	3.3	4.0		77.5	92.4	66.8	
	8.0	2.0	2.0	3.3		79.2	95.0	55.2	
	Mean	1.7	2.5	3.5	0.5	80.7	94.7	56.2	8.3
				1980					
Wells	0.0	1.8	1.8	2.0		44.2	12.5	3.3	
	4.0	1.5	1.3	1.5		41.7	23.3	0.8	
	26.0	1.5	1.5	2.5		40.0	23.3	7.5	
	37.0	1.3	1.3	2.5		55.8	17.5	6.7	
	46.0	1.5	1.5	1.8		46.7	30.8	5.0	
	77.0	1.5	2.5	3.0		53.4	31.7	4.2	
	Mean	1.5	1.6	2.2	0.5	47.0	23.2	4.6	5.6

[a]CS=continuous soybean, CSR-corn-soybean rotation, CC=continuous corn.

[b]Rated on a scale from 1-5, where 1=all plants erect, 2=slight lodging, 3=plants lodged at a 45-degree angle, 4=severe lodging, 5=all plants horizontal.

[c]Potato dextrose agar plating.

[d]Four replicates/30 pods.

Figure 1. Progression of *Phomopsis* infection of soybean hypocotyls during the 1980 growing season as affected by rotational practice and seed-borne inoculum

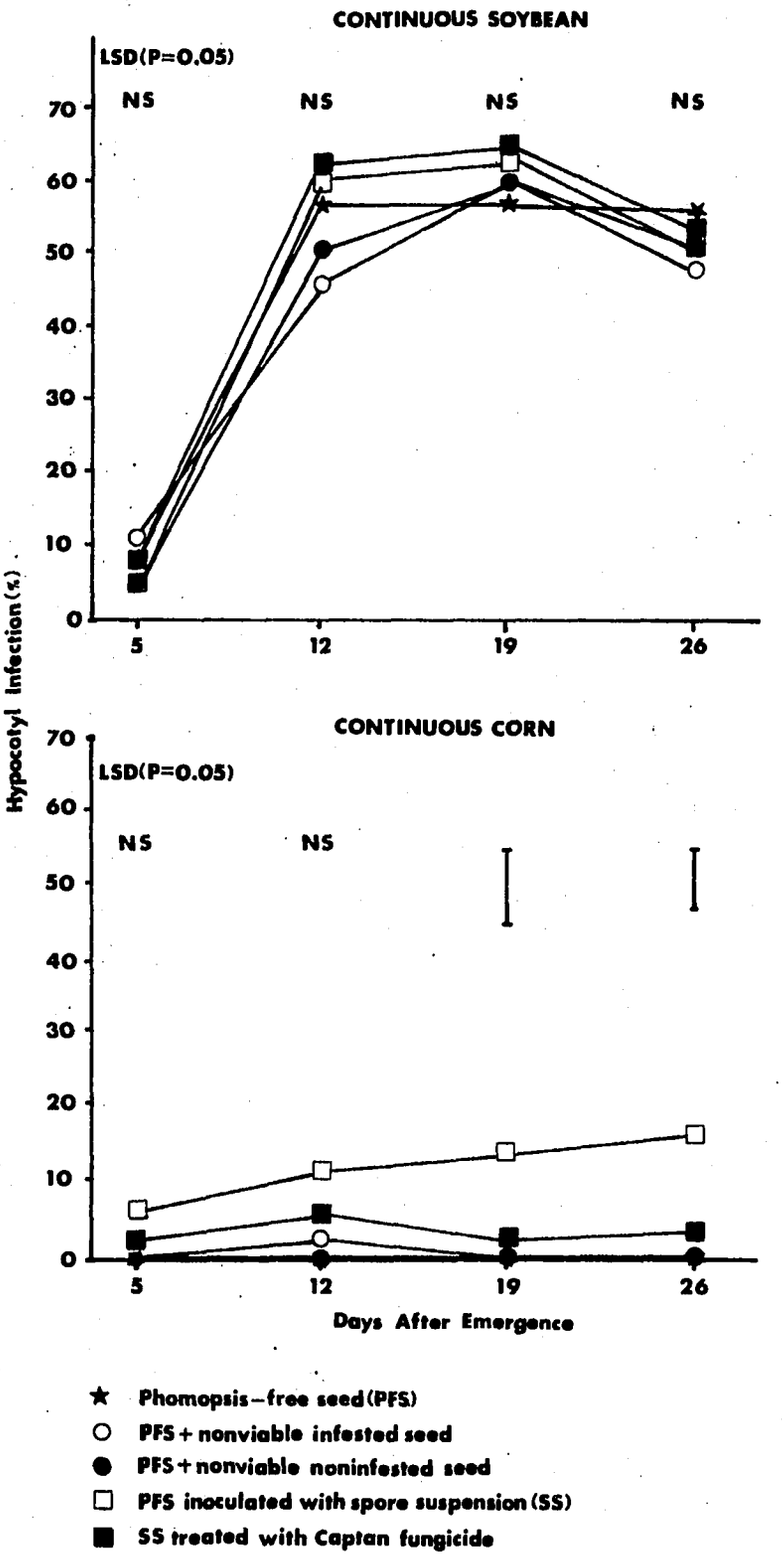
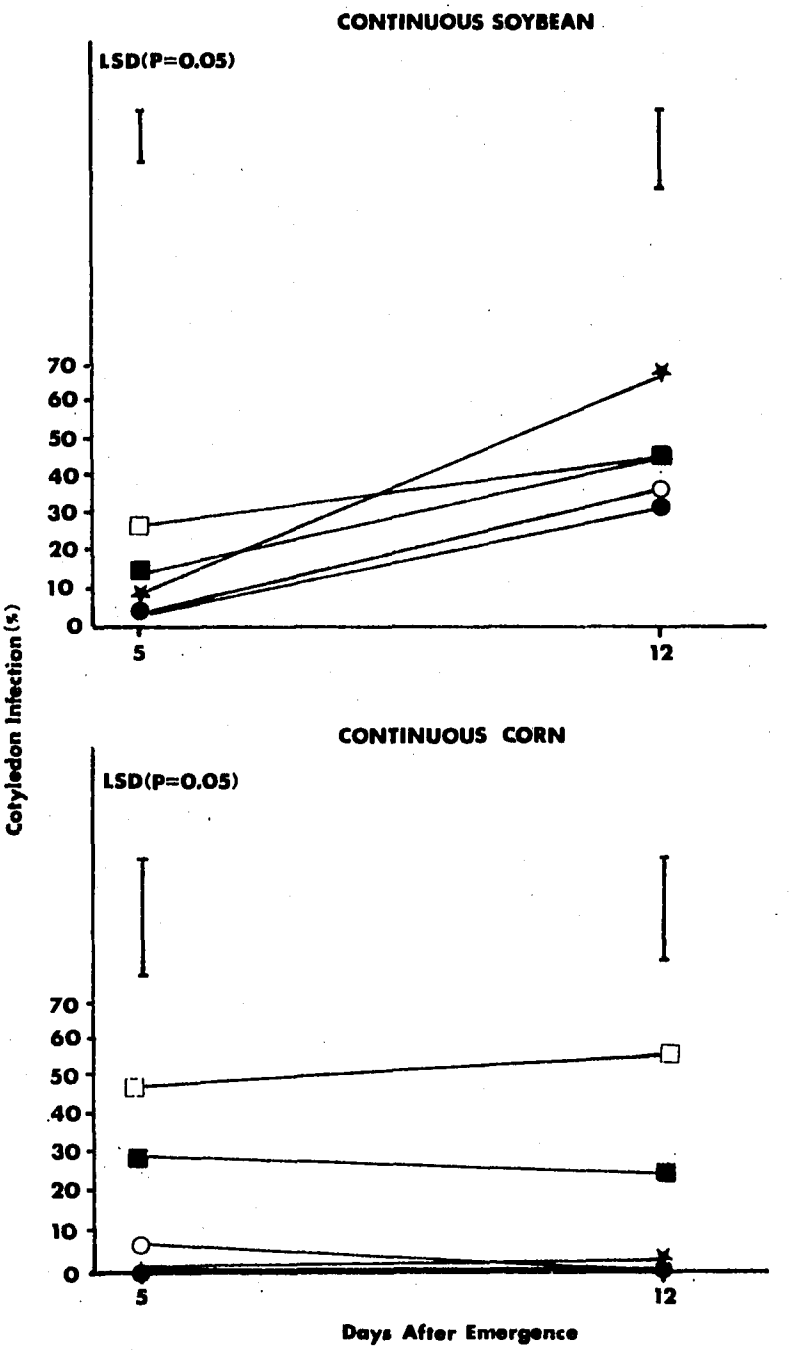

CONTINUOUS SOYBEAN
LSD(P=0.05)
NS
NS
NS
NS
70
60
50
40
30
20
10
0
5
12
19
26
Hypocotyl Infection (%)
CONTINUOUS CORN
LSD(P=0.05)
NS
NS
70
60
50
40
30
20
10
0
5
12
19
26
Days After Emergence
★ Phomopsis-free seed (PFS)
○ PFS + nonviable infested seed
● PFS + nonviable noninfested seed
□ PFS inoculated with spore suspension (SS)
■ SS treated with Captan fungicide

