Distribution and Abundance of *Heterodera glycines* and *Macrophomina phaseolina* in Ohio

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ABSTRACT

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Soybean and corn are grown on more than 60% of the arable land in Ohio. The soybean cyst nematode (SCN) is responsible for significant losses incurred by soybean growers every year. The fungus that causes charcoal rot, *Macrophomina phaseolina*, causes significant yield loss in soybeans worldwide and can also affect corn. Both organisms are soil-borne pathogens. The objective of this study was to determine the presence, distribution, and abundance of both SCN and *M. phaseolina* in soybean and corn fields across Ohio. During 2013 and 2014, composite

soil samples were collected from 370 corn and soybean fields. Samples were processed for SCN eggs/100 cm³ and *M. phaseolina* colony forming units (CFU)/g soil with standard techniques. Results from this study revealed a widespread distribution of SCN and *M. phaseolina* in both soybean and corn fields. This study represents the first survey on the distribution of *M. phaseolina* in Ohio and the findings will be used to educate producers on the potential risks posed by both SCN and *M. phaseolina*.

INTRODUCTION

In Ohio, soybean (*Glycines max* [L.] Merr.) and corn (*Zea mays* L.) are grown on more than 60% of its agricultural area (Anonymous 2013a; Anonymous 2013b). Based on cash receipts, these crops are the top commodities produced in the state. During the period from 2008 to 2012, soybean and corn production in Ohio increased by 84% (Stiers 2014); in 2013, values for corn and soybean were approximately \$2.9 and \$2.7 billion, respectively (Anonymous 2013a). Continuous soybean or a soybean-corn rotation are the two most common cropping sequences in Ohio (Barker et al. 2005).

Heterodera glycines Ichinohe, the soybean cyst nematode (SCN), is the most economically important pathogen of soybean in the United States, causing annual losses estimated to exceed \$1 billion (Koenning and Wrather 2010; Wang et al. 2003). Worldwide, *H. glycines* is responsible for significant losses incurred by soybean growers every year (Niblack et al. 2006; Wrather et al. 2001). *Heterodera glycines* has a broad host range (Poromarto and Nelson 2010); however, soybean is the major host (Venkatesh et al. 2000).

Macrophomina phaseolina (Tassi) Goid., causal agent of charcoal rot of soybean, is reported to affect more than 500 species of plants (Mihail and Taylor 1995), including corn and soybean (Pearson et al. 1986; Pearson et al. 1987; Su et al. 2001). The disease is more severe under high temperatures (28 to 35°C) and drought conditions (Gupta et al. 2012; Pearson et al. 1984). Yield losses in soybean have been reported most often in the

southern part of the United States and more recently from across the northern and central regions (Koenning and Wrather 2010; Smith et al. 2014). *Macrophomina phaseolina* can also cause stalk rot in corn when environmental conditions are conducive, which can reduce yield by at least 5% and up to 20% in the Midwest (Anonymous 1995; Dodd 1980).

As demands on worldwide crop production increase, there is a growing interest in improving cultural practices in agriculture. Meeting this challenge is further complicated by global climate change, which threatens to modify environmental conditions. Depending on the presence of plant pathogens, these modified environmental conditions may become conducive for plant diseases, and ultimately lead to yield reduction (Chakraborty and Newton 2011; Pautasso et al. 2012). *Macrophomina phaseolina* is impacting soybean in regions where previously it was thought to have limited distribution (Smith et al. 2014). Moreover, this fungus has reportedly affected important agronomic crops, such as corn and wheat (Almeida et al. 2003; Dodd 1980; White 1999). In Ohio and most of the Midwest, this fungus could constrain crop rotation tactics for integrated disease management, as the two major grain crops, corn and soybean, are both susceptible hosts. Under dry and hot environmental conditions, yield losses due to *M. phaseolina* take place (Gupta et al. 2012; Pearson et al. 1984); moreover, *H. glycines* population densities at the end of the season were reported to be greater under dry conditions (i.e., non-irrigated plots) (Koenning and Barker 1995). Both organisms are soil-borne pathogens and eradication is very unlikely once they become established in a field.

