IDENTIFICATION, DISTRIBUTION AND VECTOR BIOLOGY OF BROME

MOSAIC VIRUS OF WHEAT IN ALABAMA

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Venkata Suresh Kumar Srivatsavai

Certificate of Approval:

John F. Murphy Professor Plant Pathology	Robin N. Huettel, Chair Professor Plant Pathology
Kathy Flanders Associate Professor Plant Pathology	Stephen L. McFarland Acting Dean Graduate School

IDENTIFICATION, DISTRIBUTION AND VECTOR BIOLOGY OF BROME MOSAIC VIRUS OF WHEAT IN ALABAMA

Venkata Suresh Kumar Srivatsavai

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IDENTIFICATION, DISTRIBUTION AND VECTOR BIOLOGY OF BROME MOSAIC VIRUS OF WHEAT IN ALABAMA

Venkata Suresh Kumar Srivatsavai

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Signature of Author

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Date of Graduation

VITA

Venkata Suresh Kumar Srivatsavai, son of Subba Raju Srivatsavai and Suguna Srivatsavai was born on November 14, 1979, in Visakhapatnam, Andhra Pradesh, India. He graduated with the degree of Bachelor of Science in Agriculture Sciences in 2003 from Acharya NG Ranga Agricultural University, Naira, India. He Joined the Masters program in the Department of Entomology and Plant Pathology at Auburn University in January 2004.

THESIS ABSTRACT

IDENTIFICATION, DISTRIBUTION AND VECTOR BIOLOGY OF BROME MOSAIC VIRUS OF WHEAT IN ALABAMA

Venkata Suresh Kumar Srivatsavai

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Wheat leaves were collected from different counties in Alabama. The collected wheat leaves were tested for the presence of *Brome mosaic virus* (BMV) using direct double antibody sandwich ELISA. BMV was identified from Escambia, Mobile, Elmore, Autauga, Dallas, Henry, Macon, Baldwin, Dekalb and Limestone counties suggesting that this virus was becoming established throughout the state. Weeds growing in the vicinity of the wheat fields were collected at E.V.Smith Research Center (EVSRC) in Shorter, AL and Gulf Coast Research and Extension Center (GCREC) in Fairhope, AL during April and May of 2004 and 2005. *Oenothera laciniata* (Evening Primrose) was the

only weed species that tested positive for BMV. Soil samples were collected from one wheat variety trial at the EVSRC two times per month from October to May and at other fields in different counties throughout the state monthly during the wheat growing season. Nematodes were extracted by sugar flotation and identified. Even though plant parasitic nematodes were detected in all samples, no *Xiphinema* spp. were found in relation to any wheat field. However, *Xiphinema* sp. was found in an adjacent peanut field at EVSRC. Therefore, in Alabama, *Xiphinema* spp. were neither associated with nor likely to be the vector of BMV. *Altica foliaceae*, flea beetles were collected from wheat and *O. laciniata* plants at EVSRC and GCREC during April and May of 2005. The beetles were allowed to feed simultaneously on BMV (Oklahoma strain) infected wheat plants in one pot and uninfected wheat plants in three other pots in an insect cage in the green house. The flea beetles were able to transmit the virus from infected plants to uninfected plants indicating the vector behavior of the insects. The flea beetles collected were mostly associated with *O. laciniata*, a weed commonly found around the wheat fields. Both the *O. laciniata* and *Altica foliaceae* tested positive by ELISA for the virus suggesting that the flea beetles are a vector for BMV in Alabama, and might be involved in transmitting the virus from *O. laciniata* to wheat fields.

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I wish to dedicate this work to my parents and younger brother whose love and encouragement provide me the strength to achieve my goals.

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I. LITERATURE REVIEW

Brome mosaic virus

Brome mosaic virus (BMV) belongs to the family *Bromoviridae* and is one of the smallest RNA viruses (4). BMV is found in all the wheat growing regions of the world. McKinney et al. (41) in 1942 first observed yellow mosaic symptoms on *Bromus inermis* Leyss. growing in the wheat nursery at the Kansas Agricultural Experiment Station, Manhattan, Kansas. The virus has been reported to infect wheat in Russia, Hungary and Brazil (7,44). BMV infects several species of the family *Graminae* (35). BMV can cause severe infection on individual plants but the overall damage in the field depends upon the number of infected plants. This virus has been reported to cause economic damage in wheat in South Africa (60). The infected plants appear to be stunted with shriveled grains. The host range of BMV includes other crops such as oats, barley, rye and corn. BMV has been shown to infect rice but only under experimental conditions (33).

