RESEARCH NOTE – NOTA INVESTIGATIVA

XIPHINEMA SPP., PUTATIVE VECTORS FOR *BROME MOSAIC VIRUS* (BMV), ARE NOT ASSOCIATED WITH BMV-INFECTED WHEAT PLANTS IN ALABAMA

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ABSTRACT

Srivatsavai V. S. K., R. N. Huettel, and J. F. Murphy. 2006. *Xiphinema* spp., putative vectors for *Brome mosaic virus* (BMV), are not associated with BMV-infected wheat plants in Alabama. Nematropica 36:269-272.

Brome mosaic virus (BMV) was first detected in Alabama on wheat in 2002. Since that time it has been detected in wheat plants in all counties where wheat is grown, indicating it has become established in all wheat growing regions of the state. Wheat is an important winter crop in Alabama and is grown for both seed and animal feed. A vector for BMV has been reported to be the dagger nematode, *Xiphinema* spp. This study was conducted to determine if this nematode was associated with wheat production and the occurrence of the virus in Alabama. Soil samples were collected from a wheat variety trial and grower wheat fields throughout the state monthly during the growing season. Nematodes were extracted using the sugar flotation method and identified. The virus was detected using a BMV-specific direct double antibody sandwich ELISA. Although plant parasitic nematodes were detected in all samples, no *Xiphinem*a spp. were found in relation to any wheat field in which BMV-infected plants occurred. However, in some locations *Xiphinema* spp. were isolated from soil samples taken from adjacent fields associated with other crops which are not considered hosts for BMV. In Alabama, *Xiphinema* spp. were not found to be associated with the occurrence of BMV in wheat fields and, therefore, is not the primary vector of this virus.

Key words: Dagger nematode, distribution, transmission, *Triticum aestivum*, wheat.

RESUMEN

Srivatsavai V. S. K., R. N. Huettel, y J. F. Murphy. 2006. *Xiphinema* spp., vectores putativos del virus del mosaico del bromo (BMV), no se ecuentran asociados con plantas de trigo infectadas con BMV en Alabama. Nematropica 36:269-272.

El virus del mosaico del bromo (*Brome mosaic virus*, BMV) se detectó por primera vez en trigo en Alabama en 2002. Desde entonces, se ha detectado en plantas de trigo en todos los condados en donde se siembra trigo, indicando que está bien establecido en todas las regiones productoras de trigo del estado. El trigo es un cultivo de invierno importante en Alabama, que se cultiva para semilla y alimento de animales. Se ha registrado a *Xiphinema* spp. como posible vector de BMV. El objetico de este estudio fue determinar si este nematodo se encuentra asociado con la producción de trigo y la ocurrencia del virus en Alabama. Se recolectaron muestras de suelo de lotes de evaluación de variedades y de lotes de agricultores a través del estado durante la estación de crecimiento. Se extrajeron los nematodos por centrifugación-flotación y se identificaron. El virus se detectó utilizando una prueba de ELISA específica para BMV con doble anticuerpo. Aunque se detectaron nematodos fitoparásitos en todas las muestras de suelo, no se encontró *Xiphinema* sp. en ningún campo de trigo con plantas infectadas con BMV. En algunos sitios se aisló *Xiphinema* sp. de muestras de suelo tomadas en campos adyacentes sembrados con otros cultivos no considerados hospedantes de BMV. En Alabama, no se encontró que *Xiphinema* spp. estuvieran asociadas con la ocurrencia de BMV en cultivos de trigo, y por ello no es el vector primario de este virus.

Palabras clave: nematodo daga, distribución, transmisión, *Triticum aestivum*, trigo.

In Alabama, wheat is produced on ca. 150,000 acres with the acreage increasing yearly. Wheat is often grown as a cover crop and harvested for hay and feed. In 2002, *Brome mosaic virus* (BMV) was found for the first time in Henry County in southern Alabama (Kathy Burch, personal communication; Srivatsavai *et al.*, 2005). In subsequent studies in 2003 and 2004, BMV was identified from wheat in Autauga, Baldwin, Dallas, Dekalb, Elmore, Escambia, Henry, Limestone, and Mobile counties indicating it was widespread throughout the wheat growing areas of Alabama. BMV is found in most wheat growing areas worldwide and is capable of infecting wheat and other grains, such as barley, corn, oats and rye. Plant viruses in the genus *Bromovirus* are primarily vectored by beetles with limited inefficient transmission occurring by aphids in a non-persistent manner (van Regenmortel *et al.*, 2000). Lane (1974), however, indicated that BMV was not transmitted by aphids or mites. Nematodes of the genus *Xiphinema* were shown to transmit BMV under laboratory conditions (Schmidt *et al.* 1963; cited by Lane, 1977). The transmission of BMV by *Xiphinema* sp. is also cited by Agdia® within their commercial catalogue for virus detection kits (see www.Agdia.com). Although *Xiphinema* spp. are recognized vectors for viruses in the genus *Nepovirus*, they are not typically considered vectors for viruses in the genus *Bromovirus* (Weischer, 1975; Taylor and Brown, 1997). Trudgill *et al.* (1983) considered the evidence for transmission of BMV by *Xiphinema* spp. to be inadequate. However, due to the occurrence of *Xiphinema* spp. in Alabama and the increase in production of winter wheat, this study was conducted to determine whether this nematode was associated with the occurrence of BMV in wheat plants grown under field conditions in Alabama.