During the 1980s, *H. glycines* (Hammond et al. 1981; Riedel and Golden 1988) and *M. phaseolina* (Lipps and Riedel 1984) were reported in Ohio. In 1996, results from a 4-year systematic survey to determine the presence of *H. glycines* in Ohio were reported (Willson et al. 1996). During 2009 and 2010, both *H.*

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glycines and *M. phaseolina* were detected in a survey of yield-limiting diseases of soybean in five counties in southern Ohio (Gearhart et al. 2010). Of the soybean fields sampled in 2009 and 2010, *H. glycines* counts ranged from 0 to over 3,000 eggs/100 cm³ soil (Gearhart et al. 2010). The maximum number of *H. glycines* eggs per 100 cm³ soil was more than 2,000, a population density which is potentially economically damaging (Dorrance et al. 2012). *Macrophomina phaseolina* was isolated from both symptomatic and asymptomatic soybean plants. Soybean plants with charcoal rot symptoms wilted and died prematurely in the field. Lower stems and taproots were darker in color than healthy plants and the internal grey discoloration in the vascular tissues was due to the presence of microsclerotia (Dorrance and Mills 2009; Smith et al. 2014). *Macrophomina phaseolina* was always present in symptomatic soybean plants.

Ohio farmers in some regions of the state have been commenting on low yields (A. E. Dorrance, *unpublished*). Many fields have been in continuous soybean cropping sequence which, combined with drought-like conditions, may contribute to a greater impact from these two pathogens than previously known. More than 20 years have passed since the last assessment of *H. glycines* populations and, with the recent report of *M. phaseolina* in southern Ohio, we considered it relevant to crop production to reassess the potential threat of these important soil-borne pathogens in a statewide survey. The objective of this study, therefore, was to determine the presence, distribution, and abundance of both *H. glycines* and *M. phaseolina* from soil samples collected in soybean and corn fields across Ohio. Information generated from this survey will help us develop better integrated disease management strategies and recommendations to improve production.

SAMPLING METHOD AND DATA COLLECTION

Fields were located and sampled with the help of farmers, extension educators, and researchers from The Ohio State University. Farmers of fields planted to soybean and corn volunteered to have their fields sampled. In addition, a written survey was given to the growers to determine the cropping history of their farms. Corn fields were sampled with the primary objective of identifying plant-parasitic nematodes associated with corn production (Simon 2015). Unlike *H. glycines*, plant-parasitic nematodes associated with corn are found deeper in the rhizosphere (MacGuidwin 1989; Pudasaini et al. 2006); consequently, the sampling procedures in soybean and corn fields differed in relation to both soil sample depth and timing during the crop growth stage.

Sampling soybean fields. *Heterodera glycines* is usually most abundant in the first 15 to 30 cm of the soil profile, with the greatest numbers found in the upper 15 cm (Alston and Schmitt 1987). The standard sampling procedure used in soybean fields was at the top 15 to 25 cm between soybean rows for detection and quantification of *H. glycines* (Niblack and Tylka 2008). Soybean fields were sampled when soybeans were between growth stages VE to V3 (Fehr et al. 1971; Pearson et al. 1984; Pedersen and Elbert 2004). Soybean growers responded to a questionnaire about the cropping sequence and their awareness of *H. glycines* infestation in their fields.

Sampling corn fields. Corn growers provided information about the cropping sequence of their farms. Corn fields were sampled when corn was between growth stages V3 and V6 (Abendroth et al. 2011). The protocol used to sample corn fields was modified to improve detection of plant parasitic nematodes associated with corn. The sampling method targeted a deeper

region in the soil profile, including removal of the first 15 cm of soil (Simon 2015; Tylka et al. 2011).

In both soybean and corn fields, a composite sample of 15 to 20 soil cores was obtained with a 2.54-cm-diameter cylindrical soil probe. Soil cores from within the rows of soybean and corn plants were collected in a zigzag pattern throughout an area no larger than 10 ha. Soil cores were combined in a plastic sample bag properly labeled and placed in insulated containers until arrival at the laboratory. Upon arrival at the laboratory, all samples were stored in a cold room at 4°C until processed.

