RACES OF THE PATHOGEN *PHYTOPHTHORA SOJAE* FOUND IN MICHIGAN AND FACTORS AFFECTING ROOT ROT OF SOYBEAN

By

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ABSTRACT

RACES OF THE PATHOGEN *PHYTOPHTHORA SOJAE* FOUND IN MICHIGAN AND FACTORS AFFECTING ROOT ROT OF SOYBEANS

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A study was conducted in order to establish what races of *P. sojae* are currently present in Michigan soybean fields, and to estimate yield loss to *P. sojae*, and determine the effect of the isoflavone genistein on the infection of soybeans by zoospores of *P. sojae*. Also, a possible relationship between fluorescence values of root exudates (as indicators of the amounts of genistein in exudates) and field tolerance levels of soybean varieties was examined. Additionally, a soybean field naturally infested with the soybean cyst nematode (SCN) was surveyed for possible increased infection by *P. sojae*.

Ninety isolates of *P. sojae* collected from Michigan fields (1993-1997) were tested for virulence and race status. Races 2, 3, 4, and 27 were identified, and the virulence formulae of most isolates did not match those of known races of P. sojae. Fifty five percent (55%) of the isolates tested were highly virulent defeating 7 or more Rps genes while 13% showed intermediate virulence defeating 4-7 genes each. Twenty percent were mildly virulent (defeating 1-3 genes) while 11%

were avirulent.

Genistein (5 ppm) significantly reduced disease levels in soybean seedlings inoculated with zoospores of *P. sojae*. However, there was no correlation between tolerance levels of soybean cultivars to the pathogen and fluorescence values of root exudates from the cultivars. There was significant correlation between nematode cyst counts and the presence of *P. sojae* in the non-fumigated field plots. However, no correlation was observed between the two pathogens in the non-fumigated plots.

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This dissertation is dedicated to my parents who, though not schooled, made the education of their children the center of their lives.

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Acknowledgment

I am indebted to my advisor, Dr. Gene R. Safir , whose guidance, patience and support made this work possible. My thanks also go to the other members of my guidance committee including Dr. L. Patrick Hart for his patient reading of the drafts and revisions, and his insightful suggestions, Dr. Dennis Fulbright for his mentoring friendship and encouragement. Special thanks to Dr. Ken Poff whose tact and concern often revived my spirits.

My family provided the necessary springboard for continued enthusiasm. My children , KipChumba, Kibor, and Jerotich, were a source of unfailing inspiration. It not possible to express enough gratitude to my wife, Andrea, without whose contanstant love and encouragement this work would not have been possible.

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TABLE OF CONTENTS

LIST OF TABLES

Figure 2
n

Figure 2

Figure 2
P

Figure 2
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Figure 3

Figure 3

Figure 3

Figure 4
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LIST OF FIGURES

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Chapter 1

INTRODUCTION

Soybeans in industry and the economy

The soybean (*Glycine max* L.) was introduced to North America in 1765 (Hymowitz and Harlan, 1983) but it remained a minor hay crop until it was developed as an oil seed crop. Processing of soybeans for oil and meal began in the 1930's and in 1938, production for processing exceeded production for hay (Thatcher,1947). Soybean seed is high in protein, making it highly adaptable to the nourishment of both man and animal. Soybean meal is a major source of protein in animal feed while soybean oil is used in cooking oil, margerine, paints, varnishes, adhesives and many other products. Soybean oil is also highly valued because it contains no cholesterol and has the lowest levels of saturated fats of nearly all vegetable oils. It may also serve as a source of energy in the future. Some soybeans are not crushed and are used for human consumption in such products as full-fat soy flour, soy milk, tofu, miso and temphe (Scott and Aldrich, 1970). There is great potential for expanding further the use of soybeans in

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food and industrial products which could improve the profitability of soybeans. Future research on soybean includes finding new uses for presently grown soybean varieties, developing new varieties that have altered levels of important components such as the major fatty acids that comprise the soybean oil (for specific markets), and protection against disease. Also environmental concerns for chemical contamination of surface and ground water from agricultural practices, may in future, place soybeans at an advantage over other field crops due to its low-input status. Thus soybean is expected to remain an important crop in the United States for many decades to come.

In Michigan, production of soybeans started in the early 1900s. By 1930, 1,000 acres were grown in the state. In 1997, Michigan farmers produced a record yield of 42 bushels per acre on 1.15 million acres with a market value of $249 million. Owing to market projections, acreage under soybeans continues to increase and Michigan now ranks eleventh among producing states with 2.3 percent of the U.S. production. As Michigan farmers adapt new soybean varieties for both the traditional and product specific markets, disease problems may pose new challenges that will require attention and more research efforts. Although most soybeans grown in Michigan have been relatively free of disease and insect problems,

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Phytophthora root rot, white mold, and the soybean cyst nematode (*Heterodera glycines* Inchinohe) have been identified in the state and can be yield-limiting factors in any one season or on any one field/farm.

Phytophthora root and stem rot of soybean (PRR) caused by *Phytophthora sojae* is one of the most important diseases of soybean in the North Central region of the Midwest. According to the North Central Soybean Disease Committee (NCR-137), losses due to PRR represent roughly 15% of the total soybean disease losses in the region (Approximately 192 million dollars/annum) (Doupnik,B.1993). Nationwide, approximately 16 million hectres are infested (Schmitthenner., 1985). The Soybean-Phytophthora disease interaction is believed to be a very young symbiosis with many possibilities for genetic manipulation through cultural practices and host genetics (Schmitthenner. 1985)

Genetic control of Phytophthora root and stem rot in soybean has been classified in four (not necessary exlusive), ways including: 1) resistance-conferring whole-plant immunity to incompatible races (compatible races cause disease) (Kaufman and Gardemman,1958); 2) tolerance (schmitthenner and Walker, 1979); 3) rate reducing resistance (slow rotting) (Tooley and Grau, 1982); and 4) root resistance conferring low infection but not immunity to incompatible races in the root

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A compatible race can infect, colonize and possibly kill a susceptible host plant. The same race is incompatible with a host which has either whole plant or root resistance specific for that race. In the case of whole plant resistance, the plant is immune to incompatible races because it has a hypersensitive response which restricts pathogen growth and kills plant tissue around the infection site (Kaufman and Gardemman, 1958). Resistance to root rot in the inoculum layer technique (Walker and Schmitthenner, 1984c) results in lesion formation but very limited growth of the pathogen compared to more extensive rot for soybeans with whole plant or root resistance (Schmitthenner and Kilien 1986). Rate-reducing resistance is used almost exclusively in epidemiology to describe reduction in rate and amount of sporulation and lesion size in pathogenesis. In Phytophthora root rot, 'slow rotting' is a more appropriate characterization of slow rate of rotting and does not indicate reduced colonization or reproduction (Walker and Schmitthenner, 1984c).

Whole plant resistance to *Phytopthora sojae* was first identified in 1955 (Suhovecky and Schmitthenner, 1955), and easily recognized by hypersensitive reaction to infection upon inoculation of sensitive hypocotyl tissue (Kaufmann and

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Gerdemman, 1958). Thirty nine races of *Phytopthora sojae* and 13 genes (some are alleles) at seven loci ($Rps1_a$, $Rps1_b$, $Rps1_c$, $Rps1_d$, $Rps1_k$, Rps_2, Rps3a, $Rps3_b$, $Rps3_c$, Rps_4, Rps_5 Rps_6 and Rps_7) which condition differential resistance to the physiological races in soybean, are known (Athow, 1987; Ploper et al, 1985; Leyton et al, 1986).

History of the Disease.

Phytophthora root and stem rot (PRR) of soybean was first observed as a disease of unknown etiology in Indiana in 1948 and in northwestern Ohio in 1951. The disease was originally thought to be caused by species of *Fusarium* or *Diaporthe*. *Phytophthora* was first associated with root and stem rot of soybean in North Carolina and Ohio in 1954 (Schmitthenner, 1985). The first reports on the disease in the United States were published in 1955 (Scotland, 1955; Suhovecky, 1955; Suhovecky et al, 1955), and identified as a disease caused by *Phytophthora coctorum* (Leb and Cohn) Schroeter. Later, Kaufman et al (1958), found an undescribed species of *Phytophthora* associated with root and stem rot of soybean in Illinois. They published a comprehensive report of the disease and proposed the name *Phytophthora sojae* for the causal agent. PRR has been reported in most parts of the North central region of the United States and is a limiting factor in

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Symptoms

Phytophthora root and stem rot may be found in soybeans at any stage of plant development. Seed rot and pre-emergence damping-off can occur in flooded fields, and is often misidentified as water damage (Anderson,1986). When conditions are favorable, seed rot, damping-off, and seedling rot and stem rot may cause losses and yield reductions of up to 100% in susceptible soybean cultivars.

Under flooded conditions, seeds often rot before emergence thereby reducing stands. After emergence, young plants are very susceptible to infection and often wilt and die. Symptoms on older seedlings depend on the relative susceptibility or tolerance of the cultivar. In low tolerance-cultivars, at the primary leaf stage, affected plants turn yellow and wilt, and seedlings are killed gradually. On the other hand in high tolerance cultivars, the damage may be restricted to roots and seedlings appear only stunted (Anderson, 1986).

In older plants of low tolerance cultivars, symptoms consist of yellowing between veins and along margins of lower leaves. Upper leaves become chlorotic and the plant wilts completely. Wilted leaves commonly remain attached to the

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plant. In the field, affected plants usually occur in groups in a row rather than singly. Wilting symptoms occur when the lateral roots and the taproot are destroyed (Anderson,1986). A dark brown discoloration progresses up the stem, often as high as ten nodes before the plant wilts, and internally the cortex and the vascular tissues are discolored. In older plants of high tolerance cultivars, symptoms are confined to the lateral roots. Plants are not killed by the pathogen but are stunted with mild chlorotic symptoms similar to those associated with nitrogen deficiency or severe flooding. Occasionally these symptoms are accompanied by long, narrow, sunken brown lesions that progress up one side of the stem. These mild symptoms are referred to as hidden damage and may reduce yield by as much as 40%. Hidden damage is readily evident if plants with isogenic resistance are subject to disease control treatments for comparison in the same field. Progressive light brown lesions with yellowish margins, characterize the leaflets of young susceptible plants. In older plants, lesions are severely restricted, a phenomenon referred to as age-related resistance.

Favourable environmental conditions

Environmental factors greatly influence the infection and disease severity of Phytophthora root and stem rot of soybean.

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The most important of these factors are soil type, soil compaction, soil moisture and soil temperature. Conditions favourable for infection occur most often in heavy, compacted clay soils with poor drainage. Disease incidence and the number of dead plants increases with soil compaction while the total number emerged is reduced (Moots et al, 1988). Extended periods of high soil moisture, rainfall or standing water highly favour disease development. The disease is most severe in years with heavy rainfall early in the growing season, and is most destructive in low, poorly drained portions of the field (Kittle et al, 1979). In greenhouse experiments (Klein, 1959), the percentage of diseased plants increased with the length of soil wetness before planting. The optimum soil temperature for infection ranges from 27°c to 33°c for seedlings and young plants, and 25°c to 30°c for older plants, but infection can occur at soil temperatures as low as 15°c (Kittle et al, 1979). Greatest root loss by soybean plants in soils infested with *P. sojae* occurred at lower temperatures than at optimum. At low temperatures, *P. sojae* may have greater metabolic activity and hence the ability to attack and destroy roots than the plant is able to regenerate them. As soil temperature increases above 15°c, growth of soybean roots out-phase disease development or *P. sojae* becomes less active or both.

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Disease cycle

Phytophthora sojae (Kaufmann and Gardemman) is homothallic; sexual reproduction takes place in a single thallus, and therefore mycelium and sporangia are diploid. Meiosis occurs in antheridia and oogonia and karyogamy takes place in the oogonium, which forms a diploid oospore. Oospores germinate by germ tubes which result in the formation of single terminal sporangia (conidia)(Ribeiro, 1983). Sporangia are simple and indeterminate. Typically, sporangia are obipyriform (42-65 x 32-53 um) and non papillate. Sporangia extrude zoospores into a thin, delicate membranous vesicle which quickly expands and raptures. Zoospores sometimes remain trapped in the sporangium and germinate from within. Sporangia may also germinate directly thus functioning as conidia. Empty sporangia commonly proliferate internally, forming new sporangia within the old. Sporangia readily develop in dilute extract of lima bean agar or other media. They can also be induced to form on solid medium if washed repeatedly with water or Chen-Zentmyer salt solution.

The optimal temperature for zoospore production is 20°c (the minimum is 5°c). Zoospores which form the primary inoculum are bluntly pointed at both ends and biflagellate. One of the flagella (tinsel) is short and directed anteriorly

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while the other (whiplash) which is four to five times larger is directed posteriorly. After a motility period which may last for several days, zoospores encyst and germinate directly by producing germ tubes which usually swell to form appresoria when they come in contact with a solid surface or, rarely, miniature sporangia form at their tips.

Sexual organs (antheridia and oogonia) which develop abundantly on a single thallus, are produced in cornmeal, lima bean and V-8 agars. The antheridia are mostly paragynous with occasional amphigyny. The oogonia (about 36.9 μm in diameter) are thin-walled spherical structures in which oospores are formed. Thick-walled dormant oospores develop when antheridia fertilize the oogonia. At the end of the dormant stage, the smooth inner wall of the oospore erodes and the central refractive body is absorbed. When oospores germinate, the inner walls are completely absorbed, and germ tubes are produced, which develop into hyphae or terminal sporangia, depending on the availability of moisture.

Taxonomy

Phytophthora sojae is placed in the phythiaceae, a family of the Oomycetes, a group of organisms that were for a long time identified with fungi. The Oomycetes have been recognized as being significantly different from fungi (Pringsheim, 1958,

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Kreisel, 1969, and Shaffer,1975). However due to a number of factors (Barr, 1992), including traditional as well as practical considerations, there has been a tendency for mycologists to classify the Oomycetes as true fungi. Kreisel (1969) and Shaffer (1975) were the first to exclude Oomycetes from the true fungi. In recent years, more workers have adopted this approach, as it has become increasingly apparent that while these organisms are morphologically similar to fungi, and exhibit absorptive nutrition, they do not have close phylogenetic relationship with fungi. The Oomycetes have been grouped with heterokont organisms such as the subdivision, Pseudofungi, phylum Heterokonta of the kingdom Chromista (Cavalier-Smith, 1986, 1987), or the subdivision Heterokontimycotina of the kingdom Heterokonta (Dick, 1976, 1990a). Another approach has been to include them in the protoctista (Margulis et al. 1989), a group composed of organisms that are not monophyletic. Patterson and Sogin (1992) placed the phylum *Oomycota* in a new kingdom, *Stramenopila*. The characteristics that set the *Oomycota* organisms apart from the true fungi and also delineate the phylum, include among others, asexual reproduction by means of biflagellate zoospores, diploid thallus in which meiosis occurs in the gametangia, oogamous reproduction by gametangial contact, cell wall composition (mostly B-glugans), and various

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ultra-structural features of the oospore (Sogin, 1992). In modern botanical works, the Oomycetes and other organisms that are linked by their basic eukaryotic cell structure and their lack of the distinguishing features of plants, animals, or fungi are placed in the Kingdom Protista. This is due to differences in morphology, pathogenicity, and growth rate with related species of *P. coctorum* and *P. megasperma*. In 1959, Hildebrand changed the name of the soybean pathogen to *P. megasperma* Drechs. *var. sojae* Hildeb. This name remained valid until 1980, when Kuan T. and Erwin D.C. reclassified the fungus as *P. megasperma f. sp. glycinea*, following extensive studies of the host range and oospore size. They concluded that oogonial size of isolates of *P. megasperma* from various hosts overlap and was, therefore, not a suitable trait for varietal separation. However, isolates of *P. megasperma* from soybean and alfalfa had sufficiently distinct host range to place them into two *forma specialae* (*P. megasperma f. sp. glycinea* and *P. megasperma f. sp. medigaginis*) and to separate them from *P. megasperma* found in other hosts. Later, Kaufman et al (1958), found an undescribed species of Phytophthora associated with root and stem rot of soybeans in Illinois. They published a comprehensive report on the disease and proposed the name *Phytophthora sojae* for the causal agent.