Figure 2. Progression of *Phomopsis* infection of soybean cotyledons during the 1980 growing season as affected by rotational practice and seed-borne inoculum


CONTINUOUS SOYBEAN
LSD(P=0.05)
70
60
50
40
30
20
10
0
5
12
CONTINUOUS CORN
LSD(P=0.05)
70
60
50
40
30
20
10
0
5
12
Cotyledon Infection (%)
Days After Emergence
★ Phomopsis-free seed (PFS)
○ PFS+nonviable infested seed
● PFS+nonviable noninfested seed
□ PFS inoculated with spore suspension (SS)
■ SS treated with Captan fungicide

however. There was no increase in seedling infection in plots containing nonviable infested seed compared to that in the noninoculated treatments

Further experimentation on the effect of nonviable infested seed on disease development showed that when either two or four of these seeds were planted adjacent to a *Phomopsis*-free seed in noninfested soils in trays in the greenhouse, no seedling infection was detected 21 days after planting (Table 14, Appendix Table 6). When the same seed-inoculum treatments were planted in trays with two different amounts of infested soybean residue, extensive seedling infection by *Phomopsis* developed by 21 days after planting, but there was no difference in infection between the treatments with infested seed and that with no infested seed.

Quantification of Soil-borne Inoculum

When a spore suspension of *Phomopsis* was used as the source of inoculum in a pre-sterilized potting medium in the greenhouse, roots and shoots were extensively colonized by the fungus on seedlings either treated or untreated with a herbicide (Table 15). Some colonization of roots and shoots was detected when infested soybean crop residues were incorporated into the potting mix, but infection levels were much lower than those obtained using the spore suspension.

In an attempt to bio-assay *Phomopsis* inoculum in soils by growing soybean seedlings in a pre-sterilized potting medium infested with soil and residue samples, more infected seedlings were found in potting media infested with soil and residue from the CS field in 1980 than in those infested with the CSR or CC fractions (Table 16). This held when either the crop residue or soil particle fractions were tested. The differences were

Table 14. Soybean seedling infection by *Phomopsis* under greenhouse conditions as affected by *Phomopsis* infested soybean crop residue and nonviable infested seed

Residue (g/l. soil)	Nonviable infested seed[a]		Seedling infection (%)
0.0	0		5.0[b]
	2		0.0
	4		0.0
		Mean	1.7
2.5	0		20.0
	2		25.0
	4		25.0
		Mean	23.3
7.5	0		25.0
	2		15.0
	4		45.0
		Mean	28.3
LSD (P=0.05)[c]			12.8

[a]Number of *Phomopsis* infested seeds planted adjacent to each *Phomopsis*-free seed.

[b]Two replicates/10 plants.

[c]LSD for comparing crop residue treatment means.

Table 15. Soybean seedling infection by *Phomopsis* under greenhouse conditions as affected by time of sampling, herbicide application, and inoculum source

Days after planting	Herbicide application	Seedling infection (%)			
		Roots		Shoots	
		Residue[a]	Spore susp.[b]	Residue	Spore susp.
9	NO	0.0[c]	24.0	4.0	28.0
	YES[d]	8.0	88.0	0.0	44.0
23	NO	16.0	56.0	4.0	12.0
	YES	4.0	36.0	0.0	4.0

[a]Residue = 25 g/l soil.

[b]Spore susp. = spore suspension, 1.3 x 10^7 conidia/ml.

[c]One replicate/25 plants.

[d]Roundup (41% a.i.) applied to runoff, plants sampled 7 days later and plated on potato dextrose agar plates.

Table 16. Soybean seedling infection by *Phomopsis* in the bio-assay as affected by rotational practice, time of sampling, and source of inoculum, during the 1980 growing season

Rotational practice	Sampling time	g. Residue/ l. soil	Seedling infection (%) Residue[a] fraction	Soil fraction
Continuous soybean	June 18	12.6	17.5[b]	5.0
	July 30	7.4	7.5	0.0
	Sept 14	55.6	0.0	0.0
Corn-soybean	June 18	0.8	5.0	2.5
	July 30	0.4	0.0	0.0
	Sept 14	71.1	7.5	0.0
Continuous corn	June 18	35.3	0.0	0.0
	July 30	8.3	0.0	0.0
	Sept 14	49.1	2.5	0.0

[a]Residue separated from soil using a series of screens and a gravity separation table.

[b]Four replicates/10 plants.

easily seen for the June 18 sampling, but could not be detected for the later sampling times. Actual amounts of residue bore little relationship to infection levels. It should be pointed out, however, that no attempt was made to distinguish soybean and corn residue.

In the spring of 1981, when only soybean crop residue on the soil surface in fields used in the 1980 field experiments was used in a bio-assay, seedling infection was greatest when residue from the CS field was used and lowest with residue from the CC field (Table 17). These data were well-correlated with the disease severity in these fields in 1980. An inoculum index which took into account both the severity of infection on seedlings exposed to the same quantity of crop residue and the amount

residue in the fields indicated that the inoculum level in the CS field was much greater than that in the other two fields.

Table 17. Quantitative estimate of *Phomopsis* inoculum on soybean crop residue by a bio-assay

Rotational practice	1980/R8 Plant infection (%)[a]	Stem rating[b]	Seedling infection (%)[a] Root	Hypocotyl	Total	g Residue /m^2	Bio-assay Index[c]
Continuous soybean	98.3[d]	1.7	9.2[e]	25.0	31.2	103.3	3223
Corn-soybean	73.1	1.0	4.2	11.7	15.0	13.2	198
Continuous corn	72.6	0.6	0.0	3.3	3.3	36.5	120

[a]Potato dextrose agar plating.

[b]Stem rating index, where 0 = no visible pycnidia observed, 1 = 1-25% stem area colonized, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.

[c]Bio-assay index = total % of seedlings infected by *Phomopsis* X g residue/m^2.

[d]Four replicates/60 plants.

[e]Three replicates/40 plants.

DISCUSSION

The consistent trend, whereby *Phomopsis* infection of soybean stems, pods, and seeds was greatest at the CS field, less at the CSR field, and least at the CC field, and the lack of a relationship between seed-borne inoculum and disease severity provided good circumstantial evidence that soil-borne inoculum is the major source of inoculum for the disease. These findings are in agreement with those of Kmetz (1975), who found that infection by *Phomopsis* sp. was greater when soybeans followed soybeans than when soybeans followed corn. The conclusion is further substantiated by the disease development pattern, in which infection was always greater in the lower part of the plant irrespective of rotational practice. Lower parts were closer to soil-borne inoculum and present longer during the season, thus increasing the chance of infection. Seed-borne inoculum could also produce a similar disease pattern in relation to height, but differences would have been seen across seed treatments instead of across rotations if seed had been an important inoculum source. This was never observed. The disparity in amount of soil-borne inoculum between the three rotational practices is further emphasized by the relationship between lodging and pod infection. Despite the fact that lodging, which should favor infection by bringing pods close to the soil, was greatest in the CC field, pod infection was least severe in that field. This suggested that soil-borne inoculum was extremely low, compared to that in the CS and CSR fields. This low level of infection could be expected after continuous cropping of corn for 10 years (Table 2) which should have reduced soybean crop residues to a negligible level. However, despite low

infection levels in the earlier part of the growing season, stem infection at that location did average 73.3% and 72.6% by R8 in the 1979 and 1980 growing seasons, respectively. It should be pointed out, however, that, although the total number of plants infected at the CC field was comparable to the level of infection at the other locations by harvest maturity, especially in 1980, the extent of stem colonization by *Phomopsis* was never as great at that location as determined using the stem rating index. The experimental sites in the CC field were approximately 30-45 m from a field which was in a corn-soybean rotation and it is probable that some soybean crop residue or air-borne spores of *Phomopsis* had been disseminated from that field by wind to the CC field.

Differences in soil and leaf potassium detected between rotational practice fields does not appear to have had a confounding influence on seed infection by *Phomopsis* in either growing season. Schmitthenner and Jeffers (Ohio Agric. Res. and Dev. Center, Wooster, personal communication) found increased seed infection only when soils had very low values (100-150 Kg/ha) of available potassium, or if plants showed symptoms of potassium deficiency. In this study, soils tested no lower than 165 Kg/ha and plants never expressed visual potassium deficiency. In addition, upper leaf potassium concentration was never lower than 1.27%. As reported by Jones (1967) and Ohlrogge (1960), less than 1.25% and 0.7% potassium concentration in upper foliage, respectively, is needed for plants to be in a deficiency situation. Average potassium concentration in leaves, in the present study, also was comparable to the findings of Hanway and Weber

(1971), who reported that average concentration in leaves was approximately 1.0% in nonfertilized and 1.5% in potassium fertilized plots, under Iowa conditions. Average concentration was 0.86% at the nonfertilized CS field and 1.36% and 1.46% at the potassium fertilized CSR and CC fields, respectively, in this study.

