ASSOCIATION OF *PHYTOPHTHORA SOJAE* WITH *HETERODERA GLYCINES* AND NUTRIENT STRESSED SOYBEANS

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ABSTRACT

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Soybean cyst nematode (SCN, *Heterodera glycines*), a serious pest of soybeans, has been found in 30 counties in Michigan. *Phytophthora sojae*, a serious and opportunistic pathogen of soybeans, has also been found in many of the fields. This study was conducted to assess the incidence of *P. sojae* in 12 selected soybean cultivars grown under low (fumigated) and high (non-fumigated) SCN conditions and nutrient stress. A total of 70 soil and root and stem samples (same plant) from each nematicide treatment were analyzed for SCN population densities and *P. sojae*. Incidence of *P. sojae* was highly correlated with SCN population densities in untreated plots; whereas, both organisms were low in the treated plots and poorly correlated. Leaf N and K were significantly higher in nematicide-treated plots while the reverse was true for Ca, Al, and Fe. Leaf P and Mg contents were similar in both nematicide treatments. The results suggest that the incidence of *P. sojae* may be increased by SCN and nutrient stress. Hence, management recommendations need to consider biotic and abiotic factors.

Key words: Glycine max, Heterodera glycines, interactions, *Phytophthora sojae*, soil fumigation, soybean.

RESUMEN

Kaitany, R., H. Melakeberhan, G. W. Bird, and G. Safir. 2000. Asociación de *Phytophthora sojae* con *Heterodera glycines* el estrés nutricional en los frijoles de soya. Nemtropica 30:193-199.

El nematodo del quiste de la soya (SCN, *Heterodera glycines*), una plaga seria de los frijoles de soya, ha sido encontrado en 30 condados de Michigan. *Phytophthora sojae*, un patógeno oportunista de los mismos, también ha sido encontrado en muchos de los campos. Este estudio se realizó, para evaluar la incidencia de *P.sojae* en 12 variedades de soya selectas, crecidas en bajas (fumigadas) y altas (no-fumigadas) condiciones de SCN y de estrés nutricional. Un total de 70 muestras de suelo, raíces y tallos (de la misma planta) por cada tratamiento con nematicida, fueron analizadas, en relación a las densidades poblacionales de SCN y de *P.sojae*. La incidencia de *P.sojae,* estuvo altamente correlacionada con las densidades poblacionales de SCN en parcelas no tratadas; mientras que ambos organismos fueron escasos en las parcelas tratadas y pobremente correlacionados. El N y K de la hoja, fueron significativamente altos en las parcelas tratadas con nematicida mientras lo contrario ocurrió para el Ca, Al y el Fe. Los contenidos de P y Mg en la hoja, fueron similares en ambos tratamientos con nematicida. Los resultados sugieren que la incidencia de *P.sojae* pudiera ser aumentada por SCN y el estrés nutricional. Por lo que las recomendaciones de manejo, necesitan considerar los factores bióticos y abióticos.

Palabras claves: Glycine max, Heterodera glycines, interaccións, *Phytophthora sojae*, fumigación de suelo, soja.

INTRODUCTION

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is a major pest of soybeans worldwide. Present in most soybean-producing states, it causes an estimated $1.4 billion crop loss annually in the USA (Wrather *et al.*, 1996). The SCN accounts for *ca.* 54% of crop losses due to infectious diseases. Rotation and the use of resistant cultivars are among the common ways of controlling SCN (Young, 1994). Use of susceptible cultivars is particularly significant if selection pressure is to be avoided.

Phytophthora sojae Kaufmann and Gerdemann, causal agent of root and stem rot of soybean, is an opportunistic pathogen (Kittle and Gray, 1979; Moots *et al.*, 1988). Although yield losses may vary by state, *P. sojae* accounts for approximately 8% of crop loss annually in the USA (Doupnik, 1993; Wrather *et al.*, 1996). Like SCN, *P. sojae* has multiple races, and the host-parasite interactions vary by race and the prevailing conditions (Adeniji *et al.*, 1975; Riggs and Schmitt, 1988). Also the impact of SCN and *P. sojae* are worse in the presence than in the absence of abiotic stresses (Kittle and Gray, 1979; Melakeberhan, 1999; Moots *et al.*, 1988).

Soybean cyst nematode is found in 30 soybean-producing Michigan counties, and highly virulent isolates of *P. sojae* have been found in many of the same fields. However, it is not known whether or not the presence of *P. sojae* is associated with SCN and non-biotic stress-inducing factors in Michigan. This study was conducted to determine any associations among SCN, *P. sojae* and nutrient stresses in selected soybean cultivars adapted to Michigan conditions.