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Ecology and epidemiology

P. sojae survives as oospores in crop residue and soil for many years without growing competetively enough to colonize soil debris (Schmittenner, 1985). The fungus cannot be demonstrated in soil immediately after freezing or storage for long periods at 3°c, indicating that mycelium, sporangia and zoospores do not survive cold temperatures. If overwintered soil is incubated for one week at 25°c under suitable moisture conditions, the fungus can be readily demonstrated using the leaf disk bait technique (Schmitthenner, 1985). This demonstration indicates that *P. sojae* survives as resistant oospores that germinate when dormancy is broken and when temperature and moisture are suitable. It is not known exactly what factors break the dormancy of oospores or what minimum soil saturation times and temperatures favour the germination of oospores. It is known that extended rotations with non-host crops does not eliminate the pathogen (Schmitthenner, 1985). Work by Stella Avila (MS Thesis, 1992) showed ability by *P. sojae* to infect non-host crops without the manifestation of disease. This may further explain the failure of crop rotation as a strategy in the management of the disease. The association and interaction of *P. sojae* with other fungi may increase or decrease the

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severity of phytophthora root rot. Mycorrhizal fungi decrease the infection of soybean roots by increasing plant vigor and competing for site while *Phythium*, *Rhizoctonia*, and *Fusarium* may increase the severity of root rot. Infection of soybean plants by the northern root-knot nematode (*Meloidogyne hapla* Chitwood) also increases the severity of root rot.

Physiologic races and reistance

Two types of susceptible reactions to *Phytophthora* are found in soybeans; race-specific and nonrace-specific (general) reactions. Race-specific reactions are controlled by 14 (Rps) genes at seven loci. More than thirty races of *P. sojae* have been described, and isolates have been found that do not fit any of the described races. The races can be distinguished on eight soybean cultivars. Among susceptible cultivars, many quantitative differences in disease reaction can be found. The least susceptible reactions are referred to as field resistance, tolerance, or rate reducing resistance. Tolerance levels can be evaluated by differential plant loss, vigor and yield in infested soils or in greenhouse and growth chamber environments.

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Disease Management

Control of *Phytophthora* stem and root rot initially utilized only race specific resistance. However, continued appearance of new virulent races in response to continued deployment of resistant host cultivars has led to several documented failures of specific resistance (Schmitthenner, 1985). Because of the potential for race shifts, it has become increasingly apparent that control of PRR cannot continue to utilize race-specific resistance effectively without a better understanding for the *P. sojae*-soybean interaction and deployment of other forms of host resistance to the pathogen.

Other methods important in controlling PRR include tolerance[1], fungicides, (primarily metalaxyl), cultural practices such as tile drainage, moldboard ploughing, but none of these have singly proven to be entirely effective. Based on the above observations, an integrated pest management (IPM) approach has been advocated in the control of PRR (Schmitthenner, 1985). Under this IPM strategy, it has been demonstrated that various combinations of the methods stated above can lead to effective control of PRR. Currently, breeders try to incorporate resistance to the most important

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[1] Tolerance denotes slow-rotting and root resistance of soybeans to compatible races of *P. sojae* (Mussel, 1980 and Schmittenner and Walker, 1979).

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Isoflavonoids and Field Tolerance

Isoflavonoids (compounds produced and exuded by plant roots) have been shown to affect the infection characteristics of soybean roots by non pathogens (Nair et al., 1991, Siqueira et al., 1991). The production of these compounds varies with plant age and variety (Osman and Fett, 1983) Siqueira et al., 1991b) and may be an important factor in early season infection. There is also evidence that certain isoflavonoids that are found in and exuded by soybean roots are capable of attracting the zoospores of *P. sojae* and inducing their encystment and germination in vitro (Morris and Ward, 1992). Research by Graham (1989) and Reviera-Vargas, et al. (1993) suggested that certain isoflavonoids may reduce *P. sojae* hyphal growth at low concentrations. It is likely that isoflavonoid stimulation and or attraction of *P. sojae* operates independently of race specific gene resistance by acting as a prerequisite for germination and initial colonization. Possibly, these compounds may be directly related to differences in susceptibility and tolerance to *P. sojae* among soybean varieties. In this regard, there is evidence that susceptible alfalfa roots attract *P. sojae* more

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There is an increasing evidence of pathogen specificity in relation to tolerance of soybean cultivars to *P. sojae* (Thomson et al., 1988). In addition, these authors reported that some *P. sojae* isolates may differ in virulence whereas others differ in tolerance. Tolerant soybean cultivars that have been selected are not equally tolerant to all races of *P. sojae*. Research by Graham (1989) suggested that certain isoflavonoids such as genistein may have activity against *P. sojae* hyphal growth in vivo when glyceollin production is low. Taxis of zoospores of *P. sojae* to soybean isoflavones, to which other Phytophthora spp. did not respond, has been demonstrated (Rivera-Vergas, 1993). Conjugates of genistein and several other aromatic metabolites are selectively secreted into root and seed exudates (Graham, 1991). Morris and Ward (1992) suggested that *P. sojae* has developed a mechanism for recognition of the same chemical signals as *B. japonicum*, although not for the purpose of symbiosis. At 10 ug/ml, the isoflavonoid genistein inhibited radial (hyphal) growth and reduced asexual reproduction of isolates of *P. sojae* in culture (Vedenyapina et al., 1996). Work by G. R. Safir and T. L. Wacker (unpublished) found that adding genistein at concentrations as low as 5 ppb to a plant growth solution can reduce infection of soybean seedlings by

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zoospores of *P.sojae*. Thus it this possible that field tolerance of soybean to *P. sojae* may be controlled to a large extend by root isoflavonoid exudation characteristics and by the presence of certain isoflavonoids within the roots.

The research being reported here is part of multi-state (Kansas, Illinois, Indiana, Iowa, Michigan, Minnesota, Nebraska, North Dakota, Ohio, and South Dakota) effort whose specific objective is to assess the population and structure of *P. sojae*, using classical, molecular, and biochemical techniques. The information resulting from this research will greatly increase our understanding of the basis for race-specific resistance as well as enhance our ability to identify races rapidly and enable growers to manage the disease more efficiently.

The specific objectives for this part of the project are: 1) Monitor the races or virulence structure of P. sojae populations in Michigan soybean fields. Information on the variability of *P. sojae* as it relates to race and virulence structure would be a key component of any IPM program which utilizes plant resistance. This information would be valuable to soybean breeders as well as agronomists and farmers who must make practical decisions concerning the management of PRR. 2)Characterize the nature of field resistance (tolerance) to P. *sojae*. The specific goal is to determine the effect of

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exogenously applied genistein (4',5,7-trihydroxy isoflavonoid) on Phytophthora root rot in soybean seedlings, and compare the fluorescence levels of soybean root exudates to field tolerance. Given the effects genistein has on the zoospores of P. sojae (Vedenyapina et al. 1996), it is possible that there is correlation between field tolerance and the fluorescence levels of root exudates. 3) Investigate possible synergistic relationship between soybean cyst nematode and *P. sojae* in soybeans. *Since P.sojae* is essentially a stress pathogen, it is possible that nematode activity in soybeans increases susceptibility to infection.

In Chapter 2, race determination and virulence structure of *P. sojae* isolates were determined, and the effect of exogenously applied genistein on the infection and development of *Phytophthora* root rot in soybean seedlings was evaluated. This work reports the first information on the effect of exogenously applied genistein on the soybean disease. In Chapter 3, findings on the fluorescence levels of root exudates from soybean varieties containing both race specific resistance and various levels of field tolerance to *P. sojae* are reported. Also included in this chapter is the effect of exogenously applied genistein on the development of PPR. In Chapter 4, possible correlation between soybean cyst nematode infestation and infection by *P.sojae* on soybeans is examined.

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CHAPTER 2

RACE DETERMINATION OF ISOLATES OF *PHYTOPHTHORA SOJAE* FROM MICHIGAN FIELDS AND ESTIMATION OF ECONOMIC IMPACT OF PRR ON YIELD

Abstract

Knowledge on the race composition of *P. sojae* (Kaufmann and Gerdemann) that occur in any one field or soybean growing area is important in the management of Phytophthora root and stem rot of soybeans. In this study, plant and soil samples were obtained from Michigan soybean fields through scouting and in collaboration with growers and extension service agents. A total of one hundred and fifty field isolates of P. sojae were obtained, and ninety were evaluated for virulence using differential soybean cultivars.

Fifty five per cent (55%) of the isolates tested were highly virulent, and defeated seven or more Rps genes each. Ten (13%) defeated four to six genes each while 20% or eihteen defeated one to four genes each. Eleven of the isolates were avirulent as they did not attack any of the Rps genes

including the susceptible variety Williams (rps). These results show the potential of P. sojae to impact yield in soybean production in the state.

Introduction

Monitoring of race or virulence structure of a pathogen population is a key component in any management program which utilizes specific resistance in the control of disease. Race specific genetic resistance has been a major element in the control of PRR. However, the appearance of new virulent races in response to continued deployment of resistance host cultivars has led to several documented failures of specific resistance (Schmitthenner, 1985). Race characterization of *P. sojae* is based on its differential reaction to 13 genes (in 7 loci) using the hypocotyl inoculation test (Ward, 1990). Currently, there are 39 races of *P. sojae* known. Breeders try to incorporate resistance to the most important races in their areas, if that knowledge and resistant germplasm are available. But in many cases, growers are forced to rely on varieties in which resistance does not exist or inadequate knowledge on *P. sojae* races exist to allow informed choices of resistant germplasm (Schmittenner, 1985).

Other methods important in controlling PRR have included tolerance (field resistance), fungicides (primarily

metalaxyl), and cultural practices such as moldboard plowing, and tile drainage, but none of these have by themselves proven to be entirely effective (Schmittenner, 1985). Based on the above observations, Schmitthenner (1985) has advocated an integrated pest management (IPM) approach to control of PRR. Under this IPM strategy it has been demonstrated that various combinations of the methods stated above can lead to effective control of PRR. In order to make host resistance an effective component of the IPM program, knowledge of the availability of resistant germplasm and the virulence structure of the pathogen in target area is vital.

Although Michigan soybeans have been relatively free from PRR, potential for economic impact does exist. Occasionally, *Phytophthora* is identified in plant samples sent to the MSU plant diagnostic clinic by growers and extension service agents. Lockwood (Lockwood et al, 1985) isolated races 1, 3, 4, and 6, and involvement of *P. sojae* in the root rot complex of soybeans in the state has been proven. Thus, there is need for current information on the occurrence and virulence structure of *P. sojae* in the soybean growing areas of the state. Presently, information on the occurrence or virulence structue of *P. sojae* and its impact on yield in Michigan fields is not sufficient enough for farmers to make informed decisions in the selection of available resistant varieties.

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The first objective of this project was to obtain isolates of *P. sojae* from Michigan soybean fields and determine their virulence characteristics and race structure. The second objective was to locate a soybean field which contains Phytophthora root/or stem rot and then to estimate the potential economic impact of *P. sojae* on yield.

Results from this project will increase our knowledge on the status of *P. sojae* and the occurrence of PRR in Michigan. It is hoped that information from this project will enable breeders and growers make informed choices in the control of PRR and thus enhance its efficacy as an IPM component in low input production systems.

Materials and methods

Collection of samples

A total of 260 soybean and 20 soil samples were obtained from Michigan soybean fields through scouting and collaboration with extension service agents over a period of five growing seasons (1993-96). Plants with well defined stem symptoms were collected mostly from depressed areas in the fields. In a rainy spring, these depressions collect water and remain wet for long periods, a condition that is conducive to Phytophthora root and stem rot. Other samples were obtained from farmers through the MSU plant disease clinic. Plants were

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placed in plastic bags and stored at 4°c to maintain pathogen viability as samples were being processed. In 1995, soil samples were also collected in Eaton and Monroe counties. Each soil sample consisted a composite of soils collected from various parts of a field.

Media

The Canaday - Schmitthenner Medium (Canaday and Schmitthenner, 1982) was used to isolate Phytophthora from plant samples. The seedling bioassay method(Canaday and Schmitthenner, 1982), a technique that minimizes Pythium contamination, was used to isolate Phytophthora from the soil samples. The isolation medium consist 40 ml of V-8 juice, 2000 ppm $CaCo_3$, 20 ppm pentachloronitrobenzine, 10 ppm benomyl, 100 ppm neomycin sulfate, and 9 ppm rifampicin per one liter. Two grammes of $CaCo_3$ was added to one liter of V-8 juice and heated to 80°c, and allowed to cool to room temperature, and then centrifuged to clarify prior to incorporation in the medium. With the exception of rifampicin, all ingredients were added prior to autoclaving. After autoclaving the medium, rifampicin was added in 5 ml of 95% ethanol. Hymexizol was excluded because of its potential to inhibit sensitive strains of Phytophthora. All chemicals were obtained from Sigma Chemical Corporation; St. Louis Missouri.

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Isolation from diseased soybean plant samples

Stems and roots of plants with well defined symptoms were disinfected with 10% bleach for ten min. and thoroughly rinsed 3 to 4 times with sterile distilled water. Small sections of tissue were taken from the edges of advancing lesions and placed on the isolation medium. Necrotic tissue was also removed from plants with severe flood damage (following heavy rainfall) and placed on the medium after thoroughly surface sterilizing and washing as described above. In all cases, the plant tissues were placed under the medium in order to minimize bacterial contamination by limiting oxygen availability. As soon as hyphae of Phytophthora appeared in the medium, they were hyphal-tipped in clean areas and transferred to new medium-plates to avoid contaminating fungi.

Isolation from soil with the soybean seedling bioassay method

The Soybean Seedling Bioassay technique (Canaday, and Schmittenner, 1982) was used to isolate *P. sojae* from soil. Each soil sample was air-dried and passed through a 3 mm mesh sieve. Approximately 800 g of air-dried soil from each soil sample was placed in plastic pots (3 pots/soil sample). Pots were flooded overnight, then drained and allowed to air-dry until the moisture content approached -300 mb matrix potential (soil cracks or pulls away from side of container although it

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Production of single zoospore cultures

Fifteen pieces (1 mm diameter) of culture from the edges of actively growing colonies of *P. sojae* on dilute V-8 juice agar were placed into 25 ml of quarter strength V-8 liquid medium (50 ml V-8/L water). After 48 hrs., the culture medium was poured off and replaced with 25 ml of Aphanomyces Replacement Solution (2.94 g $CaCl_2.2h_2O$, 2.47 g $MgSO4.7H_2O$, and 0.75 g KCL/1000 ml of distilled deionized water) at Ph 7.0. The solution was changed 4 times at 5 min. intervals. The final salt solution was replaced with 20 ml of sterile de-

ionized distilled water and cultures were incubated under cool white lights at room temperature. Sporangia formed 12 hrs after cultures were flooded. Cultures were placed in the refrigerator (4°C) for 4 hours and then incubated on the bench at room temperature. Large numbers of zoospores were seen swimming freely within an hour.

Zoospore concentration was estimated by placing a 50 μl zoospore suspension on a slide and staining with 25 μl of lacto-phenol Tryphan blue solution. The suspension was covered with a 22 X 22 mm cover-slip and zoospores were counted. Through dilution series, the zoospore concentration was adjusted to $1x10^{-2}$ and plated onto a 1/4 strength V-8 agar medium (200 ml/1 liter) to generate single zoospore cultures.

Identification

Two keys, one that groups species of Phytophthora by sporangial characteristics (Waterhouse, 1963) and one that used other characteristics were used in the identification of Phytophthora isolates. Host range, growth rate and oogonal size were used to delineate *P. sojae* from *P. megasperma*. D.H. Scott of Purdue University supplied the isolate (1819B-type culture) of *P. megasperma* used in the host range study.

Test for virulence and race determination

Isolates of *P. sojae* were tested for virulence and race determination by inoculating seedlings of a differential set of soybean cultivars based on their reactions to the pathogen, using the hypocotyl injection method (Hass and Buzell, 1976). *P. sojae* cultures were grown on soft (12 g agar/L) dilute (40 ml V-8 juice/L) V-8 juice agar until the mycelium covered the plate. Strips of the cultures were cut and placed in a 10 ml syringe and forced through to make a slurry of the culture. The syringe was then reloaded with the slurried culture and a # 18 needle was put on it. Six-day old seedlings were inoculated by making a slit about one 1 cm long in the hypocotyl of the seedling just below the cotyledonary node with the needle tip. Approximately 0.2 to 0.4 ml (200 to 400 cfu/ml) of the culture slurry was deposited in the slit with the syringe. Plants were then covered with clear plastic bags for 12 hrs to prevent drying of the agar slurry before infection could take place.

The plants were incubated at 25°C in 14 hrs of light for one week. Soybean seedlings with specific resistance developed characteristic hypersensitive response which hinders growth of the pathogen by killing the plant tissue around the infection site and creating a necrotic fleck. The susceptible varieties

died or manifested distinctive lesions during this period.