Basically, there are two types of seed-borne inoculum, that from viable infected seed and that from nonviable infested seed. When seedlots containing both inoculum types were planted into fields with CS and CC histories, the pattern of disease development was essentially the same for all seed treatments at the CS field, emphasizing further the greater importance of soil-borne inoculum. However, transmission of *Phomopsis* from viable infected seed was detected at the CC field. Seed-borne inoculum in this form may be of practical significance if there is concern about introducing the pathogen into an area previously free from pod and stem blight. In established soybean cropping areas, however, where wind-blown inoculum is likely, very little disease control could be expected by planting of *Phomopsis*-free seed, even in fields not cropped to soybeans for several years. In field and greenhouse experiments, planting of nonviable infested seed adjacent to *Phomopsis*-free seed did not result in increased infection of seedlings grown from seeds free from the pathogen, suggesting negligible importance of this type of seed-borne inoculum. However, increased seedling infection in the greenhouse in treatments where infested soybean crop residues were incorporated into a pre-sterilized potting medium, provided direct evidence for the importance of soybean crop residue as an inoculum source.

Significant negative correlations between seed-borne inoculum and field emergence, especially in 1980 when level of seed-borne inoculum was greater than in 1979, substantiated the findings of other researchers (Ellis et al., 1975; Ellis et al., 1979; Kulic and Schoen, 1981; Schmitthenner and Kmetz, 1979; Wallen and Seaman, 1963).

It was clearly demonstrated that *Phomopsis* could move to germinating seedlings, either from free conidia or infested soybean crop residue applied to sterilized soil, thus showing that soil-borne inoculum could directly infect plants. Although application of herbicide nine days after planting to seedlings prior to plating was effective in detecting infection when free conidia were the source of inoculum, it was not as effective when crop residue was the inoculum source, nor was it of value when applied at 23 days with either inoculum source. Herbicide application in bio-assaying crop residue-borne inoculum would probably be of little value, therefore.

Bio-assaying of soil and residue fractions in 1980 substantiated the conclusions of the field experiments by providing further direct evidence that soil-borne inoculum was the major source of inoculum, as seedling infection was, in general, greater using soil and residue from the CS field and lowest from the CC field. In addition, the greater level of seedling infection in the residue than soil fractions suggests that much more of the inoculum is in crop residue rather than in soil particles. From June 18 to July 30, the amount of crop residue declined at all three fields, probably as a result of machine cultivation between the two sampling times. Consequently, less crop residue-borne inoculum was available

in the fractions in the July 30 sampling and seedling infection in the assay was lower than on June 18. The low level of seedling infection for the September 14 sampling date was not expected as newly fallen leaves and petioles also were included when sampling each location. It is possible that the availability of soil- and/or crop residue-borne inoculum is reduced later in the growing season.

Results of the bio-assay using only crop residue suggest that sensitivity might be improved by this method. By incorporating the severity of seedling infection and the amount of surface residue into a bio-assay index, a quantitative estimate of soil-borne inoculum was made over a broad range. Although this method shows promise, problems remain in its practical application. A major problem is in the area of crop residue sampling. In this study, rotational practice fields were sampled before field preparation during the Spring of 1981. Consequently, surface residue was readily visible and obtainable for use in the bio-assay. However, many growers in the Midwest use a corn-soybean rotation. As such, limited availability of surface residue after the field has been tilled and grown to corn for one year may pose a problem in sampling for soybean residue. In addition, inoculum in soil particles is not accounted for in the bio-assay index as only crop residue is assayed.

A possible application of the bio-assay would be the incorporation of bio-assay indices with results of the recently proposed predictive scheme by McGee (1980) to provide an integrated pest management approach to controlling *Phomopsis* seed infection. According to the scheme, which is used to determine the need for foliar fungicide application, it is estimated

that 10.2 cm of rainfall is needed between mid-August and early September to increase pod-borne inoculum to a level where severe seed infection could occur under wet fall conditions. Depending on the level of soil-borne inoculum, however, possibly more or less rain might be needed to increase pod-infection. The bio-assay could, therefore, be used to determine the various levels of soil-borne inoculum which, when combined with the corresponding amounts of rainfall, result in raising pod-borne inoculum to the critical level.

SUMMARY AND CONCLUSIONS

The main objectives of this study were to demonstrate transmission of *Phomopsis* from seed- and soil-borne sources of inoculum, to determine the relative importance of each inoculum source, and to develop methods to quantify soil-borne inoculum.

In 1979 and 1980, soybean seedlots with varying amounts of *Phomopsis* seed-borne inoculum were planted in fields with rotational histories of continuous soybean, corn-soybean rotation, or continuous corn. These were assumed to represent varying amounts of soil-borne inoculum. *Phomopsis* infection of soybean stems, pods, and seeds was found to be greatest in the continuous soybean, less at the corn-soybean rotation, and least at the continuous corn fields. No relationship was found between seed-borne inoculum and disease severity, thus providing circumstantial evidence that soil-borne inoculum was the major source of inoculum for the disease. This finding was further substantiated by the disease pattern whereby infection was greater on lower than middle or upper plant parts at all locations and relationships between lodging and pod infection in which pod infection was the least at the continuous corn field, despite lodging being the greatest at that location. The possibility that the disease pattern might be a result of soil potassium levels in the different locations was eliminated by determining that soil and leaf levels at the locations were not at low enough concentrations to influence disease development.

To investigate transmissions of *Phomopsis* from infected and infested seed in more detail, *Phomopsis*-free, viable infected, and nonviable infested seed were planted in 1980 into fields with continuous soybean and

continuous corn rotation histories. In the continous soybean field, disease progression was similar on seedlings grown from all seed treatments over the 26 days that infection readings were taken. In the continuous corn field, however, infection was greater in seedlings grown from viable infected seed compared to other seed treatments. These results further emphasized the importance of soil-borne inoculum, but also suggested that seed-borne inoculum, particularly viable infected seed, might be of importance in introducing the pathogen into areas where soybeans had not previously been grown.

A greenhouse experiment to study *Phomopsis* transmission from non-viable infested seed showed that even in sterilized soil in which single viable *Phomopsis*-free seeds were planted together with two or four non-viable infested seeds, the latter had no effect on *Phomopsis* infection of the developing seedlings. When *Phomopsis* infested soybean crop residues were also incorporated, extensive seedling infection occurred. This provided direct evidence that crop residue could act as a source of inoculum.

An attempt was made to quantify soil-borne inoculum by measuring the degree of seedling infection obtained by growing *Phomopsis*-free seeds in soils infested by crop residue and soil from fields of the three rotational practices previously described. Although disease severity followed a decreasing pattern of severity from the continuous soybean through corn-soybean rotation and continuous corn fields, this did not provide a sensitive enough bio-assay to be of practical value. This experiment did show, however, that soil-borne inoculum was primarily located in soybean crop residues, although some was found in soil particles. A more sensitive

bio-assay was obtained by including the severity of seedling infection from soybean crop residue and the amount of crop residue in the sampled field into a bio-assay index. While this method shows promise, it does not, however, take into account inoculum in soil particles. Further work is needed to refine this technique.

LITERATURE CITED

American Phytopathological Society. 1975. Compendium of Soybean Diseases. J. B. Sinclair and M. C. Shurtleff, eds. Am. Phytopathol. Soc., St. Paul, MN. 69 pp.

Anderson, J. M. and C. C. McWhorter. 1976. The economics of common cocklebur control in soybean production. Weed Sci. 24:397-400.

Andrews, E. A. 1950. Stem blight of soybeans in Michigan. Plant Dis. Rep. 34:214.

Anonymous. 1981. Annual Progress Report, Clarion-Webster and Northern Research Centers, 1980. Iowa Agric. Home Econ. Exp. Stn. 24 pp.

Anonymous. 1976. Annual Report, International Institute of Tropical Agriculture. IITA, Ibadon, Nigeria. 219 pp.

Association of Official Seed Analysts Rules Committee. 1978. Rules for testing seeds. J. Seed Technol. 3(3):1-126.

Athow, K. L. 1973. Fungal diseases. Pages 459-489 *in* B. E. Caldwell, ed. Soybeans: Improvement, production, and uses. Am. Soc. Agron., Madison, WI.

Athow, K. L. and R. M. Caldwell. 1954. A comparative study of *Diaporthe* stem canker and pod and stem blight of soybean. Phytopathology 44: 319-325.

Athow, K. L. and F. A. Laviolette. 1973. Pod protection effects on soybean seed germination and infection with *Diaporthe phaseolorum* var. *sojae* and other microorganisms. Phytopathology 63:1021-1023.

Atlas de Gotuzz, E. 1970. Microorganisms isolated from normal and spotted soybean seed, their effect on the percentage of germination. Rev. Fac. Agron. Vet. Univ. B. Aires 18:7-16.

