RESEARCH NOTE/NOTA INVESTIGATIVA

DISTRIBUTION, HOST STATUS AND POTENTIAL SOURCES OF RESISTANCE TO *VITTATIDERA ZEAPHILA*

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ABSTRACT

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Vittatidera zeaphila was described from stunted *Zea mays* (corn) roots collected in northwestern Tennessee (Obion County) in 2006. Similar cyst specimens had previously been collected in 1978 from Lauderdale County, TN, on *Eleusine indica* (goosegrass). Comparison of the 1978 specimens deposited in the USDA Nematology Collection at Beltsville, MD, and the 2006 specimens verified that they were identical. The purpose of this research was to determine the distribution of this nematode, determine the host range and determine if there was resistance which could be incorporated into commercial corn hybrids in the event the nematode was a threat to Midwest corn production. This nematode is known only from the 1978 site in Lauderdale County, TN and the two findings in Obion County, Tennessee and a field in Hickman County, Kentucky from this study. *Zea mays* and *E. indica* were the only plants supporting increase of this nematode and were good hosts, with Rf values of 6 or more. Nonhosts included common dicotyledous plants that are known hosts of cyst nematodes, a range of native grasses, and teosinte. Preliminary data on potential sources of resistance to *V. zeaphila* suggest that resistance is not a dominant trait and that there is a potential role of the cytoplasm in conferring resistance.

Keywords: goosegrass*, Eleusine indica, Zea mays,* teosinte, native grasses, switchgrass.

RESUMEN

Donald, P.A., R. Heinz, E. Bernard, D. Hershman, D. Hensley, S. Flint-Garcia, R. Joost. 2012. Distribución, susceptibilidad y fuentes potenciales de resistencia a *Vittatidera zeaphila*. Nematropica 42:91-95.

En 2006 se describió *Vittatidera zeaphila* asociado a raíces de maíz (*Zea mays*) con pobre crecimiento en el noroeste de Tennessee (Condado de Obion). Especímenes similares de este quiste se habían colectado previamente en 1978 en el condado de Lauderdale, Tennessee, en el pasto *Eleusine indica.* La comparación de los especímenes de 1978 depositados en la Colección de Nematología del laboratorio del Departamento de Agricultura (USDA) en Beltsville, Maryland con los especímenes del 2006 mostró que eran idénticos. El objetivo de esta investigación es determinar la distribución de este nematodo y su rango de hospedantes y deteminar si existe resistencia que pueda utilizarse en híbridos comerciales de maíz en caso de que este nematode se convirtiera en amenaza para la producción de maíz en Estados Unidos. Esta especie sólo se ha registrado en 1978 en Lauderdale, los dos hallazgos en Obion County, en Tennessee y en un campo en el condado de Hickman en Kentucky que reportamos en este estudio. Las dos plantas que demostraron ser buenas hospedantes son *Zea mays* y *E. indica*, con factores reproductivos (Rf) de 6 ó más. Se encontró que plantas dicotiledóneas comúnmente hospedantes de otros nematodos quiste, varios pastos nativos y teosinte no son hospedantes de este nematodo. Datos preliminares sobre fuentes potenciales de resistencia a *V. zeaphila* sugieren que la resistencia no es un caracter dominante y que es posible que el citoplasma juegue algún papel en la resistencia.

Palavras-chave: Eleusine indica, Panicum virgatum, pastos nativos, teosinte*, Zea mays*

Corn is a major crop for the US with 32 million hectares harvested in 2010. This is the largest number of harvested acres since 2007, when almost 215 million hectares were harvested (National Ag Statistics Service, 2011). Corn products can be found in many human food items, as animal feed, in biodegradable packaging, fiber, and ethanol for vehicle fuel.