Estimation of the impact of *P.sojae* on yield

P. sojae was isolated from all soybean samples from a field in Saginaw county that were provided by Dr. L. P. Hart. Initial survey showed random distribution of disease in the field, and a systematic zigzag approach was used in the collection of samples. Samples yielded highly virulent isolates from the field. Due to the high incidents of PRR in the field, it was decided that impact on yield be estimated. In 1997, Grower Service Corporation (St. Charles MI), provided stand counts (made on Sept.4) and yield estimates for selected strips of seven rows (20' combine head) within the diseased field and also for strips within an adjacent field containing the same soybean cultivar and grown under the same cultural and pest management conditions. The two fields (60 acres each) were separated by a narrow strip of corn. The adjacent field which appeared healthy throughout the growing season was not sampled extensively for Phytophthora, however *P.sojae* was not found in it (table 2.5). The soybean fields in this study were planted with soybean variety Golden Harvest 1271 (which does not have Rps genes) at a rate of 190,000-200,000 seeds /acre. The diseased field was planted on June 3 and sprayed on Jul.3 with 2.7 oz Cobra, 1/4 pinnacle, Choice

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and Act 90. The non-diseased field was planted and sprayed in similar fashion except for the Cobra application of 2.0 oz. It is difficult to say whether or not the rate difference in the application of Cobra was a factor in disease. But unlike the healthy field, the diseased field had depressed areas which had high moisture content earlier in the season, and there was a higher frequency of symptomatic plants around the depressions. The fields were harvested on Oct. 25.

Results

Isolation and identification of *P. sojae*

A total of 150 field isolates of *P. sojae* were obtained from diseased soybean plant samples from Barrien, Clinton, Eaton, Ingham, Ionia, Jackson, Lenawee, Monroe, Oakland, Saginaw, and Shiawassee counties in the 1993-96 growing seasons (Table 2.1). Isolates were also obtained from soil samples from Eaton and Monroe counties.

The oogonial size of isolates on V-8 agar ranged from 26-44 μm and were similar to those described for *P. sojae* (Kuan and Erwin 1980). The sporangia were pyriform and nonpapillate and virulent isolates infected only susceptible soybean seedlings but did not cause disease in alfalfa and dry beans {(variety black magic) (figure)}. Average radial growth rate for isolate cultures was 3.6 mm/day and was less than that of

Table 2.1 Rea
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Table 2.1 Reaction of soybean resistance genes to the Michigan field isolates of *P.sojae*. Six-day old soybean seedlings were inoculated by making a 1 cm long slit in the hypocotyls and depositing 0.2 to 0.4 ml. of agar slurry containing 200 to 400 cfu/ml of *P.sojae.* Resistant varieties developed characteristic hypersensitive response while susceptible lines died or manifested distinctive lesions within one week.

R= resistant; S= susceptible

		Isolates of *Pytophthora sojae* from Michigan fields																
		MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	SMU
Source	Gene	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
Williams		S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Williams 82		S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Harosoy		S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Harosoy 63		S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Corsoy		S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Corsoy 79		S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams		S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1a*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1b*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1c*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1d*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps2*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps3a*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps3b*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps3c*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps4*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps5*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps6*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps7*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps8*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-1*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-2*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-3*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-4*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-5*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-6*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-7*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-8*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-9*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-10*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-11*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-12*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-13*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-14*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-15*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-16*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-17*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-18*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-19*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-20*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-21*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-22*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-23*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-24*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-25*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-26*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-27*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-28*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-29*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-30*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-31*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-32*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-33*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-34*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-35*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-36*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-37*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-38*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-39*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-40*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-41*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-42*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-43*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-44*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-45*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-46*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-47*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-48*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-49*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-50*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-51*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-52*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-53*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-54*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-55*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-56*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-57*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-58*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-59*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-60*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-61*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-62*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-63*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-64*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-65*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-66*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-67*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-68*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-69*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-70*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-71*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-72*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-73*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-74*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-75*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-76*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-77*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-78*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-79*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-80*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-81*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-82*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-83*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-84*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-85*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-86*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-87*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-88*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-89*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-90*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-91*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-92*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-93*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-94*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-95*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-96*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-97*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-98*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-99*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
Adams	*Rps1k-100*	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S

		Isolates of *Pytophthora sojae* from Michigan fields																
Source	Gene	MSU 01	MSU 02	MSU 03	MSU 04	MSU 05	MSU 06	MSU 07	MSU 08	MSU 09	MSU 10	MSU 11	MSU 12	MSU 13	MSU 14	MSU 15	MSU 16	SMU 17
Williams	rps	S	S	S	R	R	R	R	S	S	S	R	R	R	S	S	S	S
Harlon	Rps_{1a}	R	S	S	R	R	R	R	S	S	S	R	R	R	S	S	S	S
Harosoy 13xx	Rps_{1b}	S	R	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R
Williams 79	Rps_{1c}	R	R	S	R	R	R	R	S	R	R	R	R	R	S	S	S	S
PI 103	RPS_{1d}	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R
Williams 82	Rps_{1k}	R	R	S	R	R	R	R	S	R	R	R	R	R	R	S	R	R
L76-1988	Rps_{2}	R	R	R	R	R	R	R	S	S	S	R	R	R	R	S	R	S
L83-570	Rps_{3a}	R	R	R	R	R	R	R	S	R	S	R	R	R	R	S	R	S
PRX 146-36	Rps_{3b}	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S
PRX 145-48	Rps_{3c}	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
L85-2352	Rps_{4}	R	R	R	R	R	R	R	R	R	S	R	R	R	R	S	R	R
L85-3059	Rps_{5}	R	R	R	R	R	R	R	R	R	S	R	R	R	R	S	R	S
Harosoy 62xx	Rps_{6}	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
Harosoy	Rps_{7}	S	S	S	R	R	R	R	S	S	S	R	R	R	S	S	S	S

		MSU 18	MSU 19	MSU 20	MSU 21	MSU 22	MSU 23	MSU 24	MSU 25	MSU 26	MSU 27	MSU 28	MSU 29	MSU 30	MSU 31	MSU 32	MSU 33	SMU 34
Williams	rps	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Harlon	Rps_{1a}	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Harosoy 13xx	Rps_{1b}	R	R	R	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Williams 79	Rps_{1c}	R	R	R	S	S	R	R	R	R	S	R	R	R	R	S	R	R
PI 103	RPS_{1d}	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	R
Williams 82	Rps_{1k}	R	R	R	S	S	S	S	S	S	S	S	S	S	R	S	S	R
L76-1988	Rps_{2}	R	R	R	R	R	S	S	S	S	S	S	R	S	R	S	S	R
L83-570	Rps_{3a}	R	R	R	R	S	S	S	S	S	S	S	S	R	R	S	R	R
PRX 146-36	Rps_{3b}	R	R	R	R	S	S	S	S	S	S	S	R	S	R	R	R	R
PRX 145-48	Rps_{3c}	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S
L85-2352	Rps_{4}	R	R	R	R	R	S	S	S	S	S	S	R	S	R	S	S	R
L85-3059	Rps_{5}	R	R	R	R	R	S	S	S	S	S	S	S	S	R	S	S	R
Harosoy 62xx	Rps_{6}	R	R	R	R	R	S	S	S	S	S	S	S	S	R	S	S	R
Harosoy	Rps_{7}	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S

		Isolates of Pytophthora sojae from Michigan fields																
		MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	SMU
Source	*Gene*	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51

		Isolates of *Pytophthora sojae* from Michigan fields																
Source	Gene	MSU 35	MSU 36	MSU 37	MSU 38	MSU 39	MSU 40	MSU 41	MSU 42	MSU 43	MSU 44	MSU 45	MSU 46	MSU 47	MSU 48	MSU 49	MSU 50	SMU 51
Williams	rps	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Harlon	Rps_{1a}	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Harosoy 13xx	Rps_{1b}	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Williams 79	Rps_{1c}	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R
PI 103	RPS_{1d}	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	R
Williams 82	Rps_{1k}	S	S	R	S	S	R	S	R	R	R	R	R	R	R	R	R	R
L76-1988	Rps_{2}	R	S	S	S	S	S	S	S	S	S	S	R	R	S	S	R	S
L83-570	Rps_{3a}	R	R	R	S	R	R	R	R	R	R	R	R	S	R	R	R	R
PRX 146-36	Rps_{3b}	R	R	R	S	R	R	R	R	R	R	R	R	S	R	R	R	R
PRX 145-48	Rps_{3c}	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
L85-2352	Rps_{4}	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	S
L85-3059	Rps_{5}	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R
Harosoy 62xx	Rps_{6}	S	S	R	R	R	R	R	R	S	S	S	S	R	R	R	R	R
Harosoy	Rps_{7}	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

		MSU 52	MSU 53	MSU 54	MSU 55	MSU 56	MSU 57	MSU 58	MSU 59	MSU 60	MSU 61	MSU 62	MSU 63	MSU 64	MSU 65	MSU 66	MSU 67	SMU 68
Williams	rps	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Harlon	Rps_{1a}	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
Harosoy 13xx	Rps_{1b}	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
Williams 79	Rps_{1c}	R	S	R	S	R	S	R	R	S	R	S	R	R	R	S	S	R
PI 103	RPS_{1d}	R	S	R	R	S	S	R	S	R	R	S	R	S	R	R	S	R
Williams 82	Rps_{1k}	R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	S	R
L76-1988	Rps_{2}	S	R	R	R	R	R	S	S	R	R	D	R	R	R	R	R	R
L83-570	Rps_{3a}	S	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R
PRX 146-36	Rps_{3b}	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	R	R
PRX 145-48	Rps_{3c}	S	S	S	S	R	S	S	S	S	R	S	R	S	S	S	S	S
L85-2352	Rps_{4}	S	R	R	R	R	S	S	S	R	R	S	R	S	R	R	R	R
L85-3059	Rps_{5}	S	R	R	R	S	S	S	R	R	S	R	S	R	R	S	R	
Harosoy 62xx	Rps_{6}	S	S	R	R	R	R	S	S	R	R	S	R	S	R	S	S	R
Harosoy	Rps_{7}	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S

Isolates of *Pytophthora sojae* from Michigan fields

MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	MSU	SMU

		Isolates of *Pytophthora sojae* from Michigan fields																
Source	Gene	MSU 69	MSU 70	MSU 71	MSU 72	MSU 73	MSU 74	MSU 75	MSU 76	MSU 77	MSU 78	MSU 79	MSU 80	MSU 81	MSU 82	MSU 83	MSU 84	SMU 85
Williams	rps	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Harlon	Rps_{1a}	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Harosoy 13xx	Rps_{1b}	R	S	S	S	S	R	R	S	S	R	R	S	R	R	R	R	S
Williams 79	Rps_{1c}	S	R	R	S	S	S	S	S	S	R	S	S	R	S	S	R	R
PI 103	RPS_{1d}	S	R	R	S	S	S	R	R	S	R	R	S	R	S	R	R	S
Williams 82	Rps_{1k}	R	R	S	S	R	R	S	R	R	R	R	S	R	S	R	R	S
L76-1988	Rps_{2}	R	S	R	R	S	S	R	S	S	R	R	R	S	S	R	R	R
L83-570	Rps_{3a}	S	S	R	R	S	S	R	R	S	R	R	R	R	S	R	R	R
PRX 146-36	Rps_{3b}	S	R	R	R	R	R	R	S	S	R	R	R	R	S	R	R	R
PRX 145-48	Rps_{3c}	S	R	R	R	S	S	S	R	R	S	R	S	R	S	S	R	R
L85-2352	Rps_{4}	S	R	R	R	S	R	R	R	S	R	R	S	S	S	R	R	R
L85-3059	Rps_{5}	S	S	R	S	S	S	R	S	S	S	R	S	S	S	R	R	R
Harosoy 62xx	Rps_{6}	S	S	R	R	S	S	S	R	S	R	R	S	R	S	R	R	R
Harosoy	Rps_{7}	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S

		MSU 86	MSU 87	MSU 88	MSU 89	MSU 90	RACE[@] 01	RACE[@] 03	RACE[@] 04	RACE[@] 07	RACE[@] 25
Williams	rps	S	S	S	S	S	S	S	S	S	S
Harlon	Rps_{1a}	S	S	S	S	S	R	S	S	S	S
Harosoy 13xx	Rps_{1b}	S	S	S	R	R	R	R	S	R	S
Williams 79	Rps_{1c}	R	S	S	R	R	R	R	S	R	S
PI 103	RPS_{1d}	S	S	S	R	S	R	R	R	R	R
Williams 82	Rps_{1k}	S	S	S	R	S	R	S	S	S	S
L76-1988	Rps_{2}	R	R	S	R	S	R	R	R	R	S
L83-570	Rps_{3a}	S	R	S	R	R	R	R	R	S	R
PRX 146-36	Rps_{3b}	S	S	R	R	R	R	R	R	R	R
PRX 145-48	Rps_{3c}	S	S	S	R	S	R	R	R	S	R
L85-2352	Rps_{4}	R	S	R	R	S	R	R	R	S	R
L85-3059	Rps_{5}	R	S	R	S	S	R	R	R	S	R
Harosoy 62xx	Rps_{6}	R	S	S	S	R	R	R	R	S	R
Harosoy	Rps_{7}	S	S	S	S	S	S	S	S	S	S

Table 2.2 Virulence levels of field isolates of *P.sojae*.. Isolates were ranked according to the number of Rps genes defeated :

Low virulence.....1-4 Rps genes.
Intermediate virulence.....4-6 Rps genes.
High virulence.....7-12 Rps genes.

* Percent of the 90 isolates tested.

Low virulence

Isolate	Number of genes def.
MSU 01	2
MSU 02	2
MSU 14	3
MSU 16	3
MSU 31	4
MSU 34	4
MSU 54	3
MSU 55	4
MSU 56	3
MSU 60	4
MSU 61	2
MSU 63	3
MSU 65	4
MSU 68	3
MSU 71	3
MSU 79	4
MSU 83	4
MSU 84	3

Percentage 20%*

High virulence

MSU 03	5
MSU 09	5
MSU 21	5
MSU 47	6
MSU 50	5
MSU 51	5
MSU 66	6
MSU 75	6

Intermediate virulence

Isolate	Number of genes def.
MSU 78	5
MSU 81	5
MSU 85	5
MSU 89	5

Percentage 13%

High virulence

MSU 08	9
MSU 10	9
MSU 15	8
MSU 17	7
MSU 22	8
MSU 23	12
MSU 24	12
MSU 25	12
MSU 26	12
MSU 27	13
MSU 28	12
MSU 29	9
MSU 30	11
MSU 32	12
MSU 33	10
MSU 35	9
MSU 36	10
MSU 37	8
MSU 48	7
MSU 49	7
MSU 52	8
MSU 53	8

High virulence

Isolate	Number of genes def.
MSU 57	7
MSU 58	7
MSU 59	9
MSU 62	12
MSU 64	7
MSU 69	10
MSU 70	7
MSU 72	7
MSU 73	11
MSU 76	7
MSU 77	11
MSU 80	10
MSU 82	11
MSU 86	8
MSU 87	11
MSU 88	10
MSU 90	9

Percentage 55%

Table 2.3 Ranked performance of Rps genes against isolates of *P. sojae*.

* Following hypocotyl inoculations, gene is defeated when 50% or more of inoculated material are killed.

Source	Gene	Percent of isolates* defeating the gene
PRX 146-36	$Rps3_b$	20
L83-570	$Rps3_a$	24
Harosoy 13xx	$Rps1_b$	27
Williams 82	$Rps1_k$	32
Harosoy 62xx	RPS6	35
L76-1988	Rps2	39
L85-2352	RPS4	40
L85-3059	RPS5	45
Williams 79	$Rps1_c$	46
PI 103	$Rps1_d$	46
PRX 145-48	$Rps3_c$	55
Harlon	$Rps1_a$	78
Harosoy	RPS7	79

Figure 2.1 Typical empty parch in a depressed area of a field affected by *P. sojae* near St. Charles in Saginaw county. Plants with fully manifested symptoms of Phytophthora root rot were concentrated around the empty spot.

Images in this dissertation are presented in color.

Figure 2.2 Field isolates of *P. sojae* were evaluated for host range by comparing them to *P. megasperma* in soybeans, dry beans (black magic) and alfalfa seedlings. *P. sojae* isolates killed only the soybean seedlings while *P. megsperma* did not attack any of the plants as it was found to be avirulent.

Images are presented in color.

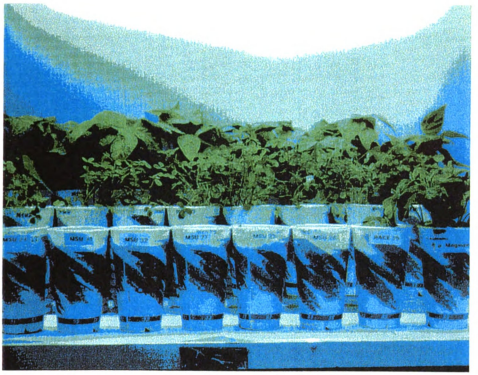

Figure 2.3. For identification, growth rates of field isolates of *P.sojae* were compared to that of *P. megasperma*. *P. megasperma* (center)covered the plate in six days while it took *P. sojae* isolates ten to twelve days to cover plates.