Backman, P. A., R. Rodriguez-Kabana, J. M. Hammond, and D. L. Thurlow. 1979. Cultivar, environment, and fungicide effects on foliar disease losses in soybeans. Phytopathology 69:562-564.

Balles, J. A. 1980. The effects of the field production environment, as influenced by planting date and cultivar on soybean (*Glycine max* (L.) Merr.) seed quality. M.S. thesis, University of Kentucky, Lexington.

Bernaux, P. 1979. Identification of some soybean diseases in Cameroon. Agronomie Tropicale 34:301-304.

Brandon, H. 1979. Point system guides producers' decisions on soybean foliar-applied fungicides. Delta Farm Press 36. 4 pp.

Bretches, K. 1979. Point systems may boost odds for fungicide profits. Progressive Farmer 94:44-46.

Bretz, T. W. 1944. Diseases observed on soybeans in Missouri. Plant Dis. Rep. 28:832-834.

Caviness, C. E. 1966. Spacing studies with Lee soybeans. Ark. Agric. Exp. Stn. Bull. 713. 19 pp.

Chagas, D. and O. D. Dhingra. 1979. Effect of timing of weed control on the incidence of seedborne fungi in dry bean seeds. Fitopatologia Brasileira 4:423-426.

Chamberlain, D. W. and L. E. Gray. 1974. Germination, seed treatment, and microorganisms in soybean seed produced in Illinois. Plant Dis. Rep. 58:50-54.

Crall, J. M. 1950. Soybean diseases in Iowa in 1949. Plant Dis. Rep. 34: 96-97.

Crittenden, H. W. and L. V. Svec. 1974. Effect of potassium on the incidence of *Diaporthe sojae* in soybean. Agron. J. 66:696-697.

Dhingra, O. D. and J. F. da Silva. 1978. Effect of weed control on the internally seedborne fungi in soybean seeds. Plant Dis. Rep. 62:513-516.

Dhingra, O. D. and T. Sediyama. 1979. Effect of planting and harvesting time on seed infection of soybean by *Phomopsis sojae* and *Fusarium semitectum*. Fitopatologia Brasileira 4:467-472.

Do Amaral, J. F. 1951. Principal diseases of cultivated plants in the state of Sao Paulo and their control. Biologico 17:179-188.

Dunleavy, J. 1957. Variation in pathogenicity of *Diaporthe phaseolorum* var. *sojae* to soybean. Iowa St. Coll. J. Sci. 32:105-109.

Ellis, M. A. and J. B. Sinclair. 1975. Movement of methyl 2-benzimidazole-carbamate into soybean seeds after foliar application of benomyl. Phytopathology 65:1387-1389.

Ellis, M. A. and J.B. Sinclair. 1976. Effect of benomyl field sprays on internally-borne fungi, germination, and emergence of late-harvested soybean seeds. Phytopathology 66:680-682.

Ellis, M.A., M. B. Ilyas, F. D. Tenne, J. B. Sinclair and H. L. Palm. 1974a. Effect of foliar applications of benomyl on internally seedborne fungi and pod and stem blight in soybean. Plant Dis. Rep. 58: 760-763.

Ellis, M. A., C. C. Machado, C. Prasartsee and J. B. Sinclair. 1974b. Occurrence of *Diaporthe phaseolorum* var. *sojae* (*Phomopsis* sp.) in various soybean seed lots. Plant Dis. Rep. 58:173-176.

Ellis, M. A., M. B. Ilyas and J. B. Sinclair. 1975 Effect of three fungicides on internally seed-borne fung germination of soybean seeds. Phytopathology 65:553-556.

Ellis, M. A., S. R. Foor and J. B. Sinclair. 1976. Effect of benomyl sprays on internally-borne fungi and germination of delay-harvested soybean seeds. Phytopathologisch Zeitschrift 85:159-162.

Ellis, M. A., E. H. Paschal and E. J. Ravalo. 1978a. Effects of growing location on internally-seedborne fungi (*Phomopsis*, *Lasiodiplodia theobromae*, *Fusarium semitectum*, *Alternaria tenuissima*, *Aspergillus*), seed germination, and field emergence of pigeon pea in Puerto Rico. J. Agric. Univ. P. R. 62:355-360.

Ellis, M. A., O. Zambrano and E. H. Paschal. 1978b. Evaluation of seed treatment compounds on good and poor quality soybean seeds under tropical conditions, 1977. Fungicide-nematicide test results of 1977. Vol. 33:173.

Ellis, M. A., E. H. Paschal, P. E. Powell and F. D. Tenne. 1979. Internally seedborne fungi of soyabean in Puerto Rico and their effect on seed germination and field emergence. Tropical Agriculture 56: 171-174.

Ensminger, M. P. and T. S. Abney. 1978. Effect of foliar fungicides on fungal colonization and seed quality of soybeans in the mid-West. Phytopathology News 12:144.

Ersek, T. 1978. Pod and stem blight of soybeans in Hungary (short communication). Acta Phytopathologica Academiae Scientiarum Hungaricae 13:365-367.

Fehr, W. R., C. E. Caviness, D. T. Burmood and J. S. Pennington. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merr. Crop Sci. 11:929-931.

Fenne, S. B. 1949. Alfalfa and soybean diseases in Virginia, 1948. Plant Dis. Rep. 26:383.

Fett, W. F. 1978. Volunteer soybeans: Survival sites for soybean pathogens between seasons in southern Brazil. Plant Dis. Rep. 62:1013-1016.

Gardner, M. W. 1928. Indiana plant diseases, 1927. Proc. Indiana Acad. Sci. 38:143-157.

Gerdemann, J. W. 1954. The association of *Diaporthe phaseolorum* var. *sojae* with root and basal stem rot of soybean. Plant Dis. Rep. 38: 742-743.

Girard, J. C. 1979. Detection of two diseases on soybean seeds from Senegal. Agronomie Tropicale 34:305-307.

Green, D. E., I. E. Pinnel and L. E. Cavanah. 1964. Effect of planting date and maturity on soybean seed quality. Agronomy Guide 165-168.

Hammond, J. M. and P. A. Backman. 1978. Soybean seed treatment trials, 1977. Fungicide-Nematicide Test Results of 1977. Vol. 33:174.

Hanway, J. J. and C. R. Weber. 1971. N, P, and K percentages in soybean (*Glycine max* (L.) Merrill) plant parts. Agron. J. 63:286-290.

Harter, L. L. 1917. Pod blight of the lima bean caused by *Diaporthe phaseolorum*. J. Agr. Res. 11:473-504.

Harter, L. L. and E. C. Field. 1912. *Diaporthe*, the ascogenous form of sweet potato dry rot. Phytopathology 2:121-124.

Hepperly, P. R. and J. B. Sinclair. 1978a. Soybean seed treatments, 1977. Fungicide-Nematicide Test Results of 1977. Vol. 33:175.

Hepperly, P. R. and J. B. Sinclair. 1978b. Quality losses in *Phomopsis*-infected soybean seeds. Phytopathology 68:1684-1687.

Hepperly, P. R. and J. B. Sinclair. 1980. Association of plant symptoms and pod position with *Phomopsis sojae* seed infection and damage in soybean. Crop Sci. 20:379-381.

Hepperly, P. R., M. C. Shurtleff, B. J. Jacobsen and J. B. Sinclair. 1978. Pod and stem blight of soybean. Report of plant diseases no. 509. Cooperative Extension Service, Univ. of Illinois, Champaign-Urbana.

Hepperly, P. R., G. R. Bowers, Jr., J. B. Sinclair and R. M. Goodman. 1979. Predisposition to seed infection by *Phomopsis sojae* in soybean plants infected by soybean mosaic virus. Phytopathology 69:846-848.

Hepperly, P. R., B. L. Kilpatrick and J. B. Sinclair. 1980. *Abutilon theophrasti*: Wild host for three fungal parasites of soybean. Phytopathology 70:307-310.

Hildebrand, A. A. 1954. Observations on the occurrence of the stem canker and pod and stem blight fungi on mature stems of soybean. Plant Dis. Rep. 38:640-646.

Hildebrand, A. A. 1956. Observations on stem canker and pod and stem blight of soybeans in Ontario. Can. J. Bot. 34:577-599.

Hildebrand, A. A. and L. W. Koch. 1947. Soybean diseases in Ontario and effectiveness of seed treatment. Phytopathology 37:114-124.

Hill, H. C., N. L. Horn and W. L. Steffens. 1981. Mycelial development and control of *Phomopsis sojae* in artificially inoculated soybean stems. Plant Dis. 65:132-134.

Horn, N. L., F. N. Lee and R. B. Carver. 1975. Effects of fungicides and pathogens on yields of soybeans. Plant Dis. Rep. 59:724-728.

Huber, D. M. 1979. Central corn belt. Pp. 1-35. *In* R. D. Rouse, ed. Pest management strategies vol. II. Office of Technology Assessment, Congress of the United States, Washington, D.C.

Hulea, A., I. Rosca and S. Bunescu. 1973. Fungi pathogenic on soybean recently detected in Rumania. Probleme de Protectia Plantelor 1:177-190.