Zea mays domestication occurred between 9,000 and 10,000 years ago (Doebley, 2004). Through molecular techniques *Z. mays* has been shown to be derived from *Z. mays* subsp. parviglumis (teosinte), a native grass from Mexico and Central America (Matsuoka *et al.*, 2002). Corn, like any crop that is grown over large acreages, has plant- parasitic nematode problems. Corn is important for management of soybean cyst nematode (*Heterodera glycines* Ichinohe) as it is a nonhost for *H. glycines* and is used in rotation with soybean to starve out the nematode (Niblack 2005). Symptoms of nematode damage are most obvious and severe on sandy soils but damage can occur in all soil types. Nematode symptoms are reflective of problems with root function, and they include yellowing and/or stunting of plants generally in patches as opposed to single scattered plants. The damage may mimic drought or nutrient deficiency. When roots are examined it may be difficult to distinguish nematode damage from herbicide damage, insect feeding, compaction and various other causes of root trauma. Plant-parasitic nematodes documented in the United States to attack corn roots include species of *Belonolaimus, Pratylenchus, Meloidogyne, Dolichodorus*, and *Heterodera*. Among cyst nematodes, *H. zeae* is the only species documented to parasitize corn. U.S. Federal quarantine was in effect for *H. zeae* from 1981 until 1996. The nematode was found to be of little economic importance in the U.S. and the quarantine was dropped. The optimal temperature for emergence of the second-stage juvenile from the egg was reported as 25°C, which limited its impact to warm corn production areas (Hutzell and Krusberg, 1990). Recently a cyst nematode, not matching the morphological characteristics of *H. zeae*, was found on roots of stunted corn in Tennessee and described as *Vittatidera zeaphila* Bernard, Handoo, Powers, Donald & Heinz (Bernard *et al.*, 2010). This nematode was collected in the late 1970s by Dr. E. Bernard in a tomato field in western TN reproducing on goosegrass (*Eleusine indica* (L.) Gaertn.). Specimens from this collection were sent to the late Morgan Golden and retained in the USDA Nematode Collection as a potential undescribed species. Specimens from the 1970s and 2006 Obion County, TN collection were used for the description of the nematode (Bernard *et al.*, 2010).The objectives of this research were to 1) determine distribution of *V. zeaphila* in corn production areas of Tennessee and Kentucky; 2) determine its host range; and 3) determine if resistance to the nematode is present in corn.

*Distribution***.** Soil samples were collected from corn fields in middle and west Tennessee in 2007. Samples collected in late May were 2.5 cm-dia soil cores to a depth of 12 cm. Samples collected during June through September were 10 cm-dia cores to a depth of 10 cm. All soil samples were composite samples collected in the active root zone of corn plants in corn production fields. Fields were selected at 1.6-km intervals along county roads in 14 corn-producing counties which had more than 6070 hectares of corn planted in the county. Additional samples were collected based on field history of corn production for several years or from silage production fields, which tend to be grown as a corn monoculture. These field locations were obtained with the cooperation of University of Tennessee Agricultural Extension Agents. Corn production fields operated by a food processing company were also sampled. These irrigated fields were in rotations of corn, wheat, and edible beans. All other fields were dryland production. A total of 200 soil samples were collected and analyzed for presence of *V. zeaphila*. Soil was processed to extract cysts using a semi automatic elutriator (Byrd 1976). Because of similarities in size of *H. glycines* and *V. zeaphila* cysts, bioassays were performed in the greenhouse to confirm presence of *V. zeaphila*. Cysts recovered from the soil samples were added to the remainder of that soil sample to fill a 15-cm-dia clay pot and the pot was placed in a greenhouse at 27 °C. Three corn seed were planted in the soil; corn roots were removed from the pot at 35 days after planting and placed on a 180-µm-pore sieve. The roots were forcibly washed with a stream of water. Contents from the sieve were examined microscopically at 40X for presence of cysts and or white females. Samples were considered positive when white females or cysts were found on the bioassay corn roots in two successive replications of the bioassay. Two samples from Obion County, TN, and one sample from Hickman County, KY, were positive for *V. zeaphila*.