MATERIALS AND METHODS

Eleven SCN susceptible soybean cultivars, consisting of PN 9171 (Pioneer), GL 2415 (Great Lakes), Ciba 3311 (Ciba Seeds), CAL 6180 (Callahan), A2722 (Asgrow), D260 (ICI Seeds), Corsoy (Public), Conrad (Public), J-251 (Mycogen), NC250 (Andersons), and CX 252 (Dekalb), and one resistant (Jack) cultivar were grown in a field with a history of poor soybean yields associated with SCN (Table 1). Based on analyses of 16 randomly selected soil samples, the field was divided into high, 904 ± 468, and low, 224 ± 115 numbers of eggs and second-stage juveniles per 100 cm^3 soil. To further lower the SCN population density, the low infested area was fumigated with 420.9 l/ha of Telone II[R] and treated with 3.36 kg/ha of Temik 15 G[R] in the previous fall and spring, respectively. The field also received 3.36 kg/ha of Temik 15 G[R] at planting in 1997. Both nematicide-treated and untreated fields received 224 kg/ha of 0:0:60 N:P:K fertilizer at planting.

A total of 84 plots (12 cultivars and 7 replications) were planted in each of the nematicide treatments in early June 1997. Each plot was 12.2 m long by 3.1 m wide and consisted of 4 rows 0.76 m apart. Treatments were arranged in a randomized complete block design. In late July, a plant and its rhizosphere soil from the center two rows, of 70 plots in each of the nematicide treatments, were randomly collected. Cysts were extracted from 100 cm^3 soil and counted (Melakeberhan, 1999). 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 and assessed for the presence of *P. sojae* infestation (Canady and Schmittenner, 1982). 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 *P. sojae,* when present, was readily observable at 50X under the dissecting microscope.

Table 1. Cultivar, nematicide and cultivar x nematicide interaction effects on the concentrations of N, P, K, Mg, and Ca (%) and Al and Fe (ppm) per gram dry leaf weight from 12 soybean vultivars in nematicide-treated (+) and untreated (-) plots.

Source	N		P		K		Mg		Ca		Al		Fe	
Cultivar (CV)	—	+	—	+	—	+	—	+	—	+	—	+	—	+
PN 9171	4.35 b	5.15 *	0.26 b	0.25	1.06	1.23 *	0.78 ab	0.56	2.35	1.86 *	296 b	50 *	309 ab	124 *
GL2415	4.63 b	5.12 *	0.26 b	0.25	1.36	1.43	0.69 b	0.45	1.85	1.34 *	293 b	75 *	317 ab	137 *
Ciba 3311	4.19 b	5.01 *	0.26 b	0.24	1.34	1.43	0.75 ab	0.44	2.24	1.55 *	550 a	53 *	489 a	120 *
CAL 6180	4.42 b	5.08 *	0.26 b	0.26	1.13	1.34 *	0.83 a	0.62	2.23	1.66 *	232 b	42 *	260 bc	112 *
A2722	4.19 b	4.45 *	0.28 ab	0.29	1.29	1.48 *	0.78 ab	0.46	2.47	1.70 *	313 b	35 *	324 ab	111 *
D 260	4.74 ab	5.05	0.26 b	0.24	1.40	1.42	0.63 b	0.44	1.63	138 *	174 c	63 *	215 bc	128 *
Corsoy	4.37 b	4.89 *	0.26 b	0.26	1.19	1.37	0.80 a	0.49	2.48	1.62 *	320 b	35 *	326 ab	104 *
Conrad	4.24 b	5.03 *	0.25 b	0.26	1.30	1.58 *	0.62 b	0.37	1.89	1.44 *	166 c	38 *	211 bc	108 *
J-251	4.42 b	4.55 *	0.26 b	0.27	1.29	1.43	0.64 b	0.41	1.77	1.24 *	375 b	43 *	351 ab	114 *
NC250	4.26 b	4.90 *	0.25 b	0.24	1.32	1.43	0.65 b	0.47	1.76	1.32 *	288 b	42 *	294 b	111 *
CX 252	4.29 b	5.07 *	0.23 b	0.26	1.33	1.61 *	0.57 b	0.41	1.93	1.45 *	161 c	47 *	206 cd	118 *
Jack	5.41 a	5.90 *	0.32 a	0.36	1.68	1.69	0.50 b	0.40	1.50	1.39 *	44 d	29 *	118 d	115 *
Nematicide	4.44	5.14 *	0.26	0.26	1.31	1.45 *	0.69	0.46 *	2.00	1.50	268	46 *	285	117
Nematicide × CV	n.s.		n.s.		*		n.s.		*		*		*	

*= Significant difference between nematicide treatments within a CV or interactions of nematicide × CV at $P \leq 0.05$. Means followed by the same or no letters within a nematicide treatment are not significantly different. n.s. = Not significant.