Ilyas, M. B., O. D. Dhingra, M. A. Ellis and J. B. Sinclair. 1975. Location of mycelium of *Diaporthe phaseolorum* var. *sojae* and *Cercospora kikuchii* in infected soybean seeds. Plant Dis. Rep. 59:17-19.

Jacobsen, B. J. 1979. Foliar fungicides for soybean seed production. Pp. 13-22. *In* J. S. Burris, ed. 1979 Proceedings of the Seed Technology Conference, Ames, Iowa, August 23-24, 1979, Seed Science Center, Iowa State University.

Jeffers, R. L. and A. F. Schmitthenner. 1976. Soybean seed quality and yield as affected by potassium nutrition and benomyl applications. Agron. Abstr. 1976:94.

Jones, J. B. 1967. Interpretation of plant analysis for several agronomic crops. Pp. 49-58. *In* Soil testing and plant analysis, Part II. Plant analysis. SSSA Special Publ. Series No. 2. Soil Sci. Soc. Am., Madison, WI.

Kilpatrick, R. A. and E. E. Hartig. 1955. Effect of planting date on incidence of fungus infection of Ogden soybean seeds grown at Walnut Hills, Fla. Plant Dis. Rep. 39:174-176.

King, T. H. 1948. Pod and stem blight on soybeans in Ohio. Plant Dis. Rep. 32:193.

Kmetz, K. T. 1975. Soybean seed decay: Studies on disease cycles, effects of cultural practices on disease severity and differentiation of the pathogens *Phomopsis* sp., *Diaporthe phaseolorum* var. *sojae* and *D. phaseolorum* var. *caulivora*. Ph.D. Dissertation. Ohio State University, Columbus.

Kmetz, K. T., C. W. Ellett and A. F. Schmitthenner. 1974. Isolation of seedborne *Diaporthe phaseolorum* and *Phomopsis* from immature soybean plants. Plant Dis. Rep. 58:978-982.

Kmetz, K. T., C. W. Ellett and A. F. Schmitthenner. 1975. Identification of *Phomopsis* and *Diaporthe* isolates associated with soybean seed decay by colony morphology, symptom development and pathogenicity. Proc. Am. Phytopathol. Soc. 2:61. (Abstr.)

Kmetz, K. T., A. F. Schmitthenner and C. W. Ellett. 1978. Soybean seed decay: Prevalence of infection and sympton expression caused by *Phomopsis* sp., *Diaporthe phaseolorum* var. *sojae*, and *D. phaseolorum* var. *caulivora*. Phytopathology 68:836-840.

Kmetz, K. T., C. W. Ellett and A. F. Schmitthenner. 1979. Soybean seed decay: Sources of inoculum and nature of infection. Phytopathology 69:798-801.

Koch, L. W. and A. A. Hildebrand. 1944. Soybean diseases in southwestern Ontario in 1943. Can. Plant Dis. Surv. Ann. Rept. 23:29-32.

Koehler, B. 1944. New developments in soybean disease studies. Soybean Dig. 4(8):6-7.

Kulik, M. M. and J. F. Schoen. 1981. Effect of seedborne *Diaporthe phaseolorum* var. *sojae* on germination, emergence, and vigor of soybean seedlings. Phytopathology 71:544-547.

Kurata, H. 1960. Studies on the fungal diseases of soybean in Japan. Bull. Nat. Inst. Agric. Sci., Tokyo, Ser. C. 12:1-154.

Larsh, H. W. 1944a. Diseases observed on soybeans in Arkansas. Plant Dis. Rep. 28:956-957.

Larsh, H. W. 1944b. Summary report of plant diseases in Oklahoma. 1943. Plant Dis. Rep. Suppl. 149:317-326.

Lehman, S. G. 1922. Pod and Stem blight of the soybean. J. Elisha Mitchell Sci. Soc. 38:13.

Lehman, S. G. 1923. Pod and stem blight of soybean. Ann. Missouri Bot. Bard. 10:111-169.

Liu, S. T. 1948. Seed-borne diseases of soybean. Bot. Bull. Acad. Sinica. 2:69-80.

Luttrell, E. W. 1947. *Diaporthe phaseolorum* var. *sojae* on crop plants. Phytopathology 37:445-465.

McGee, D. C. 1979. Fungicides and the disease cycle. Pp. 1-10. *In* J. S. Burris, ed. 1979 Proceedings of the Seed Technology Conference, Ames, Iowa, August 23-24, 1979, Seed Science Center, Iowa State University.

McGee, D. C. 1980. New predictive scheme for foliar fungicide use on soybeans. Iowa Seed Science 2(2):9-10. Seed Science Center, Iowa State University.

McGee, D. C. and C. L. Brandt. 1979. Effect of foliar application of benomyl on infection of soybean seeds by *Phomopsis* in relation to time of inoculation. Plant Dis. Rep. 63:675-677.

McGee, D. C., C. L. Brandt and J. S. Burris. 1980. Seed mycoflora of soybeans relative to fungal interactions, seedling emergence, and carry over of pathogens to subsequent crops. Phytopathology 70:615-617.

Mannerucci, G. F. and P. Gambogi. 1978. *Phomopsis* sp. on soybean seed in Toscany, Italy. Informatore Fitopatologico 28:7-11.

Martyn, E. B. 1933. Preliminary list of diseases of economic plants in British Guiana. Kew Roy. Gard., Kew Bull. Misc. Inf. 1933:107-110.

Mascarenhas, H. A. A., M. A. C. Miranda, O. C. Bataglia, O. F. Tiselli, N. R. Braga and J. Soave. 1976. Effect of potassium fertilization on soybean infection by *Diaporthe phaseolorum* (Cke. and Ell.) Sacc. var. *sojae* (Lehman) Wehm. Summa Phytopathologica 2:230-234.

Nath, R., S. B. Mathur and P. Neergaard. 1970. Seedborne fungi of mungbean (*Phaseolorum aureus* Roxb.) from India and their significance. Proc. Int. Seed Testing Assn. 35:225-241.

Nicholson, J. F., O. D. Dhingra and J. B. Sinclair. 1972. Internal seedborne nature of *Sclerotinia sclerotiorum* and *Phomopsis* sp. and their effects on soybean seed quality. Phytopathology 62:1261-1263.

Ohlrogge, A. J. 1960. Mineral nutrition of soybeans. Advance. Agron. 12:229-263.

Pady, S. M. 1944. Notes on the plant disease survey in Kansas, August to November, 1943. Plant Dis. Rep. Supp. 149:313-316.

Patino, H. C. 1967. Diseases of oleaginous annuals in Columbia. Agricultura Trop. 23:532-539.

Peterson, J. L. and R. F. Strelecki. 1965. The effect of variants of *Diaporthe phaseolorum* on soybean germination and growth in New Jersey. Plant Dis. Rep. 49:228-229.

Petrak, E. and H. Sydow. 1936. A small contribution to the knowledge of the fungus flora of Japan. Ann. Mycol. 34:237-251.

Petty, M. A. 1943. Soybean disease incidence in Maryland in 1942 and 1943. Plant Dis. Rep. 27:347-349.

Probst, A. H. 1945. Influence of spacing on yield and other characters in soybeans. J. Am. Soc. Agron. 37:549-554.

Ross, J. P. 1975. Effect of overhead irrigation and benomyl sprays on late-season foliar diseases, seed infection, and yields of soybean. Plant Dis. Rep. 59:809-813.

Roy, K. W. and T. S. Abney. 1977. Antagonism between *Cercospora kikuchii* and other seedborne fungi of soybeans. Phytopathology 67:1062-1066.

Sasaki, S. 1929. A *Phomopsis* disease of soybeans. Ann. Agric. Exp. Sta. Gov.-Gen. Chosen 4:1-28.

Schmitthenner, A. F. and K. T. Kmetz. 1979. Role of *Phomopsis* sp. in the soybean seed rot problem. Pp. 355-366. *In* F. T. Corbin, ed. Proceedings World Soybean Research Conference II. Westview Press, Boulder, Colorado.

Shanor, L. and C. F. Taylor. 1944. *Diaporthe sojae* on cowpeas. Plant Dis. Rep. 28:81.

Shortt, B. J., A. P. Grybauskas, F. D. Tenne and J. B. Sinclair. 1981. Epidemiology of *Phomopsis* seed decay of soybean in Illinois. Plant Dis. 65:62-64.

Shoshiashvili, I. 1940. With reference to studies on soybean and groundnut diseases. Bull. Georgian Expt. Sta. Plant Prot. Ser. A. Phytopathology 2:271-283.

Siddiqi, M. A. 1971. Report of senior plant pathologist, Malawi, 1969-1970. 10 pp.

Smith, T. J., H. M. Camper, M. T. Carter, G. D. Jones and M. W. Alexander. 1961. Soybean performance in Virginia as affected by variety and planting date. Virginia Agric. Exp. Stn. Bull. 526.