Host status. Vittatidera zeaphila from the 2006 finding was maintained at the University of Missouri as a culture in sand in a water bath at 27.5 C, increased on corn, and harvested every 28 to 30 days prior to these studies. Plants for host studies were grown in 450-mL plastic beakers filled with steam sterilized sand. Seed of the species listed in Table 1 were planted and seedlings allowed to emerge for one to two weeks, depending on plant species. Five holes evenly distributed around the seedling provided wells for the inoculum at a rate of 10,000 eggs in 25 mL water in each beaker. Each beaker was placed in a water bath set at 27.5°C. The plants were harvested 28 to 30 days after initiation of the experiment. The above-ground plant portion was removed at the soil line. The root ball was soaked in water in a small bucket and the root ball cut into quarters vertically. Half of the root system was spread over nested 710-µm-pore and 180-µm-pore sieves placed over a bucket. The root mass was washed forcibly three times while turning the root system. The process was repeated with the other half of the root system. The contents of the 180-µm-pore sieve were washed over the

stack of 710-µm over 180-µm-pore sieves three more times. The contents on the 710-µm-pore sieve were gently rinsed. The 180-µm-pore sieve contents were placed over stack of 75-µm over 25-µm-pore sieves. The contents from the 180-µm-pore sieve were ground with a large rubber stopper and were rinsed often. This process was continued until the debris passed through the sieve. The contents remaining on the 25-µm-pore sieve were rinsed into a centrifuge tube. The suspension was spun for 5 minutes at 441 G. Water was removed from the centrifuge tube with a syringe until 5 to 10 mL remained. The pellet was mixed thoroughly with a syringe and added to a graduated centrifuge tube with 40% sucrose. The suspension was centrifuged for 5 minutes at 441 G. A syringe was used to carefully remove the gradient layer of clean eggs. The eggs were placed on a 25-µm-pore sieve and the eggs rinsed free of sucrose. An aliquot was used to determine egg population density. Host status was determined by comparison of number of eggs collected from the root system in relation to the inoculum level at the beginning of the experiment. A commercial hybrid corn line, Asgrow RX715RRZ, was included with each test to serve as a susceptible control. Initial studies were not replicated but served to determine the breadth of the host range and to include all crop plant hosts of cyst nematodes as well as potential native grass hosts (Table 1). The host range of *V. zeaphila* was further investigated with 12 switchgrass (*Panicum virgatum* L.) lines (Table 2). Tennessee has a statewide iniative to make Tennessee the leading biofuel production state. Increased switchgrass production was anticipated and in a proactive approach, multiple switchgrass lines were tested for their reaction to *V. zeaphila*. Only corn and goosegrass were identified as good hosts. This result is consistent with the original collections of this nematode in Tennessee (Bernard *et al.* 2010). It is interesting that the hosts found in this study are not native to the same geographic area. Corn is native to Latin America and goosegrass is native to Eurasia. Poor hosts included barley, oat, switchgrass, teosinte, and June grass (Table 1). Many native grasses and all the dicotyledonous plants tested were nonhosts for *V. zeaphila*. Some reproduction was seen on certain switchgrass lines (Table 2). Both lowland and upland switchgrass lines were included; however, there were no significant differences in reproduction between lines or ecotypes.

Potential sources of resistance in corn. All corn hybrids examined in the host status experiments above were determined to be good hosts of *V. zeaphila*. To further examine the range in response to *V. zeaphila*, 28 genetically diverse inbred lines were evaluated in a single replicate, along with the susceptible check hybrid. The reason for evaluating these particular inbred lines is that these populations already exist for mapping of resistance genes, should resistant sources be identified. Response ranged from complete resistance (zero eggs recovered from B97) to greater susceptibility than the susceptible control (Table 3). A set of eleven potentially resistant lines were chosen for reevaluation (data not shown), resulting in a correlation of 0.81 between replicates. Lines that show resistance include B73, B97, IL14H, M37W, Mo17, MS71, and NC350. Susceptible lines include CML247, Mo18W, NC358, Tzi8, and W22. Temperate lines (including stiff stalks, non-stiff stalks, and sweet corn lines) tended to be more resistant to *V. zeaphila* than tropical lines, although there are striking exceptions to this trend. Future research is needed to explore these trends more fully, and to validate these lines as sources of resistance.