Composite samples were thoroughly mixed following gentle breaking of the soil cores. From each composite sample, a 100-cm³ subsample was obtained for *H. glycines* egg extraction and quantification (Faghihi and Ferris 2000) and the population density was recorded as the number of eggs per 100 cm³ soil (Fig. 1A). From the same composite soil sample, a second subsample was obtained for *M. phaseolina* extraction and quantification. Subsamples were air-dried, ground, and 1 g soil was suspended in sterile distilled water and placed on a semi-selective medium containing potato dextrose agar (PDA), rifampicin, and tergitol (Mengistu et al. 2009). Colonies of *M. phaseolina* were counted 5 days after samples were plated and the data are presented as colony forming units (CFU) per g soil (Fig. 1B).

DISTRIBUTION AND ABUNDANCE OF HETERODERA GLYCINES AND MACROPHOMINA PHASEOLINA

During the 2013 and 2014 growing seasons, composite soil samples were collected from 370 fields in 58 counties in Ohio.

FIGURE 1

(A) *Heterodera glycines* eggs and juveniles at 40x. (B) *Macrophomina phaseolina* colony-forming units (CFU) in selective media as described in Mengistu et al. (2009).

Among the sampled fields, 143 were planted to soybean and 227 to corn (Fig. 2).

According to the information provided by the farmers on the cropping sequence history, soybean and corn fields were classified as fields in continuous soybean (S-S-S), continuous corn (C-C-C), corn-soybean (C-S), or corn-soybean-wheat (C-S-W) rotation. Fields with more than two consecutive years planted to soybean or corn were considered to be under continuous cropping sequence. Fields in which soybean and corn or soybean, corn, and wheat were planted in alternating years were considered to be in crop rotation.

The sampling procedure used to collect soil samples in soybean fields differed from the one used in corn fields; results are presented separately for each sampling method (soybean vs. corn).

HETERODERA GLYCINES

Soybean fields. The 143 soybean fields sampled were in 51 counties. More than 86% of these counties had at least one soybean field infested with *H. glycines* (Fig. 3A). The spatial distribution of *H. glycines* in fields is highly aggregated (Niblack 2005; Niblack and Tylka 2008), and therefore a field may be infested even if the soil sample results did not detect the presence of the nematode.

Among the 143 soybean fields sampled, *H. glycines* was detected in 117 fields (Fig. 4) and the population density measured in eggs per 100 cm³ soil ranged from 13 to 12,226, with a mean of 454. More than 94% of these infested fields had fewer than 2,000 eggs/100 cm³ of soil (Table 1), which is at or below the economic threshold with potential detectable yield reduction (Dorrance et al. 2012).

As a guideline, *H. glycines* levels below 2,000 eggs/100 cm³ of soil are considered low to moderate (Anonymous 2014; Dorrance et al. 2012). Of this group, many fields (over 30%) had *H.*

glycines levels between 200 and 2,000 eggs/100 cm³ soil. In Ohio, population densities at or above 200 eggs/100 cm³ of soil may result in yield loss on susceptible soybean cultivars; similarly, *H. glycines* levels at or above 2,000 eggs/100 cm³ soil may result in yield reduction on *H. glycines*-resistant soybean cultivars (Anonymous. 2014; Dorrance et al. 2012; Niblack et al. 1992; Wang et al. 2003).

Fields in several counties across Ohio had *H. glycines* population densities between 2,000 to 5,000 eggs/100 cm³ soil

FIGURE 2

Approximate locations of fields sampled in a survey conducted in 2013 and 2014 to determine the distribution and abundance of *Heterodera glycines* and *Macrophomina phaseolina* in Ohio.

FIGURE 3

Soybean fields sampled in a survey during 2013 and 2014. (A) Distribution of *Heterodera glycines* in soybean fields. (B) Distribution of *Macrophomina phaseolina* in soybean fields.