Spilker, D. A. 1977. The effect of foliar potassium and phosphorus, and temperature and humidity during maturation on the reduction of soybean seed quality by *Phomopsis* sp. M.S. Thesis. Ohio State University, Columbus.

Svec, L. V., A. K. Andrews and H. W. Crittenden. 1976. Soybean (*Glycine max* (L.) Merr.) yield and disease incidence with potassium fertilization. Communications Soil Sci. Plant Analysis 7:727-741.

Threiner, J. T., T. Kommedahl and R. J. Klug. 1959. Hybridization between radiation-induced mutants of two varieties of *Diaporthe phaseolorum*. Phytopathology 49:797-801.

Turnipseed, S. 1979. Soybeans in the southeast. Pp. 1-57. *In* R. D. Rouse, ed. Pest management strategies, Vol. II. Office of Technology Assessment, Congress of the United States, Washington, D.C.

Walker, E. A. 1944. Diseases observed on soybeans in New Jersey, Delaware, and Maryland. Plant Dis. Rep. 28:1006-1008.

Walla, A. H. 1977. 1975 Southern states soybean disease loss estimates. Southern Soybean Disease Workers, Soybean Disease Loss Estimate Committee. Plant Dis. Rep. 61:42.

Wallen, V. R. and T. F. Cuddy. 1960. Relation of seed-borne *Diaporthe phaseolorum* to the germination of soybeans. Proc. Assoc. Off. Seed Anal. 50:137-140.

Wallen, V. R. and W. L. Seaman. 1963. Seed infection of soybean by *Diaporthe phaseolorum* and its influence on host development. Can. J. Bot. 41:13-21.

Wehmeyer, L. E. 1933. The genus *Diaporthe* Nitschke and its segregates. Univ. of Michigan Press, Ann Arbor. 349 pp.

Weimer, J. L. 1947. Disease survey of soybean nurseries in the South. Plant Dis. Rep. Suppl. 168:27-53.

Welch, A. W. and J. C. Gilman. 1948. Hetero- and homo-thallic types of *Diaporthe* on soybeans. Phytopathology 38:628-637.

Whitehead, M. D. 1966. Stem canker and blight of birdsfoot-trefoil and soybeans incited by *Diaporthe phaseolorum* var. *sojae*. Phytopathology 56:396-400.

Whitney, G. 1978. 1976 Southern United States disease losses. Pp. 1-3. *In* Proceedings Southern Soybean Disease Workers Fifth Annual Meeting. Ft. Walton Beach, Florida, March 21-23, 1978.

Wilcox, J. R. and T. S. Abney. 1971. Association of pod and stem blight with stem breakage in soybeans. Plant Dis. Rep. 55:776-777.

Wilcox, J. R., F. A. Laviolette and K. L. Athow. 1974. Deterioration of soybean seed quality associated with delayed harvest. Plant Dis. Rep. 58:130-133.

Wolf, F. A. and S. G. Lehman. 1920. Notes on new or little known plant diseases in North Carolina in 1920. N. Carlina Agric. Exp. Stn. Ann. Rep. 43:55-58.

Wolf, F. A. and S. G. Lehman. 1926. Diseases of soybeans which occur in North Carolina and the Orient. J. Agric. Res. 33:391-396.

ACKNOWLEDGEMENTS

Sincere appreciation is extended to my major professor, Dr. Denis C. McGee, for his guidance, support, and patience throughout my graduate career at Iowa State University. His help along with that of the other committee members, Dr. Joseph S. Burris, Dr. Hideo Tachibana, Dr. Lois H. Tiffany, and Dr. Detroy E. Green, is greatly appreciated.

I would also like to thank the seedsmen of Iowa and Dr. August F. Schmitthenner at Ohio State University for their donation of seed material.

I wish to extend a very warm and sincere debt of gratitude to my wife, Debby, for her assistance in field and laboratory work and for the preparation of the figures in the text of this dissertation. For her patience and understanding, I dedicate this dissertation to my loving wife.

Finally, I would like to thank the staff of the Seed Science Center for their field and laboratory assistance.

APPENDIX

Table 1. 1979 field experiment (excluding plant height analysis)

Model	df[a]	Stem infection				
		V4/APDA	V4/Roundup	R2/APDA	R2/Roundup	R8/visible
Total	71					
1) Location	2	48959.72**	35094.17**	60197.18**	1392.21**	1301.39**
Error (a)	9	159.91	187.18	610.77	19.17	153.24
2) Variety	2	138.89	126.41	600.02	589.82	536.49
1*2	4	161.81	130.57	474.79	264.56*	111.81
Error (b)	18	206.02	117.65	481.75	68.31	129.63
3) Seed-borne inoculum	1	12.50	3.21	1.10	1.23	12.50
1*3	2	37.50	270.54*	195.52	34.39	29.17
2*3	2	16.67	42.14	22.85*	28.62	29.17
1*2*3	4	147.92	93.22	1.33	84.33	64.58
Error (c)	27	64.35	74.39	85.87	128.56	80.09

[a]Error term for location = error (a), variety = 1*2, 1*2 = error (b), seed-borne inoculum = 1*3, 1*3 = error (c), 2*3 = 1*2*3, 1*2*3 = error (c).

*Significant at the 0.05 level of probability.

**Significant at the 0.01 level of probability.

Pod infection			Seed infection			
(Flower) R2	R7	R8	R7	R8	Emergence	Lodging
506.14**	8325.92**	9118.90*	1640.46	380.48*	9369.85**	18.50**
57.82	256.02	163.09	87.92	61.13	236.61	0.63
29.06	158.55	190.28	571.00	435.38	850.89	4.67**
39.08	37.99	440.73*	149.14	111.85	126.39	0.04
33.55	52.06	119.26	56.71	43.80	110.87	0.40
14.49	200.67	35.42	0.52	52.53	86.68	1.68
31.54	60.11	207.58	37.18	23.34	89.18	0.22
20.29	93.42	26.52	28.91	8.26	892.39**	0.39
22.23	47.24	25.76	18.94	17.83	28.26	0.68
32.90	67.65	75.64	20.69	12.66	52.06	0.25

Table 2. 1980 field experiment (excluding plant height analysis)

Model	df[a]	Stem infection				
		V4/APDA	V4/ Roundup	Rc/APDA	R3/ Roundup	R8/ visible
Total	71					
1) Location	2	23450.00**	8468.06**	3704.77**	11164.25**	5197.53*
Error (a)	9	296.30	221.30	166.20	761.61	856.33
2) Seed-borne inoculum	5	393.33	325.56	129.17	591.74	426.92
1*2	10	138.33	144.72	65.83	758.86	218.61
Error (b)	45	281.85	174.63	183.98	373.95	508.75

[a]Error term for location = error (a), seed-borne inoculum = 1*2, 1*2 = error (b).

*Significant at the 0.05 level of probability.

**Significant at the 0.01 level of probability.

	Pod infection			Seed infection			
R8/stem rating	R3	R7	R8	R7	R8	Emergence	Lodging
7.77**	4.74**	6159.25**	10826.89**	321.67**	457.93**	3459.43**	3.43*
0.41	0.36	62.72	73.35	16.27	55.47	39.74	0.58
0.37	0.89	308.96	162.27	5.07	39.52	2448.48**	1.19
0.27	0.89	199.80**	122.68	8.39	15.61	48.68	0.51
0.14	1.46	55.70	80.93	7.22	18.78	45.81	0.34

Table 3. 1979 field experiment (including plant height analysis)

Model	df[a]	R8 pod infection	R8 seed infection
Total	215		
1) Location	2	37307.46**	1141.28*
Error (a)	9	468.69	182.97
2) Variety	2	557.26*	1309.19
1*2	4	1313.91*	335.14
Error (b)	18	371.75	131.56
3) Seed-borne inoculum	1	116.45	157.25
1*3	2	325.98	70.95
2*3	2	87.99	25.18
1*2*3	4	96.45	53.40
Error (c)	27	220.51	37.85
4) Plant height	2	20938.89	2390.19
1*4	4	3577.23**	419.23**
2*4	4	81.20	507.00
3*4	2	2.50	86.62
1*2*4	8	321.05*	136.45**
2*3*4	4	61.63	28.92
1*3*4	4	23.38	47.09
1*2*3*4	8	128.46	25.61
Error (d)	108	134.37	34.33

[a]Error term for location = error (a), variety = 1*2, 1*2 = error (b), seed-borne inoculum - 1*3, 1*3 = error (c), 2*3 = 1*2*3, 1*2*3 = error (c), plant height = 1*4, 1*4 = error (d), 2*4=1*2*4, 3*r=1*3*4, 1*2*4 = error (d), 2*3*4 = 1*2*3*4, 1*3*4=error (d), 1*2*3*4 = error (d)

*Significant at the 0.05 level of probability.