Reaction to *V. zeaphila* was then evaluated in hybrids, the form of corn grown by producers. Hybrid seed was created between B73 (female) and eight inbreds (male) that showed either resistance or susceptibility to *V. zeaphila*. These eight hybrids were screened for reaction to *V. zeaphila*. Generally, crosses involving a susceptible parent (i.e. CML247, NC358 and Tzi8) resulted in susceptible hybrids. The exception is the resistant hybrid between B73 and Mo18W, even though Mo18W was the most susceptible line in the inbred study. Crosses of resistant inbreds with B73 yielded hybrids that were more susceptible than either of their inbred parents. This is the opposite of what was observed for corn rootworm feeding damage, using the same set of diverse inbred lines and hybrids (Flint-Garcia *et al.*, 2009). In the rootworm study, hybrids were always found to be more resistant than their inbred parents. The results in the current study indicate that resistance to *V. zeaphila* is not a dominant trait. The reciprocal hybrid of B97 (female) x B73 (male) was created to investigate the possibility of cytoplasmic (i.e., maternal) inheritance of resistance from B97. We observed a differential response of the reciprocal hybrids where using B73 as the female resulted in resistance (Rf = 0.7) and using B97 as the female resulted in susceptibility (Rf = 33.0). This finding points to a potential role of the cytoplasm in conferring resistance. Further tests are required to determine if the single replication results are reproducible for the hybrid study and to explore the nature of resistance more deeply.

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Table 1. Reproduction of *Vittatidera zeaphilia* on common cyst nematode hosts, native grasses and a variety of corn types in greenhouse trials.

Plants tested	Rf[z]	Host Status
Strawberry Popcorn (*Zea mays*)	6	Host
Goosegrass (*Eleusine indica*)	10	Host
Indian Popcorn (*Zea mays*)	10	Host
Field corn (*Zea mays*)	14	Host
Pop corn (*Zea mays*)	19	Host
White food grade corn (*Zea mays*)	19	Host
Indian corn (*Zea mays*)	28	Host
Annual ryegrass (*Lolium multiflorum*)	0	Nonhost
Brown Tip Millet (*Brachiaria fasciculate*)	0	Nonhost
Foxtail Millet (*Setaria italic*)	0	Nonhost
Gamagrass 'Highlander' (*Tripsacum dactyloides*)	0	Nonhost
Grain sorghum (*Sorghum bicolor*)	0	Nonhost
Indian Grass (*Sorghastrum nutans*)	0	Nonhost
Little Bluestem (*Schizachyrium scoparium*)	0	Nonhost
Proso Millet (*Panicum miliaceum*)	0	Nonhost
Sorghum Sudan grass (*Sorghum bicolor* var. *Sudanese*)	0	Nonhost
Sugar beet (*Beta vulgaris*)	0	Nonhost
Tall Bluestem (*Andropogon*)	0	Nonhost
Tall fescue (*Festuca arundinacea*)	0	Nonhost
Tobacco (*Nicotiana tabacum*)	0	Nonhost
Tomato (*Lycopersicon esculentum*)	0	Nonhost
Triticale (*Triticosecale*)	0	Nonhost
Wheat (*Triticum aestivum*)	0	Nonhost
Soybean 'Williams' (*Glycine max*)	0	Nonhost
Barley (*Hordeum vulgare*)	1	Poor Host
June grass (*Poa pratensis*)	1	Poor Host
Oat (*Avena sativa*)	1	Poor Host
Switchgrass (*Panicum virgatum*)	0 to 1	Poor Host
Teosinte	Less than 1	Poor host
t-Statistic ($P < 0.01$)	2.8	

[z] Rf (reproductive factor) = Total egg production/initial inoculum (10,000 eggs/pot).

Table 2. Reproduction of *Vittatidera zeaphila* on selected switchgrass cultivars in greenhouse trials.