Images are presented in color.

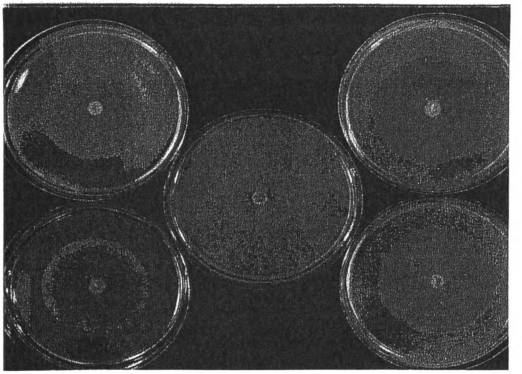

Figure 2.4 Number of highly virulent isolates obtained increased over the sampling seasons. In 1993 an average of only 3 Rps genes were defeated by an isolate. In 1997 an average of seven Rps genes were defeated per isolate. This may be attributed to wider area covered in subsequent years of sampling.

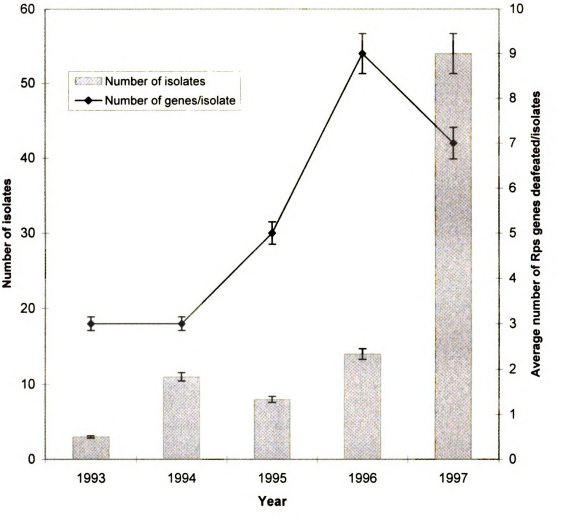
Number of isolates
Number of genes/isolate
Number of isolates
Average number of Rps genes deafeated/isolates
Year
0
10
20
30
40
50
60
0
1
2
3
4
5
6
7
8
9
10
1993
1994
1995
1996
1997

Table 2.4 Year, county of origin and virulence formulae of *P. sojae* isolates.

* Virulence formulae = list of the Rps genes defeated by isolate.
@ NM = virulence formulae do not match those of the known races of *P. sojae*

1993

Isolate	County	Source	Virulence formulae*	Race
MSU 01	Eaton	plant	1b , 7	2
MSU 02	Ionia	plant	1a, 7	3
MSU 03	Shiawassee	plant	1a, 1b, 1c, 1k, 7	25
1994				
MSU 04	Eaton	plant	Avirulent	
MSU 05	Eaton	plant	Avirulent	
MSU 06	Eaton	plant	A virulent	
MSU 07	Ingham	plant	Avirulent	
MSU 08	Barrien	plant	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 7	NM @
MSU 09	Barrien	plant	1a, 2, 3c, 5, 7	NM
MSU 10	Saginaw	plant	1a, 2, 3a, 3b, 3c, 4, 5, 6, 7	NM
MSU 11	Saginaw	plant	Avirulent	
MSU 12	Saginaw	plant	Avirulent	
MSU 13	Oakland	plant	Avirulent	
MSU 14	Eaton	plant	1a, 1c, 7	4
1995				
MSU 15	Eaton	plant	1a, 1c, 1k, 2, 3a, 4, 5, 7	NM
MSU 16	Eaton	soil	1a, 1c, 7	4
MSU 17	Ionia	plant	1a, 1c, 2, 3a, 3b, 5, 7	NM
MSU 18	Ionia	plant	Avirulent	
MSU 19	Ionia	plant	Avirulent	
MSU 20	Monroe	soil	Avirulent	
MSU 21	Monroe	plant	1a, 1b, 1c, 1k, 7	25
MSU 22	Monroe	soil	1a, 1b, 1c, 1d, 1k, 3a, 3b, 7	NM
1996				
MSU 23	Ingham	plant	1a, 1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7	NM
MSU 24	Ingham	plant	1a, 1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7	NM
MSU 25	Clinton	plant	1a, 1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7	NM
MSU 26	Monroe	plant	1a, 1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7	NM
MSU 27	Jackson	plant	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7	NM
MSU 28	Monroe	plant	1a, 1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7	NM
MSU 29	Ingham	plant	1a, 1b, 1d, 1k, 3a, 3c, 5, 6, 7	NM
MSU 30	Monroe	plant	1a, 1b, 1d, 1k, 2, 3b, 3c, 4, 5, 6, 7	NM
MSU 31	Shiawassee	plant	1a, 2, 3c, 7	NM
MSU 32	Ingham	plant	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7	NM
MSU 33	Lenawee	plant	1a, 1b, 1d, 1k, 2, 3c, 4, 5, 6, 7	NM
MSU 34	Lenawee	plant	1a, 1b, 3c, 7	NM
MSU 35	Monroe	plant	1a, 1c, 1d, 1k, 3c, 4, 5, 6, 7	NM
MSU 36	Monroe	plant	1a, 1c, 1d, 1k, 2, 3c, 4,5,6,7	NM

1997

Isolate	County	Source	Virulence formulae	Race
MSU 37	Saginaw	plant	1a, 1c, 1d, 2, 3c, 4, 5, 7	NM
MSU 38	Saginaw	plant	1a, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 7	NM
MSU 39	Saginaw	plant	1a, 1c, 1d, 1k, 2, 3c, 4, 5, 7,	NM
MSU 40	Saginaw	plant	1a, 1c, 1d, 2, 3c, 4, 5, 7	NM
MSU 41	Saginaw	plant	1a, 1c, 1d, 1k, 2, 3c, 4, 5, 7	NM
MSU 42	Saginaw	plant	1a, 1c, 1d, 2, 3c, 4, 5, 7	NM
MSU 43	Saginaw	plant	1a, 1c, 1d, 2, 3c, 4, 5, 6, 7	NM
MSU 44	Saginaw	plant	1a, 1c, 1d, 2, 3c, 4, 5, 6, 7	NM
MSU 45	Saginaw	plant	1a, 1c, 2, 3c, 4, 5, 6, 7	NM
MSU 46	Saginaw	plant	1a, 1c, 1d, 3c, 4, 5, 6, 7	NM
MSU 47	Saginaw	plant	1a, 1c, 1d, 3a, 3c, 7	NM
MSU 48	Saginaw	plant	1a, 1c, 1d, 2, 3c, 4, 7	NM
MSU 49	Saginaw	plant	1a, 1c, 1d, 2, 3c, 4, 7	NM
MSU 50	Saginaw	plant	1a, 1c, 1d, 3c, 7	NM
MSU 51	Saginaw	plant	1a, 2, 3c, 4, 7	NM
MSU 52	Saginaw	plant	1a, 2, 3a, 3c, 4, 5, 6, 7	NM
MSU 53	Saginaw	plant	1a, 1c, 1d, 2, 3c, 5, 6, 7	NM
MSU 54	Saginaw	plant	1a, 3c, 7	NM
MSU 55	Saginaw	plant	1a, 1c, 3c, 7	NM
MSU 56	Saginaw	plant	1a, 1d, 7	NM
MSU 57	Saginaw	plant	1a, 1c, 1d, 3c, 4, 5, 7	NM
MSU 58	Saginaw	plant	2, 3a, 3c, 4, 5, 6, 7	NM
MSU 59	Saginaw	plant	1a, 1d, 2, 3a, 3c, 4, 5, 6, 7	NM
MSU 60	Saginaw	plant	1a, 1c, 3c, 7	NM
MSU 61	Saginaw	plant	1a, 7	3
MSU 62	Saginaw	plant	1a, 1b, 1c, 1d, 1k, 2, 1k, 3b, 3c, 4, 5, 6, 7	NM
MSU 63	Saginaw	plant	1a, 5, 7	NM
MSU 64	Saginaw	pant	1a, 1d, 1k, 3c, 4, 5, 6	NM
MSU 65	Saginaw	plant	1a, 1c, 3c, 7	NM
MSU 66	Saginaw	plant	1a, 1b, 1c, 3b, 6, 7	NM
MSU 67	Saginaw	plant	1a, 1c, 1d, 1k, 3c, 5, 6, 7	NM
MSU 68	Saginaw	plant	1a, 3c, 7	NM
MSU 69	Saginaw	plant	1a, 1c, 1d, 3a, 3b, 3c, 4, 5, 6, 7	NM
MSU 70	Saginaw	plant	1a, 1b, 2, 3a, 5, 6, 7	NM
MSU 71	Saginaw	plant	1a, 1b, 1k	NM
MSU 72	Saginaw	plant	1a, 1b, 1c, 1d, 1k, 5, 7	

NM

MSU 73	Saginaw	plant	1a, 1b, 1d, 2, 3a, 3c, 5, 6, 7	NM
MSU 74	Saginaw	plant	1a, 1c, 1d, 2, 3a, 3c, 5, 6, 7	NM
MSU 75	Saginaw	plant	1a, 1c, 1k, 3c, 6, 7	NM
MSU 76	Saginaw	plant	1a, 1b, 1c, 2, 3b, 5, 7	NM
MSU 77	Saginaw	plant	1a, 1b, 1c, 1d, 2, 3a, 3b, 4, 5, 6, 7	

NM

MSU 78	Saginaw	plant	1a 3c, 5, 6, 7	NM

1997

Isolate	County	Source	Virulence formulae	Race
MSU 79	Saginaw	plant	1a, 1c, 7	NM
MSU 80	Saginaw	plant	1a, 1b, 1c, 1d, 1k, 3c, 4, 5, 6, 7	NM
MSU 81	Saginaw	plant	1a, 2, 4, 5, 7	NM
MSU 82	Saginaw	plant	1a, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7	NM
MSU 83	Saginaw	plant	1a, 1c, 3c, 7	NM
MSU 84	Saginaw	plant	1a, 4, 7	

NM

MSU 85	Saginaw	plant	1a, 1b, 1d, 1k, 7	NM
MSU 86	Saginaw	plant	1a, 1b, 1d, 1k, 3a, 3b, 3c, 7	NM
MSU 87	Saginaw	plant	1a, 1b, 1c, 1d, 1k, 3b, 3c, 4, 5, 6, 7	NM
MSU 88	Saginaw	plant	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 6, 7	NM
MSU 89	Saginaw	plant	1a, 3c, 5, 6, 7	NM
MSU 90	Saginaw	plant	1a, 1d, 1k, 2, 3c, 4, 5, 7	

NM

Table. 2.5. Estimated impact of P. sojae on yield in a field near St. Charles in Saginaw county. Stand counts and yield data were supplied by Growers service Corporation, St. Charles, MI.

* Percent reduction in stand count and yield of non-diseased field.

	Disease field	Non disease field	Percent reduction*
Planting rate	190,000 - 200,000	190,000 - 200,000	
Pest mgt.	2.7 oz Cobra, 0.25 oz Pinnacle, Choice and Act 90.	2.0 oz Cobra, 0.25 oz Pinnacle, Choice and Act 90.	
Stand count	123,000	180,000	32
Yield (bu/strip)	37.9	57.5	34

P. megasperma (6.8 mm/day). Hypocotyl inoculation of soybean varieties with different resistance (RPS) genes to *P. sojae* resulted in avirulent to highly virulent reactions with formulae that do not match those of currently known races of the pathogen (Table 2.4). These traits delineate the isolates from *P. megasperma* which has wider host range, larger oogonia (>45 μm) and a faster growth rate (Figure 2.3).

Tests for virulence and race determination

Ninety (90) of the field isolates were tested for virulence and race determination. Based on reactions to genes (RPS) for resistance to *P. sojae*, isolates were placed on 4 categories of virulence[1]. Fifty or 55% of the isolates tested defeated more than 7 RPS genes each and were categorized as highly virulent (table 1.2). Ten isolates (13%) showed intermediate virulence defeating 4-6 RPS genes while 20% showed low virulence levels defeating 1-4 genes. Eleven percent(11%) of the isolates were avirulent as they did not attack any of the genes including the susceptible variety Williams (rps).

Performance of the RPS genes in their respective soybean varieties showed 1_b, 3_a, and 3_b with the best performance,

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[1] Degree of virulence is based on number of RPS genes attacked.

resisting 70-78% of the isolates while 1_a, and 7 had the lowest performance, resisting only 12-13% of the isolates (Table 1.3).

Impact of *P. sojae* on yield

The average stand count of 10 samples from the diseased field was 123,000 plants/acre. The health field had approximately 180,000 plants/acre (Table 2.5). This translates to 32% reduction in stand count. Yield estimates from four strips within the diseased field were 35.08, 39.9, 43.8, and 32.8 Bu/strip for an average of 37.9 Bu/strip whereas the yield for the healthy field was 47.8 Bu/strip. This amounts to an approximate 21% reduction in yield.

Discussion

This study brings up to date information on the status of *P. sojae* and occurrence of PRR in soybean growing areas of Michigan. The isolates obtained include some of the races (1,3, and 4) identified by Lockwood et al (1985). Most were highly virulent (defeat most of the RPS genes) and thus show the potential to reduce yield when and where environmental conditions are favourable. The development of PRR is highly dependent on the environment, particularly moisture and

temperature. The empty patches (Figure 1) in the field were wet spots in the early part of the growing season, and disease was more intense around these areas at the time of sample collection.

In light of the highly virulent levels exhibited by the isolates, it is noteworthy that the hypocotyl injection test is a wound-inoculation technique and may bypass some natural defense mechanisms. It has also been noted (Schmittenner and Walker, 1979) that some cultivars which are killed by the hypocotyl inoculation are not severely damaged in the field and show little yield loss. As such, the technique does not provide information substantial enough to estimate performance of soybean lines under field conditions. A well-devised non-wounding inoculation method would therefore be appropriate in the deduction of such information.

According to the 1996/97 Michigan Soybean Performance Report (B.W. Diers and J.F. Boyse. Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI), RPS genes 1_a, 1_c, 1_k, 3, 6 and 7 are incorporated either singly or in combinations (1_b+3, 1_c+3 and 1_k+6) in varieties that are currently planted or being developed in Michigan. Previously, these genes have been reported to be resistant to most of the currently known races of *P. sojae*. In this study, RPS genes 1_a and 7 had the lowest resistance levels, each being

susceptible to at least 80% or more of the isolates tested (Figure 2.1). RPS genes 3_b, 3_a, 1_b, 1_k and 6 were (in that order) the most resistant. These genes resisted most of the races including the highly virulent (those defeating more than 8 genes) among them, and only MSU 23 and 27(note that MSU23, 24, 25, 26 and 28 have similar virulence formulae) out of the 90 that were tested for virulence defeated all the 5 genes (Table 2.1). Incorporating these genes singly or in combinations in soybean varieties should provide improved genetic protection against most of the races and minimize risk of yield loss. However, due to race shift and the presence of rare but compatible races of the pathogen, virulence has been known to increase and result in disease within eight years of continued deployment of varieties with a narrow line of genetic defense (Schmitthenner, 1991. Ohio Agricultural Research and Development Center, Woster, Ohio. Research Bulletin No. 1187). In recognition of this risk, more enduring non-race specific genetic protection in soybeans against *P. sojae* has become more attractive particularly when deployed as part of an IPM program. In some states, growers have their popular soybean lines screened for field tolerance to virulent races of *P. sojae* that are common in their state or growing areas. In light of the results obtained in this study, Michigan farmers may benefit from similar program if

implemented in the state.

A number of methods to screen soybeans for tolerance to phytophthora rot have been reported in the literature. The inoculum-layer method (Walker and Schmitthenner, 1984) allows plants to be screened quickly (14-28 days) in controlled environment, and allows the control of race composition. The slant-board test (Olah and Schmitthenner, 1985) allows the measurement of tolerance relatively quickly in a controlled environment. It also allows for the screened plants to be rescued and regenerated where necessary. Either of these two methods could be useful in screening soybeans grown in Michigan for tolerance to the races of *P. sojae* that are found in the state. However these methods allow inoculations with a single race of *P.sojae* per treatment and may be inefficient as they require much space, time and test material as was observed in the course of this study.