Thirty six leaves, each consisting of a fully developed trifoliate, per plot were randomly collected from the four upper most fully developed leaves at R4-stage (full pod) and analyzed for macro and micro nutrients as a potential indicator of host response to SCN (Melakeberhan, 1999). Main effects of nematicide and cultivar, and nematicide x cultivar interactions were analyzed by ANOVA and means separated by using Tukey's range test (Steele and Torrie, 1980).

RESULTS

In the non-nematicide-treated plots, numbers of cysts per 100 cm^3 soil ranged from about 10 in Jack (resistant) to over 200 in Ciba 3311, Cal 6181, Corsoy and PN 9171 (Fig. 1A). A significant ($P \leq 0.05$) positive correlation was found between nematode infestation and the incidence of *P. sojae* in soybean cultivars.

In the nematicide-treated plots, the numbers of cysts per 100 cm^3 soil ranged from about 10 in Jack, GL 2415, D 260, CX 252 and A2722 to less than 50 in Conrad (Fig. 1B). Correlation between the two pathogens was not significant. However, numbers of the two organisms are lower than in the non-treated plots.

Differences in leaf nutrient contents were not significant among the 12 cultivars when they were grown in nematicide-treated plots (Table 1). In the plots where no nematicide was applied, however, significant differences existed among the cultivars for all elements but K and Ca. Jack had the highest N in all cutlivars but D 260, and highest P in all but A2722. Corsoy and Cal 6180 had higher Mg concentration than all cultivars but PN 9171, Ciba 3311 and A2722. Jack followed by CX 252, Conrad and D 260 contained lower Al and Fe than the other cultivars (Table 1). Generally, Jack had the highest N, P and K concentrations compared with the susceptible cultivars; whereas, Jack and CX 252 contained less Ca, Mg, Al, and Fe than with the other cultivars.

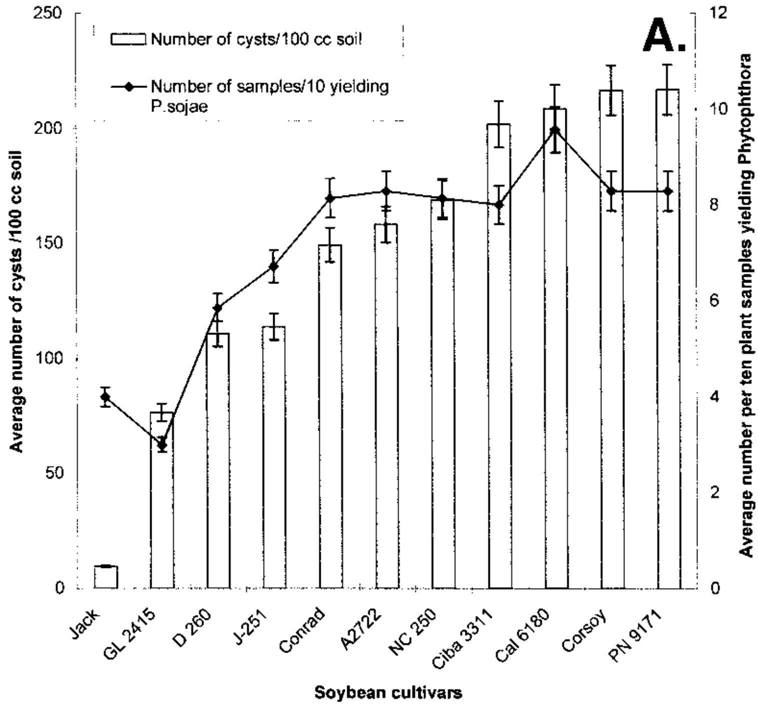
Leaves from all cultivars except D 260 had higher N levels ($P \leq 0.05$) in the low infested area than in the highly infested side of the field. Nematicide treatment had no effect on P. Potassium was significantly higher in leaves of PN 9171, CAL 6180, A2722, Conrad and CX 252 from the low infested site than from the highly infested site.