Switchgrass lines	Rf[z]
OSV-1 (SL93) lowland	0.05
OSV-2 (NSL) lowland	0.03
Noble C62 lowland	0
Noble C75 lowland	0.15
Noble C77 upland	0.09
Kanlow lowland	0.94
Alamo lowland	0.10
Shelter Switchgrass	0
Cave-in-Rock Switchgrass	0
Forestburg Switchgrass	0.02
Blackwell Switchgrass	0.02
Dakotah Switchgrass	0.05
$P > 0.58$	

[z] Rf (reproductive factor) = Total egg production/initial inoculum (10,000 eggs/pot).

LITERATURE CITED

Bernard, E. C., Z. A. Handoo, T. O. Powers, P. A. Donald, and R. D. Heinz. 2010. *Vittatidera zeaphila* (Nematoda: Heteroderidae), a new genus and species of cyst nematode parasitic on corn (*Zea mays*). Journal of Nematology 42:139-150.

Byrd, D. W., Jr., K. R. Barker, H. Ferris, C. J. Nusbaum, W. E. Griffin, R. H. Small, and C. A. Stone. (1976). Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. Journal of Nematology 8:206-212.

Doebley, J. F. 2004. The genetics of maize evolution. Annual Review of Genetics 38: 37-59.

Flint-Garcia, S. A., K. E. Dashiell, D. A. Prischmann, M. O. Bohn, and B. E. Hibbard. 2009. Conventional screening overlooks resistance sources:Rootworm damage of diverse inbred lines and their B73 hybrids is unrelated. Journal of Economic Entomology 102:1317-1324.

Hutzell, P. A. and L. R. Krusberg. 1990. Temperature and the life cycle of *Heterodera zeae*. Journal of Nematology 22:414-417.

Matsuoka, Y., Y. Vigouroux, M. M. Goodman, J. Sanchez, G. E. Buckler, and J. Doebley. 2002. A single domestication for maize shown by multilocus microsatellite genotyping. Proceedings of the National Academy of Sciences, USA 99:6080 – 6084.

National Agriculture Statistics Service 2011. http://www.nass.usda.gov/Charts_and_Maps/graphics/cornac.pdf. Accessed August 2011.

Niblack, T. L. 2005. Soybean cyst nematode management reconsidered. Plant Disease 89:1020-1026.

Table 3. Reproduction of *Vittatidera zeaphila* on the commercial check hybrid, inbred lines, and a select subset of hybrids using B73 as the female parent. Lines were inoculated with 10K *V. zeaphila* eggs at one week after planting in the greenhouse and evaluated at 30 days.

Maize Genotype	Group[y]	Rf-inbred[z]	Rf-hybrid[z]
Commercial Hybrid AsgrowRX715RRZ			14.1
B73	SS	1.2	
B97	NSS	0.0	0.7
CML103	TS	4.4	
CML228	TS	3.1	
CML247	TS	24.1	37.4
CML277	TS	13.1	
CML322	TS	15.7	
CML333	TS	6.3	
CML52	TS	11.5	
CML69	TS	5.8	
HP301	Pop	9.4	
IL14H	Sweet	1.2	
Ki11	TS	6.8	
Ki3	TS	3.4	
Ky21	NSS	11.0	
M162W	NSS	4.3	
M37W	Mixed	0.5	9.7
Mo17	NSS	1.8	12.8
Mo18W	Mixed	64.5	0.7
MS71	NSS	0.5	13.7
NC350	TS	2.3	
NC358	TS	18.0	25.2
Oh43	NSS	16.2	
Oh7B	NSS	12.0	
P39	Sweet	13.9	
Tx303	Mixed	6.3	
Tzi8	TS	20.9	27.4
W22	NSS	21.7	

[y] Three main subpopulations exist in breeding programs: stiff stalk (SS), non-stiff stalk (NSS), and tropical/subtropical (TS). Other corn groups include sweet corn, popcorn, and a catch-all "mixed" group.
[z] Rf (reproductive factor) = Total egg production/initial inoculum (10,000 eggs/pot).

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