(Table 1); such levels are considered moderate to high (Anonymous 2014; Dorrance et al. 2012). These counties included: Crawford (1 out of 2 sampled fields); Defiance (1 out of 5 sampled fields); Fulton (2 out of 7 sampled fields); and Pickaway (1 out of 3 sampled fields). For most Ohio soils, farmers would not see any above-ground symptoms at these population densities; however, significant yield reduction may occur if the initial population density at planting falls in this range (Dorrance et al. 2012; Niblack et al. 1992; Wang et al. 2003). Also, in Montgomery, a field infested with *H. glycines* (1 out of 2 sampled fields) had more than 5,000 eggs/100 cm³ soil and was considered to be at high risk for yield loss (Table 1).

Among the six fields with moderate to high *H. glycines* population densities, two were in continuous soybean cropping

FIGURE 4

Soil samples collected from 370 fields (total) and processed for *Heterodera glycines* analysis which were planted to corn (227) or soybean (143).

sequence, three were in corn-soybean rotation, and one was in corn-soybean-wheat rotation. Of the 143 soybean fields sampled in this survey, the majority (over 55%) were in a corn-soybean cropping sequence, followed by fields in corn-soybean-wheat rotation and less than 5% in a consecutive soybean sequence (Table 2). The percentage of fields infested with *H. glycines* in each of these categories ranged from 83 to 88% (Table 2).

The economic threshold for *H. glycines* has been reported to be in the hundreds of eggs per 100 cm³ soil (Dorrance et al. 2012; Niblack et al. 1992; Niblack et al. 2008). Even though many factors affect the damage threshold of *H. glycines* on soybean production, potential yield reduction can only be estimated using the initial population density (eggs per 100 cm³ soil) before or at planting (Niblack 2005).

During a growing season, *H. glycines* population fluctuates (Bonner and Schmitt 1985); therefore, sampling once during the season can be considered a snapshot of a dynamic process. Population densities reported in this study, however, could be used as a guideline to identify fields (regions) with elevated *H. glycines* numbers that could pose a potential risk to soybean production. Surprisingly, more than 75% of the growers from infested fields presented in this study were unaware they had fields infested with *H. glycines*.

Corn fields. Soil samples were collected below the top 15 cm, originally targeting nematodes that feed on corn roots. In spite of this different sampling strategy, *H. glycines* was detected in 47 of the 227 corn fields (Fig. 5A) and the population density measured in eggs per 100 cm³ soil ranged from 40 to 6,400 with a mean of 1,200 (median of 440) (Table 1). *Heterodera glycines* was not detected in 180 corn field samples; compared with soybean fields, the detection of *H. glycines* was drastically different (Fig. 4). This is most likely a consequence of the sampling procedure. Removing the first 15 cm of the soil profile (done in corn fields) will allow for a deeper soil sampling and recovery of plant parasitic nematodes associated with root system at a deeper soil profile. The upper 15 cm of soil profile, however, is the region in which *H. glycines* is reportedly more abundant (Alston and Schmitt 1987) and therefore removing this portion of the soil

Crop	Total Fields	<i>H. glycines</i> eggs / 100 cm ³ soil			
		Not Detected	1 - 2000	2000 - 5000	> 5000
Soybean ^x	143	26	111	5	1
Corn ^y	227	180	37	8	2

^x Number of soybean fields sampled at a depth of 15 to 30 cm.

^y Number of corn fields sampled at a depth between 15 to 45 cm (the first 15 cm of the soil was removed).

	<i>H. glycines</i>	C-C-C ^x	S-S-S ^x	C-S ^x	C-S-W ^x	N.I. ^y	Total Fields ^z
Soybean Fields	Not detected	—	1 (17%)	13 (16%)	2 (12%)	10	26 (18%)
	Detected	—	5 (83%)	66 (84%)	15 (88%)	31	117 (82%)
Corn Fields	Not detected	41 (79%)	—	87 (78%)	52 (83%)	—	180 (79%)
	Detected	11 (21%)	—	25 (22%)	11 (17%)	—	47 (21%)

^x Cropping sequences from fields sampled under continuous soybean (S-S-S), corn (C-C-C), corn-soybean (C-S) and corn-soybean-wheat rotation (C-S-W).