**Significant at the 0.01 level of probability.

Table 4. 1980 field experiment (including plant height analysis)

Model	df[a]	R8 pod infection	R8 seed infection	R8 stem rating
Total	215			
1) Locations	2	32458.80**	1367.63**	23.10**
Error (a)	9	220.06	167.26	1.19
2) Seed-borne inoculum	5	486.30	118.52	1.09
1*2	10	368.24	47.10	0.74
Error (b)	45	242.65	56.09	0.42
3) Plant height	2	31842.13	2142.52	13.10*
1*3	4	7099.07	761.30**	0.93**
2*3	10	88.24	48.12	0.13
1*2*3	20	145.19	25.83	0.07
Error (c)	108	100.00	46.84	0.04

[a]Error term for location = error (a), seed-borne inoculum = 1*2, 1*2 = error (b), plant height = 1*3, 1*3 = error (c), 2*3 = 1*2*3, 1*2*3 = error (c).

*Significant at the 0.05 level of probability.

**Significant at the 0.01 level of probability.

Table 5. 1980 field experiment (with simulated seed-borne inoculum)

Model	df[a]	Cotyledon infection	df	Hypocotyl infection
Total	79		159	
1) Location	1	3380.00*	1	64802.50**
Error (a)	6	436.67	6	530.83
2) Seedlot	4	2839.38	4	358.44
1*2	4	1408.13*	4	200.94
Error (b)	24	467.92	24	120.94
3) Time	1	4805.00	3	6654.17
1*3	1	5445.00**	3	5554.17**
Error (c)	6	161.67	18	120.28
2*3	4	320.63	12	46.35
1*2*3	4	291.88**	12	42.19
Error (d)	24	63.75	72	94.41

[a]Error term for location = error (a), seed lot = 1*2, 1*2 = error (b), time = 1*3, 1*3 = error (c), 2*3 = 1*2*3, 1*2*3 = error (d).

*Significant at the 0.05 level of probability.

**Significant at the 0.01 level of probability.

Table 6. Greenhouse experiment (with simulated seed-borne inoculum)

Model	df	Mean sq
Total	17	
Block	1	555.56*
1) Residue	2	1205.66**
2) Seed	2	155.56
1*2	4	172.22
Error	8	93.06

*Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.

Table 7. Soybean stem infection by *Phomopsis* in 1979 as affected by rotational practice, variety, seed-borne inoculum, and plant growth stage

Rotational practice	Variety	Seed-borne inoculum (%)	Plant growth stage: V4-V5 APDA[a]	V4-V5 Roundup[b]	R2 APDA	R2 Roundup	R8 Visible[c]
Continuous soybean	Amsoy 71	2.0	85.0%[d]	62.5	95.0	100.0	87.5
		10.0	77.5	67.5	97.5	100.0	90.0
		Mean	81.3	65.0	96.3	100.0	88.8
	Wells	0.0	82.5	80.0	95.0	100.0	97.5
		6.0	87.5	70.0	100.0	100.0	97.5
		Mean	85.0	75.0	97.5	100.0	97.5
	Beeson	1.0	87.5	82.5	97.7	100.0	90.0
		8.0	80.0	72.5	100.0	100.0	80.0
		Mean	83.8	77.5	97.4	100.0	85.0
	Overall mean		83.3	72.5	97.0	100.0	90.4
Corn-soybean	Amsoy 71	2.0	10.0	10.8	100.0	100.0	95.0
		10.0	22.5	15.8	90.0	100.0	92.5
		Mean	16.3	13.5	95.0	100.0	93.8
	Wells	0.0	17.5	5.6	70.0	95.0	97.5
		6.0	10.0	19.9	65.0	89.6	97.5
		Mean	13.8	12.7	67.5	92.3	97.5
	Beeson	1.0	2.5	8.1	82.5	95.0	100.0
		8.0	2.5	12.5	77.5	94.5	97.5
		Mean	2.5	10.3	80.0	94.7	98.8

	Overall mean		10.8	12.1	80.8	95.7	96.7
Continuous corn	Amsoy 71	2.0	2.5	0.0	5.0	97.5	72.5
		10.0	0.0	0.0	5.0	92.5	67.5
		Mean	1.3	0.0	5.0	95.0	70.0
	Wells	0.0	0.0	5.0	0.0	66.4	77.5
		6.0	0.0	0.0	2.5	79.7	82.5
		Mean	0.0	2.5	1.3	73.1	80.0
	Beeson	1.0	0.0	2.5	2.5	87.5	67.5
		8.0	0.0	2.5	5.0	87.5	72.5
		Mean	0.0	2.5	3.8	87.5	70.0
	Overall mean		0.4	1.6	3.3	85.2	73.3
LSD (P=0.05)[e]			8.5	8.9	16.1	2.9	8.1

[a]APDA = potato dextrose agar plating.

[b]Roundup = plants sprayed to runoff with herbicide to simulate senescence.

[c]Visible = stems with visible *Phomopsis* pycnidia.

[d]Four replicates/10 plants.

[e]LSD for comparing overall rotational practice means.

Table 8. Soybean stem infection by *Phomopsis* in 1980 as affected by rotational practice, seed-borne inoculum, and plant growth stage

Rotational practice	Seed-borne Inoculum (%)	Plant growth stage					
		V4-V5		R3		R8	
		APDA[a]	Roundup[b]	APDA	Roundup	Visible[c]	Index[d]
Continuous soybean	0.0	72.5%[e]	40.0	25.0	80.0	97.5	1.7
	4.0	70.0	32.5	22.5	57.5	95.0	1.4
	26.0	60.0	37.5	17.5	57.5	100.0	2.2
	37.0	62.5	57.5	40.0	68.9	100.0	1.5
	46.0	67.5	45.0	27.5	77.5	97.5	1.7
	77.0	72.5	55.0	25.0	78.9	100.0	1.9
	Mean	67.5	44.6	26.3	70.1	98.3	1.7
Corn-soybean	0.0	37.5	10.0	10.0	42.5	56.1	0.6
	4.0	27.5	25.0	10.0	57.5	62.5	0.8
	26.0	22.5	15.0	12.5	22.5	77.5	1.0
	37.0	27.5	20.0	12.5	20.0	70.0	0.6
	46.0	42.5	15.0	7.5	45.0	80.0	1.3
	77.0	52.5	22.5	12.5	41.7	92.5	1.5
	Mean	35.0	17.9	10.8	38.2	73.1	1.0
Continuous corn	0.0	0.0	0.0	0.0	5.0	62.0	0.4
	4.0	2.5	5.0	0.0	18.6	77.2	0.8
	26.0	5.0	10.0	0.0	32.5	67.5	0.6
	37.0	5.0	12.5	5.0	35.0	77.5	0.7
	46.0	7.5	12.5	2.5	37.0	75.0	0.6
	77.0	10.0	10.0	2.5	45.0	76.4	0.6
	Mean	5.0	8.3	1.7	28.9	72.6	0.6
LSD (P=0.05)[f]		10.2	9.7	8.4	18.8	19.1	0.4

[a]APDA = potato dextrose agar plating.

[b]Roundup = plants sprayed to runoff with herbicide to simulate senescence.

[c]Visible = stems with visible *Phomopsis* pycnidia.

[d]Index = stem rating index, where 0 = no visible pycnidia, 1 = 1-25% stem area colonized, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.

[e]Four replicates/10 plants.

[f]LSD for comparing rotational practice means.

Table 9. Soybean stem infection by *Phomopsis* at the R8 growth stage in 1980 as affected by rotational practice, seed-borne inoculum, and plant height

Rotational practice	Seed-borne inoculum (%)	Position on plant			LSD (P=0.05)
		Lower	Middle	Upper	
Continuous soybean	0.0	2.4[a,b]	1.7	1.1	
	4.0	1.8	1.5	1.0	
	26.0	3.3	2.0	1.3	
	37.0	2.1	1.3	1.0	
	46.0	2.3	1.7	1.1	
	77.0	2.5	1.8	1.3	
	Mean	2.4	1.7	1.1	0.2
Corn-soybean	0.0	0.8	0.7	0.3	
	4.0	0.9	0.8	0.5	
	26.0	1.4	1.0	0.6	
	37.0	0.9	0.7	0.4	
	46.0	1.6	1.4	0.9	
	77.0	1.9	1.6	0.9	
	Mean	1.3	1.0	0.6	0.1
Continuous corn	0.0	0.6	0.6	0.2	
	4.0	1.0	0.9	0.4	
	26.0	0.9	0.8	0.2	
	37.0	0.9	0.7	0.3	
	46.0	1.0	0.7	0.3	
	77.0	0.9	0.6	0.3	
	Mean	0.9	0.7	0.3	0.1
Overall mean		1.5	1.1	0.7	0.4

[a]Stem rating index, where 0 = no visible pycnidia, 1 = 1-25% stem area colonized, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.

[b]Four replicates/10 plants.