The symptoms caused by this virus vary from plant to plant. BMV causes severe yellow mosaic symptoms on *Bromus inermis*, Harvest Queen Wheat, and White Tartar oats (41). The virus causes distinct necrotic local lesions on inoculated leaves of *Chenopodium hybridum* (45). *C. hybridum* has been used as a local lesion host for the quantitative estimation of BMV in infected tissues (45). In wheat, the virus causes distinct mosaic symptoms which are expressed as light and dark green streaks along the

leaf; however, some cultivars of wheat are susceptible to BMV but remain symptomless.

BMV virions are isometric measuring about 26 nm in diameter (3). The capsid structure and coat protein sequence of this virus closely resembles that of *Cowpea chlorotic mottle virus* (CCMV) (50).

Several vectors have been reported to be involved in the spread of BMV. The vectors include nematodes, beetles, and mites (14). Flea beetles were reported to be an efficient vector for BMV of cereals in Europe (34). The nematode *Xiphinema* was able to transmit BMV under laboratory conditions (47). There are no reports indicating aphids as vectors for this virus (35).

Beetles as Vectors

Viruses in the genera *Bromovirus*, *Comovirus* and *Tymovirus* can be transmitted by beetles (21). The flea beetle, shown to serve as a BMV vector, belongs to the Order Coleoptera, Family Chrysomelidae, and Subfamily Alticinae. The virus-beetle interaction is dependent on both the species of beetle and the specific virus (62). Most of the flea beetles have a narrow host range. The host preference is determined by attractant substances in the plants (42). The majority of beetle-transmitted viruses are found in either the beetle's regurgitant or hemolymph (20,48).

Beetles e.g., bean leaf beetles which transmit viruses can become viruliferous within a short time after feeding on a virus source plant. They also can acquire virus by feeding on a solution of purified virus mixed with sucrose (36). Some beetles can become viruliferous when purified virus is injected into the hemocoel (22,46,49). The time length

during which a virus is retained in a beetle depends on the virus, beetle, plant host, and environmental conditions. Some of the vector beetles which are less active retain virus for a longer time (61)**.** Vector efficiency will largely depend upon the species of the beetle (11).

Nematodes as Vectors

Nematodes, which transmit plant viruses, belong to two sub-orders, Dorylaimina (Order Dorylaimida) and Diptherophorina (Order Triplonchida). The nematodes *Xiphinema* and *Longidorus* belong to the sub-order Dorylaimina, whereas the nematodes *Trichodorus* and *Paratrichodorus* belong to sub-order Diptherophorina (13,37,38). Hewitt *et al*. (31) first observed and identified the association between a nematode and a plant virus. They proved that *X. index* could transmit *Grapevine fanleaf virus*. Those viruses which are transmitted by *Xiphinema* and *Longidorus* belong to the genus *Nepovirus* (6,26). The Nepoviruses are isometric particles which measure 28 nm in diameter. These viruses have bipartite, single stranded RNA genomes. Both viral RNA molecules are required for complete infection (27). *Trichodorus* spp. (59) and *Paratrichodorus* spp. (58) transmit viruses belonging to the genus *Tobravirus*. Tobraviruses consists of two particles of two lengths, 180-210 nm and 45-115 nm. The viruses have bipartite, single stranded RNA genomes. Both particles are needed to cause complete infection in a plant (24). *Tobacco rattle virus* and *Pea early-browning virus* are the important viruses in this genus that are transmitted by *Trichodorus* spp. (59) and *Paratrichodorus* spp. (58).

***Xiphinema* spp.**

Most of the species of *Xiphinema* are found throughout the world but predominantly in the tropics and sub-tropics. *X. americanum* is a common species and is found widely in agricultural and forest soils of the United States (1). *X. index*, which is a vector for *Grapevine fanleaf virus*, is seen worldwide, commonly associated with grapevine (63). *Xiphinema* spp. occur in a wide range of soils but mostly prefer loam soils (24).

Xiphinema spp. are very long and slender nematodes. The posterior part of the esophagus is enlarged with a highly prominent stylet (odontostylet) in the stoma. The odontostylet is forked at its junction of the odontophore, with prominent basal flanges. The odontostylet is formed by a gland in the esophagus and reforms at each molt. The odontostylet has two guide rings at its base, with the posterior guide ring being more prominent (10).