^y No information (N.I.) about crop rotation was provided by farmer.

^z Total number of corn fields sampled at a depth between 15 to 45 cm (the first 15 cm of the soil was removed and discarded) and total number of soybean fields sampled at a depth of 15 to 30 cm.

profile will lead to underestimation of the abundance of soybean cyst nematode in a sample. Additionally, if *H. glycines* egg counts are low to moderate (<2,000 eggs/100 cm³), sampling procedures such as the one used in corn fields may result in failure to detect the soybean cyst nematode in a sample. These results highlight the importance of using an adequate sampling procedure for detection and quantification of a specific pathogen.

Heterodera glycines population densities above 2,000 eggs/100 cm³ soil were detected in 10 corn fields (Table 1). Eight had *H. glycines* egg counts between 2,000 and 5,000 eggs/100 cm³ soil and two fields had counts over 5,000 eggs/100 cm³ (Table 1). Three of these fields were in continuous corn cropping sequence, five under corn-soybean rotation, and two under corn-soybean-wheat rotation.

Among the 227 corn fields sampled, more than 77% were in crop rotation and less than 23% were in continuous corn (Table 2). The percentage of fields with detectable *H. glycines* in each cropping sequence category ranged from 17 to 22% (Table 2). Detection of *H. glycines* was similar within each cropping sequence for soybean and corn fields (Table 2). The number of fields in crop rotation was higher than that under continuous cropping sequence. Each cropping sequence category, however, had unequal number of fields (Table 2).

MACROPHOMINA PHASEOLINA

Soybean fields. *Macrophomina phaseolina* was detected in over 95% of the soybean fields sampled, and in 49 of the 51 counties (Fig. 3B). The population density ranged from 1 to 77 CFU/g soil, with a mean of 15 CFU/g soil (median of 9). The prevalence of *M. phaseolina* across soybean fields in Ohio was an unexpected finding since this pathogen is most often associated with crop disease in the southern part of the United States, where conducive environmental conditions predominate (Koenning and Wrather 2010; Smith et al. 2014).

Corn fields. A similar trend was also found in the corn fields, as *M. phaseolina* was detected in over 77% of the sampled fields (Fig. 5B). In infested fields, the population density measured in CFU/g soil ranged from 1 to 54, with a mean of 9 CFU/g soil (median of 6). *Macrophomina phaseolina* was detected in 22 of the 24 counties sampled, which means that more than 91% of the counties had at least one corn field infested with *M. phaseolina*. The distribution of *M. phaseolina* microsclerotia is reported to be concentrated in the upper 30 cm of soil profile (Bruton and Reuveni 1985). This indicates that with the sampling procedure used to recover nematodes associated with corn roots, the same soil samples can also be used to detect the presence of *M. phaseolina*; however, further sampling is needed to compare whether abundance or inoculum concentrations are the same for both sampling techniques.

CO-INFESTATION

Of all the fields sampled for this survey, many were co-infested with both *H. glycines* and *M. phaseolina*. Out of the 143 soybean fields sampled, 111 (over 77%) had detectable levels of both *H. glycines* and *M. phaseolina*. Corn fields with detectable levels of both soil-borne pathogens, were below 17% (38 corn fields). However, as stated above, this is most likely due to the sampling procedure; nonetheless, among the 47 corn fields infested with *H. glycines*, 38 had both pathogens.

CONCLUSIONS AND RECOMMENDATIONS

Major agricultural counties in Ohio, including the top ten soybean and corn producing counties in this state, were included in this survey (Anonymous 2013b), and *H. glycines* and *M.*

phaseolina were detected in all of these counties. The widespread distribution of *H. glycines* and *M. phaseolina* in both soybean and corn fields in Ohio confirms the potential risk for crop production. The fact that *H. glycines* was found in fields planted to corn was not surprising; in Ohio, corn-soybean rotation is common practice and *H. glycines* can overwinter for several years in infested fields (Niblack 2005).