The objective of screening soybean varieties for field tolerance, is to provide information to growers on the tolerance of their selected soybean lines to the races of *P. sojae* that occur in their growing areas. Since *P. sojae* may not occur in pure race forms in the field, a screening method that uses a cocktail of inoculum of *P. sojae* races instead of a single race may be more beneficial as it would require less space, time and material; less plant material and time would

be required to test soybeans against a number of *P.sojae* races. It may be possible to modify the inoculum-layer technique such that a slurry of agar containing a cocktail of inoculum is used instead of a culture of a single race of *P. sojae*

Virulent races of *P. sojae* have been identified in Michigan and, show the potential to impact on yield in soybean production as evidenced by an approximate 34% yield reduction in a field near St. Charles in Saginaw county. Incorporating RPS genes 1_b, 1_k, 3_a, 3_b, and 6 in soybean varieties with good field tolerance in conjunction with other control measures should offer more improved protection for PRR. The information obtained from this study will enable growers and plant breeders to better identify and deploy non-race-specific genetic resistance as part of an IPM program in the protection of soybeans from Phytophthora root and stem rot in the state.

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Chapter 3

Effects of the isoflavonoid genistein on the infection of soybean seedlings by zoospores of *Phytophthora sojae*, and the fluorescence of root exudates and field tolerance in soybeans.

Abstract

Compounds exuded by roots of plants have been shown to be important in plant-microbe interactions. In the case of plant pathogens, detection of specific plant molecules may be critical in the recognition and subsequent infection of the potential host , or the suppression of pathogen populations. In this study, the effects of low concentrations of the genistein on the ability of zoospores of *P. sojae* to infect seedlings of soybeans was investigated. Root exudates of soybeans of various field tolerance levels were also characterized for exuded levels of genistein.

One hundred milliliters of a 5 ppm genistein solution was added to half liter Styrofoam cups containing 500 g of wetted soil and two-day (days after emergence) old soybean seedlings. Plants were placed in the growth chamber at 20°C, 70% relative humidity and 14 hours of light. Two weeks after planting, plants were evaluated for disease severity levels. Diphenylboricacid (DPBA) was added to the samples of root

exudates and directly subjected to fluorometric analysis. Significant differences ($P \leq 0.05$) in field tolerance ratings, which varied with varieties, were observed between treatments (with and without genistein). There was no correlation between fluorescence of root exudates and the tolerance values of soybeans. These results suggest that genistein, when applied exogenously, does have an effect on the infection of soybeans by zoospores but the significance of exuded genistein in Phytophthora root rot is not clear. Differential reduction in root rot among soybean varieties can be attributed to differential interaction between individual isolates of *P. sojae* and soybean varieties, and possible differential impact of genistein on the zoospores of isolates.

Introduction

The exchange of molecular signals represents the earliest step in plant-microbe interaction (Bauer, W.D. and G. Caetano-Anolles.1990). In a complex environment such as in the soil, the detection of specific plant molecules by microbes may be critical to recognition and subsequent colonization of the potential host. In *Bradyrhizobium* and *Rhizobium* species of bacteria, expression of nodulation (nod) genes is induced by flavonoids or isoflavonoids specific to the particular hosts (Banfalvic et al. 1980; Verma, D.P.S. 1992). The induction of the nod genes leads to production of a lipo-polysaccharide (by the bacterium) which initiates formation of the nodule

structure by the plant (Lerouge et al. 1990; Verma D.P.S. 1992). The virulence (vir) genes of *Agrobacterium* tumefaciens, which mediate the transfer of DNA to the cells of the plant symbiont, are specifically induced by phenolic compounds such as acetosyringnone which are released from a wounded plant tissue (Bauer, W.D. and Caetano-Anolles1990; Zambryski, P. 1988). The response of *Agrobacterium* and *Rhizobium* species to plant signals also include chemotaxis in which the bacteria swim to towards potential colonization sites (Bauer, W.D. and G. Caetano-Anolles. 1990). It has also been suggested that the isoflavonoids formononetin (7-hydroxy,4'-methoxy isoflavone) and biochanin A (5,7-dihydroxy, 4'methoxy isoflavone) may act as signal molecules in vesicular arbuscular mycorrhiza symbiosis. (Nair et al,1991).

The zoospores of plant pathogenic Oomycetes also exhibit chemotaxis in response to certain plant compounds (Carlile, M.J. 1983; Horio et al. 1992; Morris, P.F. and E.W.B. Ward. 1992; Sekizaki, H., and R. Yokosawa. 1988; Sekizaki, H., R. Yokasawa, C. Chinen, H. Adachi, and Y. Yomane. 1993). Zoospores, motile unicellular structures that are generally released under flooded conditions and nutrient deprivation, form the predominant means by which pathogenic Oomycetes spread throughout the soil and infect plants (Carlile, M.J. 1983). Zoospores of Oomycetes achieve chemotaxis by the same strategy as bacteria ; they swim steadily by means of flagella propulsion in the presence of an attractant, but turn more

frequently in the presence of repellent compound (Carlile, M.J. 1983). Zoospores of most *Phytophthora* species are attracted to a variety of sugars and amino acids, particularly aspartate, glutamate, arginine and methionine (Carlile, M.J. 1983). Several oomycetes are attracted to specific plant signals. Isovaleraldehide, valeraldehide and anti-isovareldehide attract zoospores of *Phytophthora palmivora* at concentrations as low as 1 μM (Cameron, J.N., and M.J. Carlile. 1981; Carlile, M.J. 1983). Prunetin (4',5-aldehyde-7-methoxyisoflavone) and related compounds are potent attractants (at concentrations as low as 10 nM) of *Aphanomyces enteiches* zoospores (Sezaki, H., and R. Yokosawa. 1988; Sezaki, H., R. Yokasawa, C. Chinen, H. Adachi, and Y. Yomane. 1993), and the zoospores of *Aphanomyces cochoides* are attracted to cohliophilin A [5-hydroxy-6,7-(methylenedioxy) flavone] from the roots of its host, the spinach plant at 1 nM (Horio et al. 1992).

The zoospores of the soybean pathogen *Phytophthora sojae* (syn. *P. megasperma f.sp. Glycinea*) are attracted to the isoflavone genistein (4',5,7-trihydroxy isoflavone), which is present in soybean seeds, and is exuded by the roots of the plant (Morris, P.F. and E.W.B. Ward. 1992). This compound attracted zoospores of *P. sojae* and one spp. of *Pythium* but those of six other species of *Phytophthora* were not attracted with concentrations as high as 30 μM (Morris, P.F. and E.W.B. Ward. 1992). Apart from chemotaxis, daidzein and genistein

also cause rapid encystment and germination of zoospores of *P. sojae*. Therefore, Morris and Ward (Morris, P.F. and E.W.B. Ward. 1992) suggested that sensitive attractions of *P. sojae* zoospores to soybean isoflavones may be part of the mechanism which determine host range. Wacker and Safir (unpublished) found that genistein at concentrations as low as 5 ppm in plant growth solution can reduce infection of soybean seedlings by zoospores of *P. sojae*. At 10 μg/ml, genistein inhibited radial (hyphal) growth and reduced asexual reproduction of *P. sojae* in culture (Vedenyapina et al. 1996). Thus, it is possible that field tolerance (resistance) of soybeans to *P. sojae* may be controlled to a large extent by root isoflavonoid exudation characteristics or the properties of certain specific isoflavonoids within the roots.

Currently, little information exists on the mechanism behind the effects of isoflavonoids on microbial activity but environmental factors are believed to have a significant role (Zhang and Donald, 1996). Effects of genistein in plant-microbe interactions vary with specific organism. Sub-optimal root zone temperature (RZT) ranging from 13 to 17°C delays infection and early nodule development in *R. japonicum*, and addition of genistein overcomes some of these effects (Zhang and Donald, 1996). Also, soybeans germinated and maintained at sub-optimal RTZs have lower root genistein concentrations than those germinated and maintained at RZTs above sub-optimal (Zhang and Donald, 1996). Therefore, it is possible that

reduction of genistein concentrations in soybeans due to cool field conditions result in increased infection by *P. sojae*.

There is indication that the phenolic 4'-and 7-hydroxyl group on the aromatic rings of the isoflavone play a crucial role in chemotaxis (Tyler et al. 1996). Only isoflavones with a 4'-hydroxyl or methoxyl group attracted zoospores at concentrations below 20 nM while methylated flavones with hydrophobic B rings acted as repellants to zoospores of *P.sojae*. (Tyler et al. 1996). The process of encystment in zoospores is associated with both eflux and the uptake of calcium (Irving et al., 1984, Iser et al., 1989). This process results in substantial loss of Ca^{2}+ reserves as zoospores were reported to release up to 30% of total cellular Ca^{2}+ at encystment (Irving et al., 1984). The addition of daidzein and Ca^{2}+ at low levels (P≤0.05 mM) to the media of *P. sojae* triggered transient increase of calcium in the hyphae, and caused zoospores to encyst and germinate (Mary et al., 1999 unpublished?). Similar levels of daidzein or Ca^{2}+ alone did not significantly alter the fate of zoospores (Mary at al., 1999 unpublished). The interaction between isoflavones and Ca^{2}+ may also account for changes in hyphal morphology as observed by Rivera-Vargas et al. (Rivera-Vargas et al.1993), and Vedenyapina et al. (Vedenyapina et al.1996). The two studies reported hyphal swellings, increased branching, and twisting of hyphea of *P. sojae* grown on media containing less than 1 uM of genistein.

In this study, the effects of low concentration of genistein on the ability of zoospores of *P. sojae* to infect seedlings of soybeans of various field tolerance levels was investigated. Also the fluorescence levels of root exudates of soybeans of various field tolerance levels were studied. It is possible that at certain concentrations, genistein causes zoospores to encyst away from soybean roots and thus reduce the inoculum potential. The fluorescence characteristics of root exudates may be directly related to differences in susceptibility and tolerance to *P. sojae* among soybean varieties. This work reports what may be first information on the effects of genistein on the infection of soybean seedlings by zoospores of *P. sojae*.

Materials and Methods

The effects of the isoflavone genistein on the infection of soybean seedlings by zoospores of *P. sojae* was studied in the first experiment . In the second experiment, fluorescence readings (which served as indicators of the levels of exuded genistein) of soybean root exudates were compared to field tolerance values of respective soybean varieties. Selected isolates of *P. sojae* from soybean plants were evaluated for their ability to infect soybean seedlings in the absence and presence of genistein (4' 5, 7-trihydroxyisoflavone). The isolates MSU 23, MSU 25 and MSU 32 are single zoospore cultures of *P. sojae* obtained from Michigan soybean fields in

the 1996 growing season. The isolates were maintained on V8-juice agar (200 ml of clarified V8-juice , 2 g of $CaCO_3$ per liter, and 1.5 % Bacto agar) at 15 °C in the dark. Genistein, synthesized by and obtained from Dr. M. Nair (Michigan State University), and was tested at 0 and 5 ppm. The soybean varieties Chapman (rps), Felix (rps), Sundusky (rps), Conrad (rps), Repley (rps) and Colfax (rps) were obtained from Michigan Foundation seeds (Okemos MI, 48864) and do not have known resistance (Rps) genes to *P. sojae*. Varieties Williams 82 (Rps 1_k), Harosoy (Rps 7), PI 103 (Rps 1_d) Pella (rps), Harlon (Rps 1_a), and Sloan (rps) were provided by A. F. Schimitthenner (Ohio Agricultural Research and Development Center, Wooster,).

Experiments were repeated 3 times.

Influence of genistein on the infection of soybean seedlings by zoospores of *P.sojae*

To induce zoospore production in the *P. sojae* , ten mycilial plugs (7 mm in diameter) were transferred from the edges of actively growing cultures to sterile petri dishes and flooded with 20% clarified V8-juice agar. After 48 hours of incubation, at room temperature, the broth was removed and plugs were rinsed 5 times with distilled de-ionized H_2O and re-flooded with 10 ml of ARS (2.94 g CaCl2.2H2o, 2.47 MgSO4.$7H_2O$, and 0.75 g KCl in 1000 ml of distilled de-ionized water) salt at Ph 6.5. Cultures were further incubated at room temperature and zoosporangia formed in 12 hrs.

To accelerate and synchronize the release of zoospores by sporangia, cultures were incubated at 5°c for 30 min. Zoospores were released when cultures were returned to room temperature (24°C). One milliliter aliquots of each zoospore suspension was transferred into micropreparation wells, and 2 drops of 0.1% trypan blue solution in lactoglyceral were added to each well. The number of zoospores in the wells were counted under a light microscope at 250X. This was repeated 10X for each isolate. Final inoculum concentrations were prepared by adjusting the number of zoospores in each culture to 3 $x10^3$ /ml.

Soybean seeds were surface-sterilized with 10% bleach for 10 min. and rinsed 3 to 4 times with distilled de-ionized water. The seeds were germinated in germination paper. Genistein solutions were prepared by dissolving 3 mg of the isoflavone in 3ml of methanol and added to 997 ml of distilled de-ionized water to bring the volume to 1000 ml.

Two-day old seedlings were transplanted to ½ liter pots containing 500g wetted soil. 50 ml of the zoospore suspension were added to the pots and followed by 100 ml the of genistein solution. The seedlings were placed in the growth chamber at 20°C, 70 % relative humidity and 14 hours of light. Plants were watered daily with 50 ml of distilled de-ionized water. Two weeks after inoculation, data on tolerance to disease levels (1 = no root rot; 2 = trace of root rot; 3 = bottom third of root mass rotted; 4 = bottom 2/3 of root

mass rotted; 5 = all roots rotted, 10% of seedling kill, slight stunting of tops of plants; 6 = 50% seedling kill, moderate stunting of tops of plants; 7 = 75% seedling kill, severe stunting of tops of plants; 8 = 90% seedling kill; 9 = all seedlings dead; 10 = all seedlings killed before emergence) were collected and the dry weights of roots were determined.

Collection of root exudates

Root exudates of soybean varieties were collected using the method of T.L. Graham (1990). Surface-sterilized soybean seeds were germinated and grown in specifically designed growth chambers, which allowed the seed to imbibe slowly and the roots to be suspended in air at 100 % RH. Black polystyrine plant growing trays with wells measuring 2.0 X 2.5 cm (A.H. Hermmert Seed Co., St. Louis, MO) were cut to form grids of 5 X 6 well rectangles. Trays were sterilized by soaking in 70% ethanol for 20 - 25 min prior to use. Individual soybean seeds were loosely wrapped in moistened sterile germination paper and placed in the individual wells in trays. The seeds were oriented such that their radicles would extend out through the drainage holes at the bottom of the wells. Trays were then suspended in sterilized closed polypropylene containers (19 cm square X 21 cm deep) lined with absorbent tissue thoroughly soaked in sterile water, and incubated at room temperature for 24 hrs. Pre-washed sterile

water-soaked cotton (2 mm diameter) wicks were placed at the lower sections of actively growing roots suspended in the growth chamber. After 30 min, wicks from 30 replicate seeds were collected and centrifuged in modified centrifuge tubes that allowed the collection of exudate at a lower chamber and retention of wicks in an upper one. Samples of root exudates were subjected directly to fluorometric analysis in the presence of DPBA (diphenylboricacid) using a Sequoia Turner (model 450) fluorometer. Fluorometer filters were set at 309 nm (excitation) and 520 nm (emission) for maximum detection of genistein. Calibration was achieved with standard genistein (WR Scientific, St. Louis, MO) dissolved in methanol in the presence of DPBA.

A correlation analysis of the fluorescence levels of root exudates and the field tolerance values of soybean varieties was performed using the statistical package Minitab.

Results

Influence of genistein on the infection of soybean seedlings by zoospores *of P. sojae*

Genistein significantly increased the percentages of dry weights of roots of most of the soybean varieties across the isolates (Table 3.1). Conrad had the highest overall increase in root mass (63.0%) followed by Colfax at 56.4 %. Felix had the lowest increase of 29.2 %. The isolate MSU25 yielded the highest increase (83.7%) in combined root mass of soybean varieties. Isolates MSU23 and MSU32 yielded lower increases of

Table 3.1. Percent (%) increase in root mass (dry weights) of soybean seedling roots in the presence of genistein.

* Total increase in dry weights of all soybean varieties subject to one isolate in the presence of genistein.

@ Total increase in dry weights of individual soybean varieties subject to all three isolates of *P. sojae* in the presence of genistein.