Table 10. Soybean flower (R2) and pod (R7, R8) infection by *Phomopsis* in 1979 as affected by rotational practice, variety, seed-borne inoculum, and plant growth stage

Rotational practice	Variety	Seed-borne inoculum (%)	Plant growth stage R2	R7	R8
Continuous soybean	Amsoy 71	2.0	14.2%[a]	80.8	80.8
		10.0	7.5	82.5	80.9
		Mean	10.8	81.7	80.8
	Wells	0.0	10.0	89.2	83.3
		6.0	11.7	89.2	82.5
		Mean	10.8	89.2	82.9
	Beeson	1.0	6.8	90.9	77.5
		8.0	6.9	83.3	79.2
		Mean	6.9	87.1	78.4
	Overall mean		9.5	86.0	80.7
Corn-soybean	Amsoy 71	2.0	10.8	95.0	92.5
		10.0	8.4	95.9	95.7
		Mean	9.6	95.4	94.1
	Wells	0.0	9.2	100.0	96.7
		6.0	8.3	95.9	95.8
		Mean	8.8	97.9	96.3
	Beeson	1.0	7.5	96.7	92.4
		8.0	2.5	96.7	95.0
		Mean	5.0	96.7	93.7
	Overall mean		7.8	96.7	94.7
Continuous corn	Amsoy 71	2.0	0.0	56.7	64.4
		10.0	0.0	57.5	64.2
		Mean	0.0	57.1	64.3
	Wells	0.0	0.0	67.5	46.7
		6.0	0.0	52.5	40.0
		Mean	0.0	60.0	43.3
	Beeson	1.0	0.0	67.5	66.8
		8.0	5.0	60.8	55.2
		Mean	2.5	64.2	61.0
	Overall mean		0.8	60.4	56.2
LSD (P=0.05)[b]			5.0	10.4	8.3

[a]Potato dextrose agar plating; four replicates/30 pods.

[b]LSD for comparing overall rotational practice means.

Table 11. Soybean pod infection by *Phomopsis* at the R8 growth stage in 1979 as affected by rotational practice, variety, seed-borne inoculum, and plant height

Rotational practice	Variety	Seed-borne inoculum (%)	Position on plant			LSD (P=0.05)
			Lower	Middle	Upper	
Continuous soybean	Amsoy 71	2.0	100.0%[a]	87.5	55.0	
		10.0	100.0	95.0	47.5	
		Mean	100.0	91.3	51.3	
	Wells	0.0	100.0	92.5	57.5	
		6.0	100.0	90.0	57.5	
		Mean	100.0	91.3	57.5	
	Beeson	1.0	100.0	85.0	47.5	
		8.0	100.0	85.0	52.5	
		Mean	100.0	85.0	50.0	
	Mean		100.0	89.2	52.9	7.2
Corn-soybean	Amsoy 71	2.0	97.0	92.5	87.5	
		10.0	100.0	94.5	92.2	
		Mean	98.8	93.5	89.9	
	Wells	0.0	100.0	92.5	97.5	
		6.0	100.0	95.0	92.2	
		Mean	100.0	93.8	94.9	
	Beeson	1.0	100.0	100.0	76.7	
		8.0	100.0	97.5	87.5	
		Mean	99.6	95.3	88.9	
	Mean		99.6	95.3	88.9	4.4
Continuous corn	Amsoy 71	2.0	82.5	60.8	48.6	
		10.0	85.0	52.5	55.0	
		Mean	83.8	56.7	51.8	
	Wells	0.0	82.5	37.5	20.0	
		8.0	70.0	40.0	10.0	
		Mean	76.3	38.8	15.0	
	Beeson	1.0	86.9	60.0	55.0	
		8.0	85.0	43.3	36.1	
		Mean	85.9	51.7	45.6	
	Mean		82.0	49.0	37.5	8.2
Overall mean			93.9	77.8	59.8	N.S.

[a]Potato dextrose agar plating; four replicates/10 pods.

Table 12. Soybean pod infection by *Phomopsis* in 1980 as affected by rotational practice, seed-borne inoculum, and plant growth stage

Rotational practice	Seed-borne inoculum (%)	Plant growth stage R3	R7	R8
Continuous soybean	0.0	0.0%[a,b]	34.2	44.2
	4.0	0.8	53.3	41.7
	26.0	0.8	30.8	40.0
	37.0	1.7	30.8	55.8
	46.0	0.0	32.5	46.7
	77.0	1.7	48.3	53.4
	Mean	0.8	38.3	47.0
Corn-soybean	0.0	0. 0	25.8	12.5
	4.0	0.8	33.4	23.3
	26.0	0.0	32.5	23.3
	37.0	0.0	40.9	17.5
	46.0	0.0	17.5	30.8
	77.0	0.0	38.4	31.7
	Mean	0.1	31.4	23.2
Continuous corn	0.0	0.0	0.8	3.3
	4.0	0.0	6.7	0.8
	26.0	0.0	6.7	7.5
	37.0	0.0	6.7	6.7
	46.0	0.0	7.5	5.0
	77.0	0.0	5.0	4.2
	Mean	0.0	5.6	4.6
LSD (P=0.05)[c]		0.4	5.2	5.6

[a]Potato dextrose agar plating.

[b]Four replicates/30 pods.

[c]LSD for comparing rotational practice means.

Table 13. Soybean pod infection by *Phomopsis* at the R8 growth stage in 1980 as affected by rotational practice, seed-borne inoculum, and plant height

Rotational practice	Seed-borne inoculum (%)	Position on plant			LSD (P=0.05)
		Lower	Middle	Upper	
Continuous soybean	0.0	90.0%[a,b]	22.5	20.0	
	4.0	90.0	20.0	15.0	
	26.0	85.0	15.0	20.0	
	37.0	95.0	42.5	30.0	
	46.0	82.5	40.0	17.5	
	77.0	90.0	40.0	30.0	
	Mean	88.8	30.0	22.1	7.1
Corn-soybean	0.0	35.0	2.5	0.0	
	4.0	55.0	10.0	5.0	
	26.0	55.0	15.0	0.0	
	37.0	35.0	7.5	10.0	
	46.0	65.0	17.5	10.0	
	77.0	60.0	25.0	10.0	
	Mean	50.8	12.9	5.8	6.2
Continuous corn	0.0	2.5	2.5	5.0	
	4.0	0.0	0.0	2.5	
	26.0	12.5	5.0	5.0	
	37.0	12.5	5.0	2.5	
	46.0	7.5	2.5	5.0	
	77.0	10.5	2.5	0.0	
	Mean	7.5	2.9	3.3	3.7
Overall mean		49.0	15.3	10.4	NS

[a]Potato dextrose agar plating.

[b]Four replicates/10 pods.

Table 14. Soybean seed infection by *Phomopsis* and seed germination in 1979 as affected by rotational practice, variety, seed-borne inoculum, and plant growth stage

Rotational practice	Variety	Seed-borne inoculum (%)	Plant growth stage R7	Plant growth stage R8
Continuous soybean	Amsoy 71	2.0	8.6%[a,b]	10.3
		10.0	13.6	3.0
		Mean	11.1	6.7
	Wells	0.0	15.3	14.3
		6.0	19.6	15.7
		Mean	5.0	0.5
	Beeson	1.0	5.7	0.7
		8.0	4.2	0.3
		Mean	5.0	0.5
	Overall mean		11.2	7.4
Corn-soybean	Amsoy 71	2.0	16.6	7.3
		10.0	19.4	4.4
		Mean	18.0	5.8
	Wells	0.0	28.0	15.7
		6.0	22.1	12.0
		Mean	25.1	13.9
	Overall mean		8.5	3.5
Continuous corn	Amsoy 71	2.0	0.9	0.0
		10.0	0.8	1.3
		Mean	0.8	0.7
	Wells	0.0	0.6	0.7
		6.0	1.2	1.0
		Mean	0.9	0.8
	Beeson	1.0	0.6	0.7
		8.0	0.9	0.4
		Mean	0.8	0.5
	Overall mean		0.8	0.7
LSD (P=0.05)[c]			6.1	5.1

[a]Potato dextrose agar plating.
[b]Four replicates/approximately 75 seeds.
[c]LSD for comparing overall rotational practice means.

Table 15. Soybean seed infection by *Phomopsis* and seed germination in 1980 as affected by rotational practice, seed-borne inoculum, and plant growth stage

Rotational practice	Seed-borne Inoculum (%)	Plant growth stage R7	Plant growth stage R8
Continuous soybean	0.0	5.8%[a,b]	4.7
	4.0	8.6	8.0
	26.0	9.1	8.7
	37.0	6.7	8.7
	46.0	8.6	16.0
	77.0	5.3	7.0
	Mean	7.3	8.8
Corn-soybean	0.0	3.8	2.3
	4.0	5.6	3.1
	26.0	4.2	3.7
	37.0	5.8	0.7
	46.0	1.3	6.0
	77.0	5.2	2.7
	Mean	4.3	3.1
Continuous corn	0.0	0.0	0.0
	4.0	0.0	0.3
	26.0	0.0	0.0
	37.0	0.3	0.3
	46.0	0.0	0.7
	77.0	0.0	0.3
	Mean	0.1	0.3
LSD (P=0.05)[c]		2.6	4.8

[a]Potato dextrose agar plating.

[b]Four replicates/approximately 75 seeds.

[c]LSD for comparing rotational practice means.