Willson et al. (1996) detected the presence of *H. glycines* throughout the western and central regions of Ohio. They did not, however, identify *H. glycines* in the eastern portion of the state.

FIGURE 5

Corn fields sampled in a survey during 2013 and 2014. (A) Distribution of *Heterodera glycines* in corn fields. (B) Distribution of *Macrophomina phaseolina* in corn fields.

Twenty years later, *H. glycines* was detected in the eastern region of Ohio (Fig. 3A) and in eighteen additional counties compared with the earlier survey. Interestingly, counties in which *H. glycines* was not detected in the current survey also showed no presence of the nematode or were not sampled in the survey conducted by Willson et al. (1996). In counties with no detectable *H. glycines*, however, low numbers of fields were sampled. These counties included Auglaize (1 field sampled), Fairfield (9 fields sampled), Gallia (1 field sampled), Hocking (15 fields sampled), Meigs (2 fields sampled), Washington (1 field sampled), and Williams (1 field sampled). Note that Hocking County is in the eastern edge of the soybean production region, and only slightly over 15,000 ha are in crop production in this county.

In the previous survey conducted by Willson et al. (1996), the virulence profile of *H. glycines* from several infested soil samples was reported. They found that HG Type 0 (race 3) was the most common *H. glycines* virulence profile. To complement the information generated in this survey, an *H. glycines* (HG) Type test (Niblack et al. 2002) for field samples in which the population density exceeded a certain threshold is currently in progress. Determining the virulence profile of the nematode population in a field will allow selection of soybean cultivars with suitable sources of *H. glycines*-resistance.

To our knowledge, this is the first study that reports the distribution and abundance of *M. phaseolina* in soybean and corn fields in Ohio. *Macrophomina phaseolina* has a wide host range with economically important crops such as corn, soybean, and wheat (Almeida et al. 2003). In our study, fields planted to corn or soybean were sampled; in Ohio, these crops are commonly planted in the same field in alternate years. Concerns about potential production losses arise when *M. phaseolina* was detected in more than 95% and 77% of the soybean and corn fields sampled, respectively.

Even though host specialization was observed among *M. phaseolina* isolates from corn but not from soybean, a mixture of these different isolates is commonly found in soils (Pearson et al. 1987; Su et al. 2001). Therefore, either soybean or corn planted in infested fields could be affected. In our study, *M. phaseolina* was isolated and quantified only from soil samples. No further analysis was done in classifying isolates based on host preference.

Because of the potential for yield loss in corn due to plant-pathogenic nematodes and the availability of nematode management products for corn, many farmers are interested in obtaining information on the presence and abundance of nematodes in their corn fields. The recommended sampling procedure for nematodes associated with corn (Tylka et al. 2011) is very different from that for soybean cyst nematode in soybean (Niblack and Tylka, 2008), and it is clear from our results that soil sampling procedures for each crop/nematode combination should be followed closely. In our study, samples collected to identify and quantify root-parasitic nematodes on corn resulted in underestimation of the presence of *H. glycines*. Fields in which soybeans and corn are grown in rotation should be sampled such that important nematodes on both crops can be detected.

Finally, resistance in field crops remains the most effective way to manage diseases. Niblack (2005) recommended “rotate-rotate-rotate” to manage soybean cyst nematode: rotate to a non-host crop (corn or wheat); rotate non-host crops with resistant soybean cultivars; and rotate sources of resistance to avoid adaptation of the pathogen to resistance. The presence of *M. phaseolina* in Ohio may reduce the effectiveness of the recommendation to rotate to a non-host crop because it can infect both corn and wheat. In addition, in fields where both pathogens are present, reduction in soybean production may still take place even when cultivars

resistant to *H. glycines* are planted. Farmers in Ohio will be well served if resistance to both of these soil-borne pathogens is included in soybean cultivars targeted for the eastern soybean belt in the future.

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