	MSU23		MSU25		MSU32		All isolates[@]	
Variety	**+G-P**	**+G+P**	**+G-P**	**+G+P**	**+G-P**	**+G+P**	**+G-P**	**+G+P**
Chapman	**100** ±4.3	**65** ±4.6	**107** ±5.2	**53.6** ±3.4	**56.8** ±2.8	**40.5** ±2.7	**87.9** ±9.4	**53.2** ±23.2
Felix	**51.6** ±3.0	**3.2** ±1.2	**95.8** ±3.4	**58.3** ±3.7	**38.2** ±1.6	**26.0** ±2.3	**61.8** ±8.3	**29.2** ±29.2
Sundusky	**152.6** ±2.9	**105.3** ±7.5	**71.4** ±3.3	**50.0** ±4.5	**65.5** ±2.2	**10.3** ±1.8	**96.5** ±7.8	**55.2** ±13.8
Conrad	**95.5** ±4.1	**9.1** ±2.3	**186.7** ±2.8	**120** ±5.2	**115** ±3.6	**60.0** ±3.1	**132.4** ±10.2	**63.0** ±23.0
Colfax	**122** ±3.6	**22.2** ±2.7	**263** ±4.6	**136** ±4.3	**43** ±1.8	**10.7** ±2.6	**142.7** ±12.4	**56.4** ±9.6
All varieties*	**104.3** ±8.6	**41** ±18.7	**144** ±13.7	**83.7** ±21.1	**63.7** ±4.3	**29.5** ±12.5		

Figure 3.1. Dry weights of roots in the presence and absence of genistein. Roots of soybean seedlings were clipped off and dried at 80°C for 24 hrs before weighing. Significant($P \leq 0.05$) increases in the root mass of soybean seedlings were recorded in the presence of genistein.

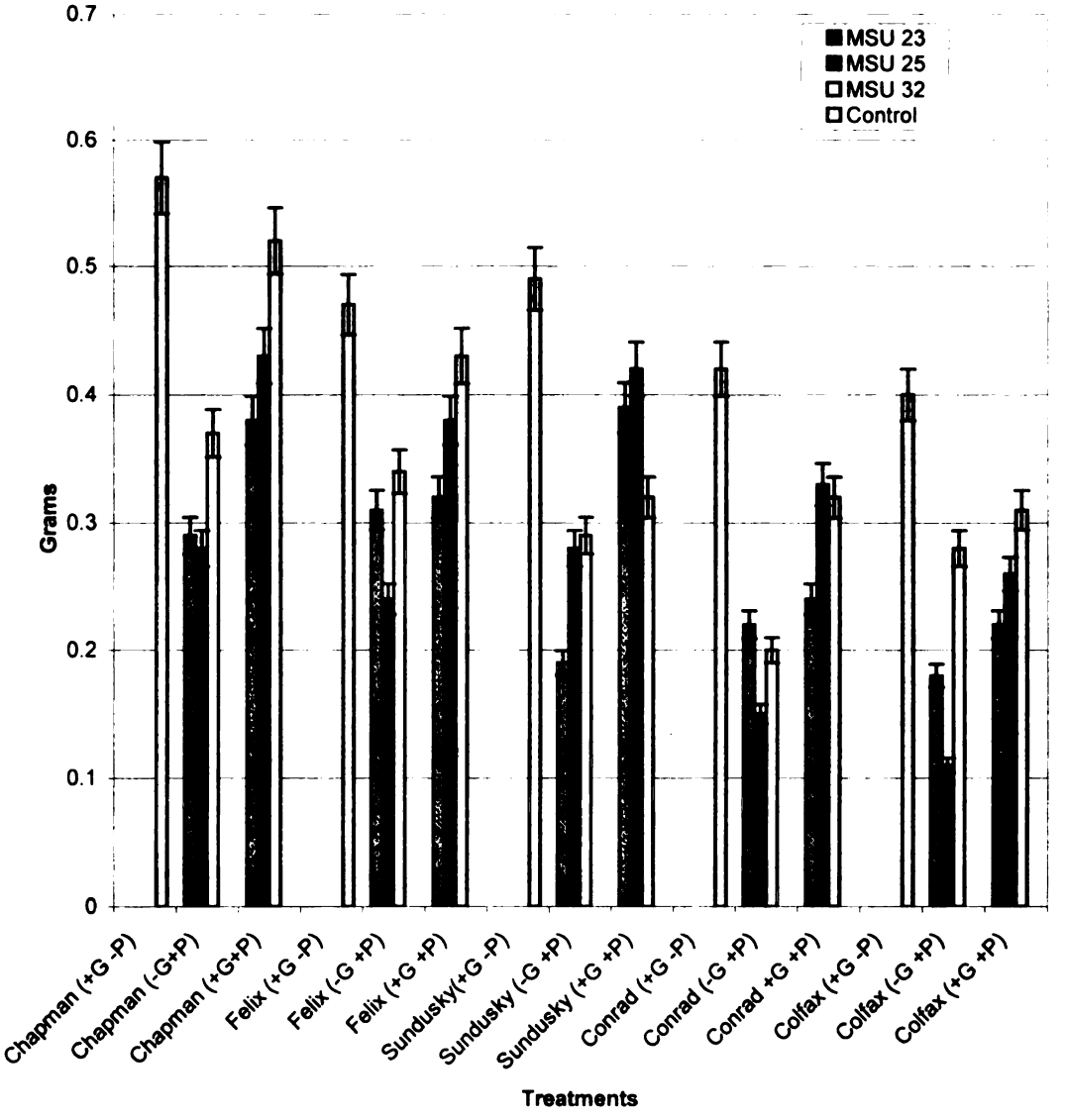

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Grams
MSU 23
MSU 25
MSU 32
Control
Chapman (+G -P)
Chapman (-G+P)
Chapman (+G+P)
Felix (+G -P)
Felix (-G +P)
Felix (+G +P)
Sundusky(+G -P)
Sundusky (-G +P)
Sundusky (+G +P)
Conrad (+G -P)
Conrad (-G +P)
Conrad +G +P)
Colfax (+G -P)
Colfax (-G +P)
Colfax (+G +P)
Treatments

Figure 3.2. Effect of genistein on field tolerance levels in soybean seedlings. Significant ($P \leq 0.05$)increase in tolerance in the presence of genistein was observed. Tolerance evaluation was based on the extend of lesions and amount of rotted root tissue.

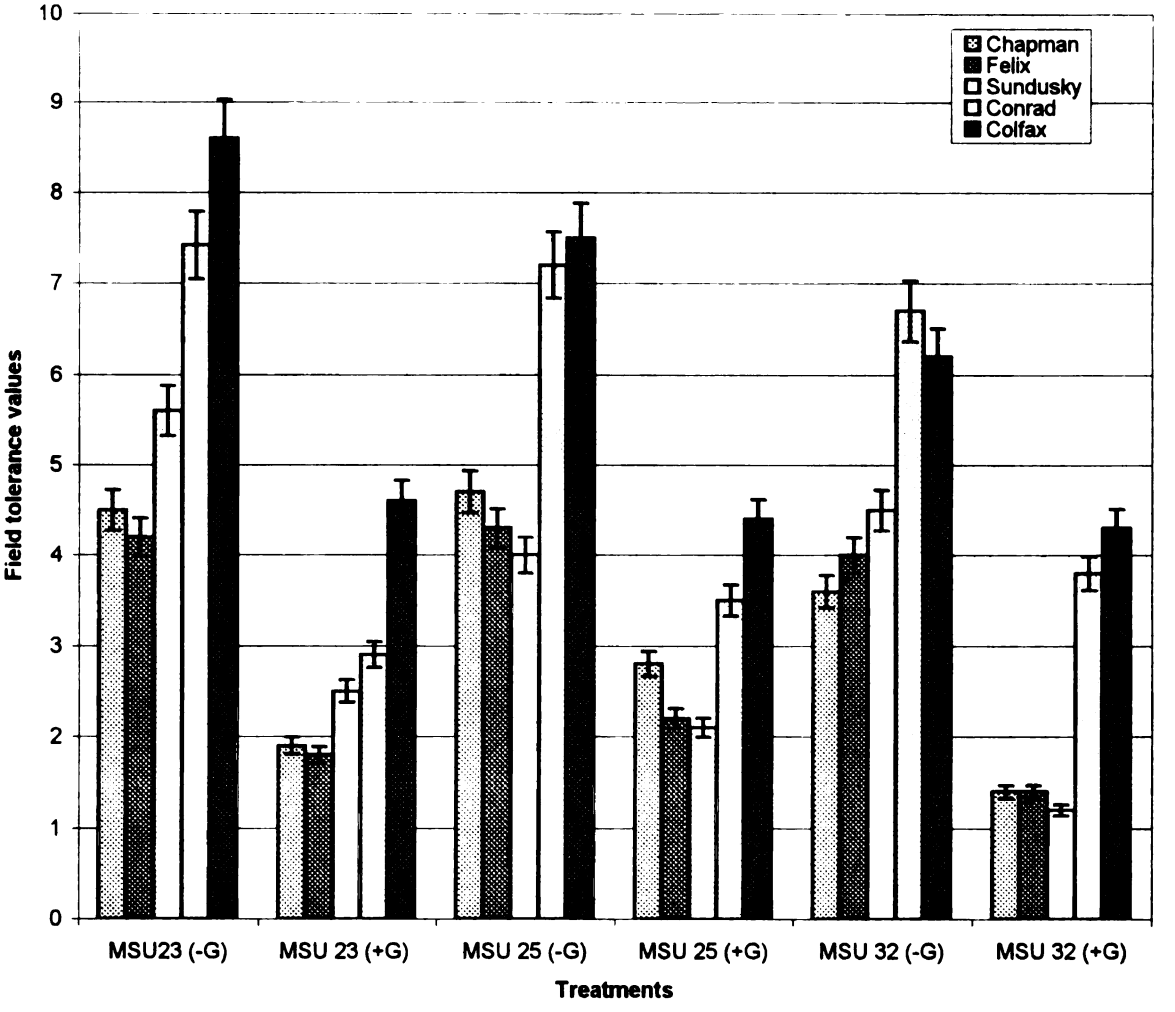
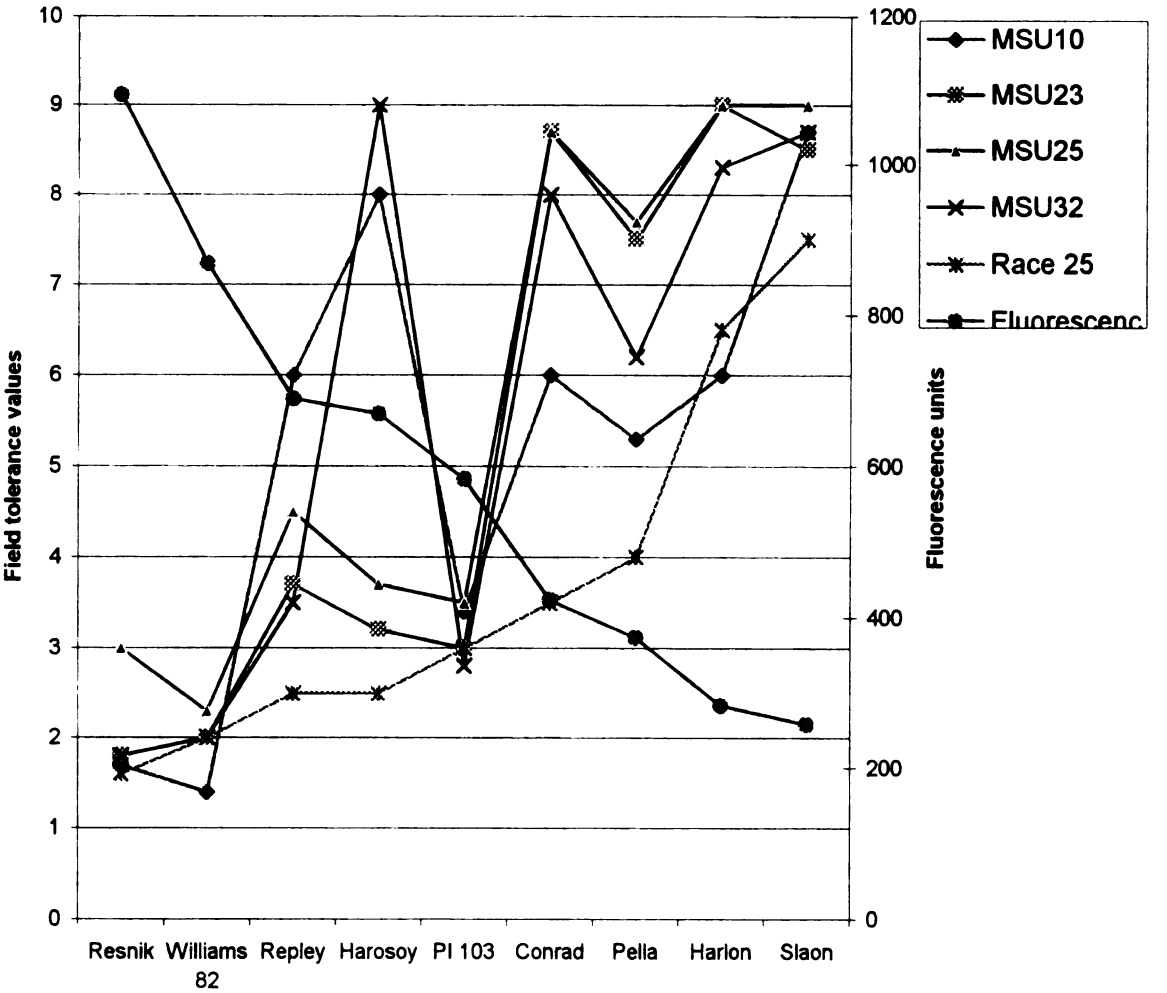

Chapman
Felix
Sundusky
Conrad
Colfax
Field tolerance values
0
1
2
3
4
5
6
7
8
9
10
MSU23 (-G)
MSU 23 (+G)
MSU 25 (-G)
MSU 25 (+G)
MSU 32 (-G)
MSU 32 (+G)
Treatments

Figure 3.3. Fluorescence of root exudates and field tolerance values of soybeans. Soybean plants were inoculated with zoospores (3000/ml) of *P. sojae* isolates. Fluorescence values were obtained by subjecting root exudates to fluorometric analysis in the presence of DPBA using a Sequoia Turner fluorometer.


Field tolerance values
Fluorescence units
MSU10
MSU23
MSU25
MSU32
Race 25
Fluorescenc
Resnik
Williams 82
Repley
Harosoy
PI 103
Conrad
Pella
Harlon
Slaon

41.1 % and 29.5 % respectively. Colfax had the highest increase of 136.4 % against MSU25 while Felix had the lowest root mass increase of 3.2 % against MSU23. Increases for individual varieties and isolates were variable, with low tolerance lines yielding higher increases in dry weights of roots.

Significant differences ($P \leq 0.05$) in field tolerance values were observed between treatments (+G-P, -G+P and +G-P) in all soybean varieties across isolates of *P. sojae* (Figure 3.1). Genistein had the greatest impact on the isolate MSU23 which resulted in increased tolerance by 3.3 points, followed by MSU25 at 2.5 points. Variety Conrad had the highest (3.7 points) overall (across isolates) increase in tolerance to *P. sojae* (Table 3.1). The greatest single increase was with MSU25 (4.5points) and the lowest was 2.9 with MSU32. Chapman had the lowest overall tolerance increase of 1.7 points, but achieved the highest single isolate increase in tolerance of 2.6 points against MSU23.

Fluorescence of root exudates and field tolerance levels of soybeans

A negative correlation between fluorescence levels of root exudates and field tolerance was observed for all the soybean varieties in the study (Figure 3.3). Resnik , Williams and PI 103 which had high tolerance levels, showed high exudate fluorescence. Conrad, Pella . Harlon and Sloan, which

had low or no tolerance to the *P. sojae* isolates, gave low fluorescence values. Varieties Repley and Harosoy had no tolerance to the isolates MSU10 and MSU32. Resnik , Williams and PI103 demonstrated high tolerance across isolates while other varieties gave highly variable results. These results varied with variety-isolate interactions.

Discussion

The results presented in this study represent what may be the first report on the reduction of *P. sojae* zoospore infection of soybean seedlings by the isoflavonoid genistein. Decreased infection of roots of soybean seedlings (recorded as increased dry weights of roots and lower disease ratings) resulted from exogenous application of genistein (5 ppm) in pot cultures of soybeans inoculated with zoospores ($1x10^3$ cfu/ml) of *P. sojae*. Genistein has previously been shown to attract zoospores of *P.sojae* (which swim against increasing concentration) and hasten their encystment and germination (Morris and Ward.1992), and may be important in the soybean-*P. sojae* interaction. Vedenyapina et al (1996) reported reduced hyphal growth and asexual reproduction by genistein at 10 μg/ml. The results of experiments we conducted in growth chambers show that at 5ppm, genistein can reduce the infection of soybean seedlings by zoospores of *P. sojae,* and significantly increase the dry weights of roots ($P \geq 0.05$) Although disease rating values (obtained through visual

examination of plant tissue) make the data rather subjective, they provide reasonable estimates on disease levels. Field tolerance values of 4.0 and below are considered good by breeders and growers (Schmitthenner, personal communication) and genistein did reduce disease to this level in all varieties that were tested.