Concentrations of Al in all cultivars, and Ca and Fe in all but Jack, from the low infested site were significantly lower than from the highly infested site (Table 1).

Overall, nematicide treatment increased leaf concentrations of N and K; whereas, the reverse was true for Mg, Ca, Al, and Fe (Table 1). Nematicide x cultivar interaction was significant in all elements but P and Mg.

DISCUSSION

Phytophthora sojae is an opportunistic plant pathogen that mainly colonizes a host that is under stress (Kittel and Gray, 1979). Although *P. sojae* and SCN were not evenly distributed, the results of the field survey support an increased incidence of *P. sojae* in SCN stressed plants. For example, the incidence of *P. sojae* in the plots without nematicide treatment was higher than in the nematicide-treated plots. This suggests that high SCN population densities may be stressing plants and increasing their susceptibility to infection by *P. sojae*. The lack of significant correlation between the incidence of *P. sojae* and SCN population densities in the nematicide-treated plots, however, may be explained by low densities of both organisms. Similar inconsistencies between SCN and other fungal pathogens have been reported (Francl and Wyllie, 1988; Schenk and Kinloch, 1974).



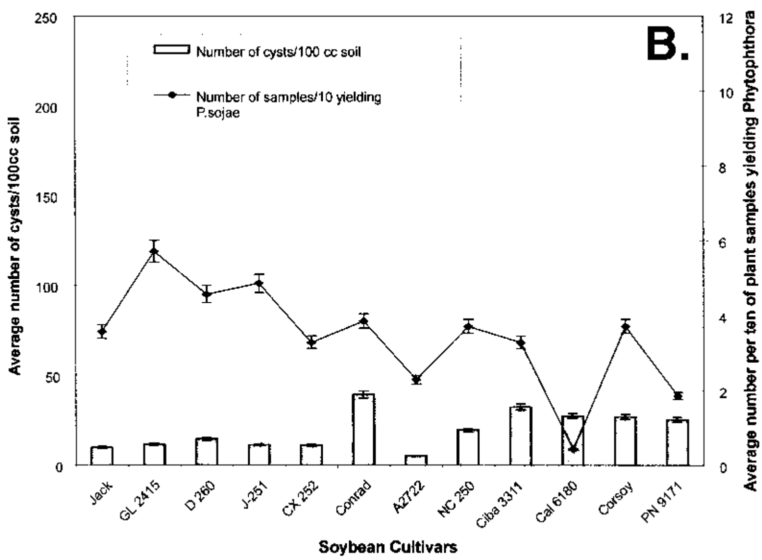


Fig. 1. The relationship between the number of *Heterodera glycines* cysts/100 cm^3 soil and the average numbers per ten plant samples yielding *Phytophthora sojae* in 12 soybean cultivars grown in untreated (A) and nematicide-treated (B) fields. Jack is resistant, the others are susceptible to *H. glycines*. For purposes of clarity, means for *P. sojae* data are connected by lines.

A major factor influencing cause-and-effect relationship analyses under field conditions is the role of environment-driven stress (Boyer, 1982), of which, soil nutrition is one. In this study, nutrient imbalance appears to have played a role in altering the health and vigor of the plants. While it appears that nematicide treatment has played an important role in the distribution of nutrients, it is worth noting how the cultivars behaved under the circumstances. For example, high Al and Fe levels in leaves of plants from plots with high incidence of *P. sojae* suggests that the concentrations of these elements might be toxic (Marschner, 1995). The low levels of Al and Fe in Jack, Conrad, D 260, and CX 252 are indications that these cultivars may not be efficient in taking up the toxic elements, indicating they have tolerance to adverse soil conditions. Jack, Conrad and D 260 also had very low incidence of *P. sojae* (Fig. 1A), a fact that seems to support the role additional stresses play in the expression of disease severity. Under these conditions, it is possible that SCN stress may have contributed to the difference in response among these four cultivars.

Differences in SCN population densities between sites and among cultivars may be related to nematicide treatment. The relationship between leaf macronutrients and SCN population densities, however, indicates the levels and types of stresses. For example, the high Mg and Ca in leaves of plants grown in the absence of nematicide treatment confirms that the increase of these elements, Ca in particular, is an indication of high stress (Melakeberhan, 1999). Overall, the study suggests that incidence of *P. sojae* may be high in the presence of SCN and nutrient stress. Therefore, SCN and *P. sojae* management recommendations need to consider both biotic and abiotic stress factors.

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