Thirty two soil samples were collected in the wheat variety trials at the E.V. Smith Research Center (EVSRC) in Shorter, AL, two times per month from pre-plant in October to harvest in May in 2004 and 2005. Samples were collected once a month in the wheat variety trials at the Gulf Coast Research and Extension Center (GCREC) in Fairhope, AL, from October to May in 2004 and 2005. In all other wheat producing counties in grower fields and variety trials at other research centers, samples were collected at least twice during the growing season in 2004 and 2005. Adjacent fields not planted in wheat were sampled in May or early fall at EVSRC and GCREC in 2004 and 2005. Over 2500 soil samples were analyzed for nematodes during this study.

A soil probe was used to obtain consistent sample size and depth. Five cores were collected in the soil probe around the periphery of the areas showing virus symptoms. Soil was collected from a depth of 20 cm in the root zone of wheat in each field, placed in plastic bags and transferred to the laboratory in coolers. Soil samples were stored in a refrigerator at 10°C until processed for nematodes.

Each sample (containing 5 cores) was mixed by hand and nematodes were extracted from 250 cm^3 of soil from each plastic bag. A soil sieving method, using a 45-µ sieve over a 25-µ sieve, was used to extract nematodes from samples. The contents of the 25-µ sieve were washed into a centrifuge tube and sugar flotation-centrifugation was used to extract the nematodes from the samples (Jenkins, 1964). Nematodes were identified to genus in all sam-

ples using a Nikon® dissecting microscope at 4×.

At each site where soil was collected, 150 leaves were collected to verify the presence of BMV. Leaves were individually wrapped and frozen at -20°C until ready for processing. The presence of BMV in plant leaf tissue was determined using an Agdia® enzyme-linked immunosorbent assay (ELISA) kit specific to BMV and according to the manufacturer's instructions (Agdia, Inc., Elkhart, IN). Briefly, microtiter plates were coated with anti-BMV immunoglobulin in 50 mM carbonate buffer, pH 9.6. Leaf samples (approximately 1 g per sample) were ground using a motorized leaf squeezer in 1 ml of extraction buffer (phosphate buffered saline, pH 7.4, containing sodium sulfite (1.3 g/L), polyvinylpyrrolidone MW 40,000 (20 g/L), powdered chicken egg albumin (2.0 g/L) and Tween-20 (20 ml/L). A 25-µl sample of sap extract was added to a microtiter plate well containing 75 µl of carbonate buffer. All subsequent steps were according to the manufacturer's instructions. ELISA reactions were allowed to develop at room temperature for 90 minutes. Samples were considered positive for the presence of BMV when the absorbance value (405 nm) was greater than the mean plus three standard deviations for comparable healthy control samples.

Plant parasitic nematodes were isolated from all soil samples collected from different wheat fields in all sampled counties. The nematodes that were identified in each of the different fields are mostly indicative of the previous crops grown (e.g., *Rotylenchus reniformis* in fields used previously for cotton). *Xiphinema* spp. were not isolated from any soil samples collected among wheat fields; however, they were isolated from samples collected in adjacent fields consisting of crops other than wheat. The plant parasitic nematodes most frequently identified from wheat fields were *Hoplolaimus* sp., *Paratrichodorus* sp., *Heterodera glycines*, *Meloidogyne* spp., *Pratylenchus* spp., *Mesocriconema* sp., and *Rotylenchulus reniformis*. A list of plant parasitic nematodes identified from each county is provided in Table 1.

BMV was detected from wheat leaf samples collected from fields in each of the counties listed in Table 1. The percentage of BMV-infected wheat samples varied from 2.0 (Limestone Co.) to 13.3 (Escambia Co.). Interestingly, BMV was also detected from Evening Primrose, *Oenothera*

Table 1. Plant parasitic nematodes isolated from soil collected from wheat fields in seven counties in Alabama.

Counties	Lance	Stubby Root	Spiral	Ring	Lesion	Root-knot	Reniform	Cyst
Macon	+	+	+	+	+	+	–	–
Escambia	–	+	+	+	+	+	+	+
Mobile	–	+	+	+	+	–	–	–
Baldwin	–	+	+	+	+	+	+	+
Limestone	–	+	+	+	+	–	–	–
Dekalb	–	–	+	+	–	–	–	–
Henry	+	+	+	+	+	+	–	–

+ Presence of nematodes.
– Absence of nematodes.

laciniata, collected along margins of some wheat fields but no *Xiphinema* spp. were found associated with it.

The absence of *Xiphinema* in all the soil samples collected from wheat fields suggests that this nematode may not be involved in the spread of BMV among wheat samples in the state of Alabama. It was not determined whether other plant parasitic nematodes, such as *Paratrichodorus* spp. were able to serve as a vector for BMV, although future studies should consider such a possibility due to their presence in many wheat fields.

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