Although there appeared to be a general inverse correlation between fluorescence of root exudates and field tolerance values of soybean varieties, high variability among soybean varieties-isolate interactions did not enable conclusive observation on the role of exuded genistein in field tolerance. However, differential reduction in root rot among soybean varieties can be attributed to differential interaction between individual isolates of *P.sojae* and soybean varieties, and possible differential impact of genistein on the zoospores of isolates. Vedenyapina et al (1996) observed strong intraspecific variation in *P. sojae* in response to genistein at concentrations as low as 0.01 to 1 μg/ml. This adaptive variation to genistein by zoospores may have a role in field tolerance. It is possible that aggressive isolates of *P. sojae* may have evolved a fitness trait to concentrations of genistein that limit the development of unadapted individuals (Vedenyapina et al). The possible trait would enable infective structures to maintain their zoosporic form and swim long enough to come in contact with plant roots. Differential interaction between soybean varieties and *P. sojae* may

explain the large variability in disease levels on Harosoy, Conrad and Pella. These varieties were also defeated by a large number of field isolates of *P.sojae* in pathogenicity tests as reported elsewhere in this study. Resnik, Williams, and PI103 displayed high tolerance and fluorescence of root exudates. These varieties were also resistant to most of the field isolates of *P. sojae* (see page 42). Results obtained from this study suggest that the composition of soybean root exudates may have an impact on Phytophthora root and stem rot of soybean.

Among the reported effects of genistein on zoospores are chemotaxis (zoospores swim against increasing concentration) and rapid encystment and germination of cystospores (Vedanyapina et al, 1996; Tyler et al, 1996; Irving et al, 1984, and Iser et al., 1989). By swimming in the direction of increasing isoflavanoid concentration, zoospores are guided to the roots of actively growing seedlings where they will aggregate, encyst and germinate. Germ tubes form appresoria and penetrate the roots at appropriate sites. It is possible that where sufficient amounts of genistein are exuded and well dispersed, most zoospores are induced to encyst and germinate away from roots and thus result in the reduction of inoculum potential. It is also probable that once germinated, zoospores of *P. sojae* which are small and delicate structures, may immediately exhaust food reserves and fail to develop in the absence of a host, and thus further limit inoculum potential.

Results obtained from this study showed that the isoflavonoid genistein does affect the potential of *P.sojae* to infect the roots of soybean seedlings. Thus, genistein may play an important role in the field tolerance of soybean varieties to the pathogen. Factors responsible for differential effects of genistein on different isolates of *P. sojae* are not clear. Vedenyapina et al (1996) reported variable effects of genistein on zoospores of different races of *P. sojae* although genistein used in the study came from one source. This observation tend to place the source of variation in intrinsic qualities of the zoospores. In this study, however, different isolates of *P.sojae* yielded variable field tolerance values for each of the soybean varieties indicating a possible second factor which resides in the host.

It is not known whether or not differences in field tolerance of soybean to *P. sojae* is due only to some intrinsic qualities of zoospores. More information is needed on the role of genistein in the variable field tolerance of soybean to *P. sojae*. Understanding the role of exuded genistein in this variability will enhance the effort by breeders and growers to identify more tolerant soybean varieties as the crop continues to grow in importance to the nation's agriculture and economy.

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Chapter 4

Survey of *P. sojae* presence in soybeans infested with the soybean cyst nematode.

Abstract

Phytophthora sojae (Kaufmann and Gerdemann) is a serious but opportunistic pathogen of soybeans. *P. sojae* is known to attack soybeans mostly when under stress from other environmental factors such as cool and wet field conditions, which put stress on plants but create favorable conditions for the release and dissemination of zoospores. Thus, it is possible that nematode feeding may augment the infection of soybeans by *P. sojae* when both organisms are present and conditions are favorable. To examine the possible impact of nematode activity on the infection of soybeans by *P.sojae,* soybean plant samples were collected from a field trial study of soybean varieties in a field naturally infested with *H. glycines.* Soybean samples were scored for the presence of *P. sojae* and mean values were compared to the average number of nematode cysts in 100 cc of soil from the rhizosphere of soybean varieties. In the non-fumigated plots, significant ($P \leq 0.05$) correlation between nematode cyst numbers and the presence of *P.sojae* were observed. In the fumigated plots, lower cyst numbers and *P.sojae* scores were observed but there

was no correlation between the two organisms. These results suggest that *H. glycines* may augment the infection of soybeans by *P.sojae* where the soybean crop is susceptible and soil conditions support both organisms.

Introduction

Of the species of nematodes known to parasitize soybeans, Soybean cyst nematode (SCN), *Heterodera glucines* Ichinoe, is a major pest of soybean (*glycine max*) in the north central United states. Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, Ohio, and Wisconsin have all reported *H. glycines* infestation (Niblack, 1993). This nematode is the major limiting factor in soybean production in the north central region of the United States (W.G. Bird and F.W. Warner, 1990). SCN probably had a long association with soybean in Asia; it was first reported in Heilongjiang province of China in 1938 (Nakata and Asuyana, 1938). The presence of the nematode was documented in 1936 from Korea (Yokoo, 1936), in 1958 from Taiwan (Hang, 1958), and in 1984 from the island of Java in Indonesia (Nishizawa, 1984).

In North America, *H. glycines* occurs in the USA and Canada. In the US the nematode was first reported in 1954 in Hanover county in North Carolina and has since spread to most soybean producing states (Winstead , Skotland and Sasser,

1955; Brewer, 1981; and Mulrooney, 1988). In Canada, SCN was first detected in Kent county in Ontario in 1957 (Anderson et al., 1988). The nematode was first detected in Michigan in 1987 in Gratiot county (W.G. Bird and F.W. Warner, 1988), and has since been reported in 25 counties in the state (G.W. Bird and F. Warner; personal communication).

Temperature, soil water, and soil texture are the most important physical factors that affect the development of nematodes. Juveniles do not develop beyond the second stage in soybean roots grown at constant temperature of 10 ^{0}C in water baths in a greenhouse, and adult females do not develop at 35 ^{0}C (Ross, 1964). The calculated basal temperature threshold is 5 ^{0}C and the thermal optimum for embriogenesis and hatch with low mortality is 24 ^{0}C (Andeson et al., 1988). Development within the egg stops at the first juvenile stage at 15 to 30 ^{0}C. Hatch occurs at 20 to 30 ^{0}C but at 36 ^{0}C the egg dies. However, soil temperature averages in excess of 34 ^{0}C during the month of July in Georgia did not lower juvenile population as expected (Hussey and Boerma, 1983). Hatch of juveniles declined in November (Ross, 1963) and this decline could be attributed to induction of dormancy by decreasing temperature (Hill and Schmitt, 1989).

For movement in the soil, nematodes require a film of water in the pore space around soil particles (Wallace, 1964).

In a study by Heatherly et al (1982), the distribution of cysts increased significantly at water potentials between -30 and - 40 kPa in sandy loam soil (49% sand, 42% silt, and 9% clay). Baker et al (Baker and Koenning, 1989) reported that at the middle of the growing season, soil water levels did not affect the number of *H. glycines*, but late in the season low soil water favored reproduction of the nematode. In the same study, yield was suppressed by approximately the same percentage in wet and dry treatments. These results suggest that SCN damage cannot be overcome by irrigation, although yield may be higher in infested fields with irrigation than in non-infested fields without irrigation. Soil water and nematode infestation effects on yield were approximately equal in a study by Young and Heatherly (1988).

Soil particle size is a major determinant of pore size , which governs the ease of nematode movement through the soil. SCN apparently does not maintain populations in fine-textured soil such as sharkey clay (Heatherly and Young, 1991). SCN number increased 60 days after planting in Dubbs containing silt loam soil maintained at - 30 kPa but declined in Sharkey clay soil kept at the same soil water levels (Heatherly and Young, 1991). Soil texture may also influence damage potential of SCN on soybean. Koenning et al (Koenning et al.,1988), reported that when sand content of soil is greater than 70% in

SCN-infested fields in Missouri, large differences in soybean yield are associated with small changes in sand content of soil. As sand content of soil decreased, differences in yield between resistant and susceptible cultivars narrowed.

Effects of tillage on SCN have been inconsistent. However population levels are lower and yields are higher in soils that receive little disturbance (no-till) compared to soil receiving conventional tillage practices (Tayler et al., 1987). In a study by Young (1987), more SCN (three-fold) females were extracted (30 days from planting) from disturbed soil earlier than from undisturbed soil cores (10 - cm-diameter by 15cm- deep). Disturbance of soil appears to have at least a short-term effect on SCN population dynamics and yield , but the factors responsible are not known.

Most soybean disease complexes that involve SCN have a soil-inhabiting fungus as the other component. Significant interactions which augment both fungal and nematode populations in soybean roots occur between *Calonectria crotalaria* Bell and SCN under greenhouse conditions (Overstreet and McGawley, 1988, 1990). Soybean plants growing under field conditions and parasitized by SCN exhibited augmented *Fusarium* wilt (Ross, 1965). Soybeans infested by SCN in the presence of *F. oxysporum* or *F. solani* have exhibited severe wilting (Roy et al., 1989). Race 3 of SCN and race 1 of

P. sojae increased seedling disease of soybean, although only additively whereas interaction between *Macrophomina phaseolina* and SCN has had variable results (Adeneji et al., 1975). In one study, SCN augmented *M.. phaseolina* activity (Todd et al., 1987). Yet in another study (FranceI and wyllie, 1988), no interaction between the two pathogens was evident. When pathogenic and *endomycorrhizal* fungi associated with soybean roots were surveyed, no consistent relationship was found (Schenck and Kinloch , 1974).

Soybean genotype is an important factor controlling the amount of disease caused by SCN. Because initial infection of soybean plants seems to be more important than subsequent infections, practices that reduce the initial SCN population densities should be effective (Wrather and Anand, 1988). Resistant cultivars are effective in reducing SCN population levels and increasing yield (Hartwig, 1981). However the value of resistance is limited by selection of new races of the nematode when the cultivars are planted frequently. Crop rotation and application of nematicides have the same effects except that these practices do not increase selection pressure on nematode population for genotypes which can reproduce on the resistant cultivars (Edward et al., 1988).

Nematicide application on soybean has been diminishing in recent years because of toxicological, environmental, and cost

factors (Johnson and Feldmesser,1987; Kinloch, 1979; and Riggs and Wrather, 1992).. Limitation of nematicide use in soybean production has also been linked to the value of the crop. From 1973 until the early 1980s, when soybean prices were conducive, use of nematicides in the southern United States was common. The combination of product removal and low prices of soybean has resulted in the rapid decline of this management tactic (Riggs and Wrather, 1992).

Biological control, the use of natural enemies to manage the population levels and minimize damage by nematodes, has been investigated (Carris and Glawe, 1989; Kerry, 1984; Morgan-Jones and Rodriguez-Kabana, 1987; Tribe, 1977, 1980). This management tactic has not achieved commercial application in the control of SCN because most of the organism studied have either exhibited limited success in trials or are impractical (Cook, 1983). About 150 species of fungi have been isolated from eight species of cyst nematodes; about 60% of these are from SCN (Carris and Glawe1989; Epps and Golden, 1967). However, most of the fungi have not been tested for parasitism and efficacy as biocontrol agents on the nematode. Also, some of the fungi are obligate parasites and cannot be cultured on artificial media (Riggs and Wrather, 1992). Field application of fungal biological control agents has further been limited by the lack of suitable carrier material and

application methods (Backman and Rodriguez Kabana, 1987; Conway, 1986, Pereira and Roberts, 1990; and Walker and Connick, 1983).

The bacterial parasite, *Pasteuria penetrans* (Sayre and Starr), continues to receive attention because of its effectiveness, resistance to adverse environmental conditions, and host specificity. A new strain of *P. penetrans* has recently been discovered in Korea, the U. S.A. (Riggs and Wrather, 1992)and Japan (Overstreet and Mcgawley, 1990), but its obligatory status limits its usefulness. Application of individual biological control agents has been further limited by the interactive competition with other soil microorganisms. However, combinations of multiple agents or the improvement in formulations, have considerable potential for biological control of SCN (Tribe,1980).

Following the recent identification of highly virulent isolates of *P. sojae* in Michigan , plant samples were collected from a field trial study of soybean varieties in a field naturally infested with *H. glycines*. The objective of the survey was to examine possible effects of SCN on *P. sojae* infection of soybean varieties. *P. sojae,* the causal agent of root and stem rot in soybeans, is an opportunistic pathogen as it attacks soybeans mostly when under stress from other environmental factors (Moots et al, 1988 and Kittle et al,

1979). Thus, it is possible that stress from nematode feeding augments *P. sojae* infection of soybeans where both organisms are present and conditions are favorable. It is hoped that information from this survey will be useful in the development and implementation of an inclusive IPM program for low input production systems.

Materials and methods

Soybean plant samples were collected from a field trial project of soybean varieties in a field naturally infested with SCN in Saginaw county. The experimental set-up was a random complete block design with 12 soybean varieties (Anderson, NC250, Asgrow seed A2722, Callahan 6180, Ciba seeds 3311, Dekalb Cx 252, Great lake GL 2415, ICI seeds D260, Mycogen J 250, Pioneer 9171, Conrad, Corsoy and Jack), two treatments (fumigated and non-fumigated) and seven replications. The nematicide Telone II (1,3-Dichloropropene) was applied at 30 gal./acre. Ten plant samples of each soybean variety were randomly collected from each replication. This gave a total of 70 samples per entry in each of the two treatments. All samples were kept in polyethylene bags and stored at 4°C to maintain fungal viability as samples were being processed.

The Canaday-Schmittenner medium (Canaday and

Schmittenner, 1982), as described elsewhere, was used in the detection and identification of *Phytophthora* from plants. Roots and stems of plants were surface-sterilized with 10% bleach for ten minutes and thoroughly rinsed 3 to 4 times with sterile distilled water . Small sections of tissue were taken from the edges of advancing lesions, where visible, and placed on the medium. To minimize bacterial contamination, plant tissues were placed under the medium to limit oxygen availability. Processed samples were incubated on benches at room temperature and observed over a period of 4 days. Fungal contamination was minimal and *Phytophthora,* when present, was readily observable at 50X under the dissecting microscope. Data was obtained as the number of samples with *Phytophthora* per soybean variety (entry). Nematode cyst numbers were determined for 100 cc of soil from the rhizosphere of each plant sample.

Data were analyzed for correlation using the statistical program Minitab.

Results

In the fumigated plots, and with the exceptions of the varieties Jack and CX 252 (data excluded), which is believed to have escaped infestation, significant (0.05) correlation between nematode infestation and the presence of *P. sojae* in

Figure 4.1. Number of cysts of *Heterodera glycines* in the rhizosphere soil volumes of soybean varieties from a field study of un-fumigated plots (in Saginaw county) were compared to the presence of *P. sojae* in the soybeans. Soybean plant tissues were surface-sterilized and incubated in low nutrient media. After two days, plants were scored for presence or absence of *P. sojae*. Significant correlation between SCN activity and *P. sojae* presence was observed.

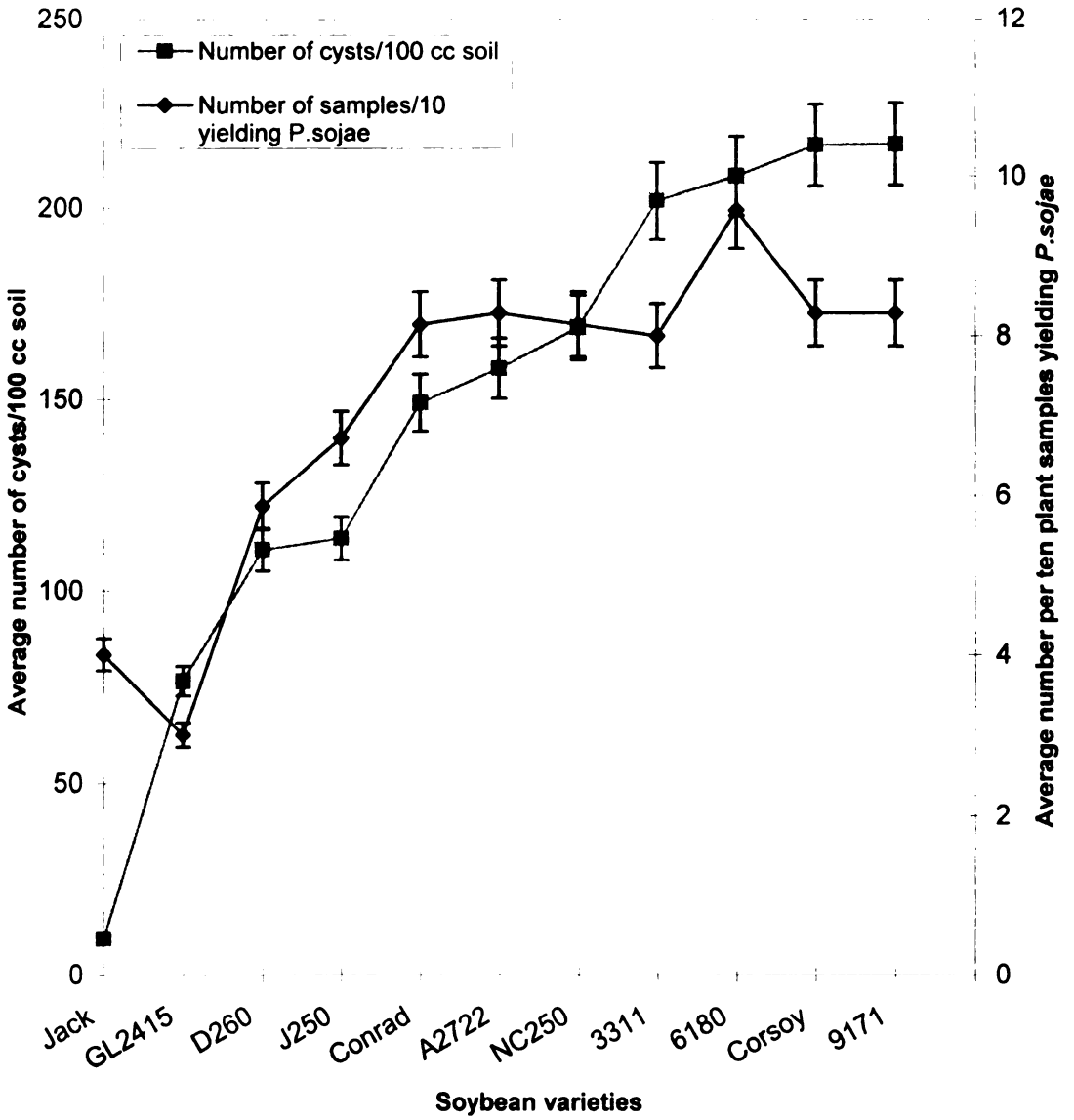
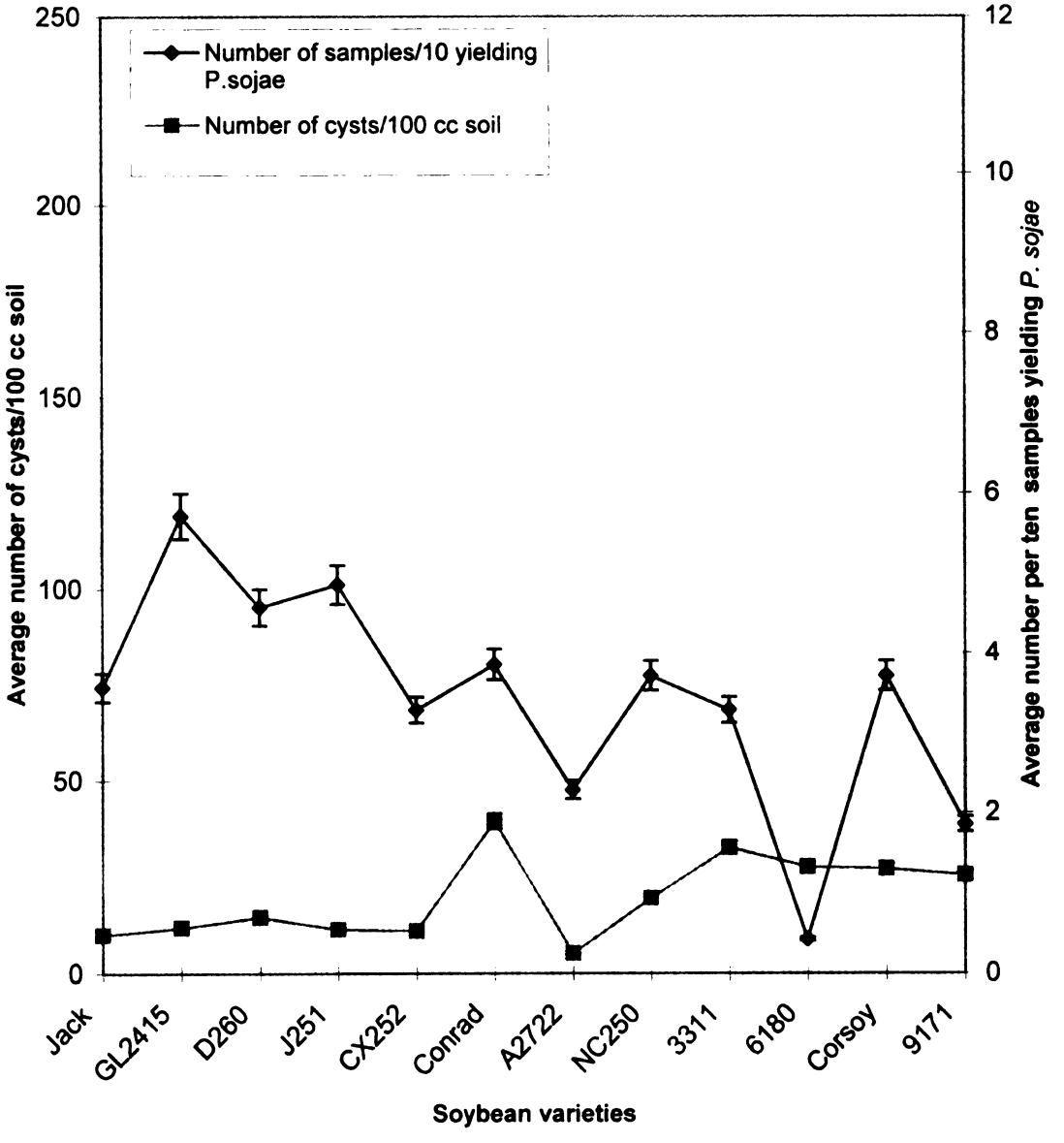

Number of cysts/100 cc soil
Number of samples/10 yielding P.sojae
Average number of cysts/100 cc soil
Average number per ten plant samples yielding P.sojae
250
200
150
100
50
0
12
10
8
6
4
2
0
Jack
GL2415
D260
J250
Conrad
A2722
NC250
3311
6180
Corsoy
9171
Soybean varieties

Figure 4.2. Number of nematode cysts and the presence of *P. sojae* in fumigated plots. Low scores of *P. sojae* and cyst nematode counts were obtained from the fumigated plots and data did not support correlation between the two pathogens.


Number of samples/10 yielding P.sojae
Number of cysts/100 cc soil
Average number of cysts/100 cc soil
Average number per ten samples yielding P. sojae
250
200
150
100
50
0
12
10
8
6
4
2
0
Jack
GL2415
D260
J251
CX252
Conrad
A2722
NC250
3311
6180
Corsoy
9171
Soybean varieties

soybean varieties was observed (figure 4.1). Variety Jack had lower nematode count but relatively high *P. sojae* presence. It is possible that CX 252 wich is tolerant to SCN but supports large populations of the nematode (Melakebern; personal communication) escaped infestation and was excluded from the analysis. All other ten varieties showed positive correlation between *P. sojae* occurrence and nematode infestation. Soybean variety 6180 had the highest incidence of *P. sojae* (9 in ten samples). Corsoy and 9171 had the highest nematode cyst populations, and also high *P. sojae* occurrence.

In the fumigated plots, lower nematode counts and *P. sojae* occurrence were observed, and data did not show any correlation between the two pathogens (figure 4.2). Varieties with the highest nematode counts (Conrad and 3311) did not have the highest incidence of *P. sojae*. The varieties with high *P. sojae* occurrence (GL2415 and J251) had lower counts of nematodes but the trend was not significant enough to indicate an inverse relationship.

Discussion

P. sojae is essentially an opportunistic plant pathogen that mainly attacks its host when under stress. It is known that wet and cool soil conditions early in the growing season favor the development of PRR in soybeans (Kittle et al.,

1979). These field conditions enable the release and dispersal of zoospores but they put soybean seedlings under stress by reducing metabolism and plant growth (Kittle et al., 1979). Nematode feeding puts stress on soybean seedlings by rendering roots inefficient in the uptake of water and nutrients(Ross, 1965).. Feeding-furrows in the roots also make it easier for pathogen propagules to enter and infect soybeans(Ross, 1965).

It is noteworthy that the results reported here were obtained from a field survey where *P. sojae* and SCN may not have been evenly distributed; thus, it is possible that some soybeans may have escaped infection by either organism. This may explain why some soybean varieties had low *P. sojae* occurrence despite high nematode cyst counts. Another possible explanation is the absence of compatible races of *P. sojae*. If compatible races are not present, wounding by nematode feeding alone may not ensure infection. Some inoculation techniques in screening procedures also create wounds in soybean seedlings but do not render them susceptible to incompatible races of *P. sojae*. SCN also has races that selectively attack certain genotypes of soybeans, and the presence of compatible races of the two organisms may be necessary for enhanced disease condition. Adeneji (1975) observed increased soybean seedling disease in the interaction

of race 3 of SCN and race 1 of *P. sojae*. Due to the race specificity factor, information on the races of both SCN and *P. sojae* occurring in a given field or growing region would be important to growers. In Michigan, this would be particularly important in areas where soil composition (structure) and field topography are likely to support SCN and *P. sojae*.

The results obtained in this survey agree in general with reports from other workers. While positive interactions between SCN and certain fungal plant pathogens have been observed (Todd et al.,1987; Rrancl and Wyllie,1988; Schenk and Kinloch, 1974), lack of interaction and inconsistency have also been reported. Interaction between SCN and Macrophomina phaseoli had variable results with increased root colonization (Todd et ,1987). In another study (Francl and Wyllie,1988), no interaction was evident. When pathogenic fungi that are associated with soybean roots were surveyed, no consistent relationship between the occurrence of specific fungi and SCN was evident (Schenk and Kinloch, 1974). Enhanced phosphorus utilization and reduction of second-stage juveniles of SCN in the presence of a vesicular arbuscular mycorrhizal (*Glomus fasiculatum*) fungus has been reported (Tylka et al., 1988). According to Roy (Roy et al. 1989), *F. oxysporum* and *F. solani* exhibited severe wilting in soybeans infested by SCN. Thus

Fusarium which is less host-specific and survives under various field conditions, may be the most significant opportunistic fungal plant pathogen in the infestation of soybeans by SCN.

The lack of typical symptoms in most of the soybean samples reported in this survey may be due to the possibility that more aggressive races of *P.sojae* infect incompatible soybean genotypes but do not cause disease in them. Stella Avila (MS thesis) reported presence of *P.sojae* in non-host crops such as wheat and dry beans in which the pathogen exist without causing disease. It is possible that Phytophthora behaves similarly in highly tolerant/resistant soybean genotypes as it is not impossible to isolate *P. sojae* from healthy-looking soybeans (*P. sojae* was isolated from healthy-looking plants in our laboratory).

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Chapter 5

Summary and Conclusions

Introduction

In this study, a survey was conducted on the occurrence and virulence of *P. sojae* in Michigan soybean fields in the 1993-97 growing seasons, and genistein (4' 5, 7-trihydroxy isoflavone), a compound that is produced and exuded by soybean roots was evaluated for potential as a molecular marker for field tolerance in soybean to *P. sojae.* The effect of genistein on the ability of *P.sojae* zoospores to infect and cause disease in soybean seedlings was also studied. A soybean field naturally infested with the soybean cyst nematode *H. glycines* , was surveyed for possible correlation between nematode activity and *P. sojae* infection, and the results are also presented in the study.

Phytophthora sojae in Michigan soybean fields

Ninety of the *P. sojae* isolates obtained from soybean fields were tested for virulence and race status. Fifty or 55% of the isolates tested defeated more than seven Rps genes each

and were categorized as highly virulent. Ten isolates (13%) showed intermediate virulence, defeating four to six Rps genes each, while eighteen (20%) of isolates were of low virulence defeating 1-4 Rps genes. Nine (11%) were avirulent as they attacked neither of the Rps genes nor the susceptible variety Williams (rps).

Rps genes $Rps3_b$, $Rps3_a$, $Rps1_b$, $Rps1_k$, and Rps_6 were (in that order) the most resistant to the field isolates. These genes resisted 70-78% of the *P. sojae* isolates while $Rps1_a$ and Rps_7 had the lowest rating resisting only 12-13% of the isolates. $Rps1_a$, $Rps1_c$, $Rps1_k$, $Rps3_a$ and Rps_7 are incorporated in varieties either being planted or developed in Michigan (B.W. Diers and J.F. Boyse. Dept. of crop and soil sciences, Michigan state university, East Lansing MI). In light of the results obtained in this study, soybean lines with $Rps1_a$ and Rps_7 need to be monitored closely for their performance in areas where *P. sojae* is known to occur or be replaced with $Rps1_b$ and $Rps1_k$. Incorporating these genes singly or in combinations in Michigan soybean varieties in conjunction with recommended cultural practices should provide improved protection against most of the *P. sojae* races that occur in the state. Due to race shift and the presence of rare but compatible races of *P. sojae*, more enduring non-race specific genetic protection in soybeans has become more attractive,

particularly when used as part of an IPM program. Genetic defense of soybeans against *P. sojae* may be further enhanced by a program where growers may have their popular soybean lines evaluated for tolerance to races that are common to their growing areas.

Effect of genistein on the infection of soybean roots

Exogenous application of 5 ppm of genistein solution to pot cultures of two-day old soybean seedlings inoculated with zoospores (3000/ml) of *P. sojae* significantly ($P \leq 0.05$) reduced infection and increased dry weights of roots. It is known that genistein hastens the encystment and germination of zoospores (Morris and Ward, 1992) and may impact the initial inoculum potential. This is particularly important since *P. sojae* is a soil-born pathogen for which secondary inoculum has little or no additive value to infection and disease progress. Zoospores which come in contact with genistein germinate away from soybean roots and the number of zoospores which come in contact with roots is reduced. Further studies are needed in order to determine the impact of genistein exuded by individual soybean lines on disease. A study examining the relationship between the concentration and dispersion of exuded genistein in the rhizosphere soil volume and disease reduction may further elucidate the role of the isoflavone in

Phytophthora root and stem rot of soybeans. Determining the effects of exuded genistein (from selected soybean varieties) on races of *P. sojae* that interact variably with the beans, will further elucidate the role of the isoflavone on field tolerance.

Fluorescence of root exudates and field tolerance values of soybeans

Fluorescence levels of root exudates and field tolerance values of soybean varieties gave variable results. Three highly tolerant varieties, Resnik, Williams 82, and PI 103 had high fluorescence values while Harlon and Sloan with low tolerance had low exudate fluorescence. Fluorescence and field tolerance values for these five varieties tended to indicate a correlation between field tolerance and fluorescence of root exudates but values for the varieties Repley, Harosoy, Conrad and Pella gave highly varied field tolerance values among the isolates of *P. sojae.*

Field tolerance values above four are considered poor by growers (A.F. Schmithenner. Personal communication) The variety Repley with fluorescence of 700 nm had good tolerance to Race 25, MSU 32 and MSU 23 but poor tolerance values of 4.5 and 6.0 to MSU 25 and MSU 10 respectively. Harosoy with fluorescence at 680 nm had low tolerance to MSU 10 and MSU 32

but good tolerance to Race 25, MSU 23 and MSU 25. Due to these observations and the highly variable tolerance values to isolates for Conrad and Pella, it was not possible to link fluorescence of root exudates to field tolerance in soybeans. Because of the variable response of soybeans to different races of *P. sojae*, finding a molecular marker that will indicate tolerance levels to various races of the pathogen may be difficult.

P. sojae* in soybeans infested with *H. glycines

With the exception of the varieties Jack and CX252, a significant ($P < 0.05$) correlation between nematode infestation of soybeans and the occurrence of *P. sojae* was observed in the non-fumigated plots. In the fumigated plots, lower nematode counts and *p. sojae* occurrence were observed and the data obtained did not support any correlation between the two pathogens.

Studies on the interactions between H. glycines and fungal pathogens have reported inconsistent results (Todd et al., 1987 and Francl and Wyllie , 1988). Adeneji (1975) observed increased soybean seedling disease in the interaction of race 3 of CSN and race 1 of *P. sojae.* Due to the race specific factor in both SCN and *P.sojae,* information on the races of both pathogens in a given field or growing area would be

important to growers. Roy (Roy et al. 1989) reported severe *Fusarium* wilting in soybeans infested by SCN. Thus non-selective opportunistic soil pathogens such as *F. solani* and *F. oxysporum* may be the most important organisms in the infestation of soybeans by SCN.

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