Xiphinema spp. feed on the roots of their hosts, with different species feeding on different parts of the roots (53). *X. diversicaudatum* feeds mostly at the root tips and causes terminal and subterminal swellings in herbaceous plants (56). *X. americanum* causes necrosis and discoloration of the cortical tissues of the roots and rarely feeds on root tips (8,18,23). Feeding occurs when the odontostylet penetrates into a cell by a rapid thrusting force (52,57). The intermittent pulsation of the esophageal bulb helps in the ingestion process for the nematode (12,15,64).

The *Xiphinema* spp. have a long life cycle ranging from several months to several years depending upon the species. The nematode generally lays eggs in spring and early

summer when the host plant is in an active growing stage. The eggs hatch within a short period of time. The larval development does not depend on the season and, hence, all stages are found throughout the year (16,19).

Specificity and Transmission process

A virus can be transmitted only by a particular nematode species. This is called its specificity. In *Xiphinema,* the virus is retained in the cuticular lining of the esophageal lumen. When the plant sap (containing virus) passes from the plant to the nematode, the virus attaches to the cuticular lining of esophagus. The attachment of the virus to the esophagus is due to the specific interaction between the viral coat protein and the retention site in the nematode (25,28).

The adult and juvenile stages are capable of transmitting viruses (39). The transmission process involves several sequential interactions among virus, nematode and plant. The time period required for nematodes to acquire the viruses from the infected plants is called the acquisition period. The transmission efficiency of the nematodes increase with increase of feeding access time on infected plants (57). The nematodes can acquire the virus immediately after a single feed on an infected plant. *X. index* can acquire *Grapevine fanleaf virus* within a feeding access period of 24 hours on infected plants (30), whereas *X. americanum* was able to acquire the virus within one hour (55).

The nematode vectors lose their infective potential if they do not have any access to plants. The viruses transmitted by *Xiphinema* may be retained for several months in their vectors (40). The viruses do not persist through a molt and cannot pass through

nematode eggs. The virus attaches extra-cellularly to the odontostylet which is shed during molt (2,17,29,54).

The transmission process involves both adsorption and dissociation. Adsorption involves the attachment of the virus particle to the cuticular lining of the esophagus. Dissociation is a process in which the virus gets detached from the retention area (cuticular lining in *Xiphinema*). During feeding, saliva is secreted by the salivary glands of the nematode. This saliva results in a pH change in the lumen of the esophagus, which leads to dissociation of the virus particle from the lumen (40).

II. IDENTIFICATION, DISTRIBUTION AND VECTOR BIOLOGY OF BROME MOSAIC VIRUS OF WHEAT IN ALABAMA

INTRODUCTION

In Alabama, wheat is produced on ca.150,000 acres with the acreage increasing yearly. It is grown as a winter 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. BMV belongs to the genus *Bromovirus*, family *Bromoviridae*. It is a cosmopolitan virus found in most wheat growing areas worldwide and capable of infecting wheat and other grains, such as barley, corn, oats and rye. The symptoms caused by this virus vary from host to host. In wheat, BMV causes mosaic symptoms which consist of light and dark green streaks along the leaf. There are contradictory reports in the literature as to how the virus is transmitted. Some literature indicates that BMV is transmitted by the nematode, *Xiphinema coxi* (47), and some have shown that it is vectored by flea beetles (34). This research was conducted to determine whether nematodes and/or beetles are involved in transmission of BMV in Alabama, to determine the distribution of the virus in wheat within the state and identify potential grass weed hosts that may serve as reservoirs for the virus.

MATERIALS AND METHODS

Wheat sampling

Wheat fields were sampled two times per month for BMV at E.V. Smith Research Center (EVSRC) in Shorter, Macon County, AL from February to May in 2004 and 2005. The fields in Escambia, Baldwin and Mobile counties were sampled once per month during the growing season (2004 and 2005). Wheat fields were also sampled in Henry, Limestone, Dallas, DeKalb, Elmore and Autauga counties once in either 2004 or 2005. Leaves were collected from plants showing mosaic (light green streaks) or yellow streak symptoms. Leaves were also collected from plants which seemed to be healthy without any viral symptoms. A total of 150 wheat leaves were collected in each wheat field at each sample date. Leaves were stored in a refrigerator at 4°C until processed for ELISA (9).

Weeds growing in the vicinity of the wheat fields were collected at EVSRC and Gulf Coast Research and Extension Center (GCREC) in Fairhope, AL during April and May of 2004 and 2005. Leaves were collected and tested by ELISA for the presence of BMV. The weed species included *Cynodon dactylon* (Bermuda grass), *Andropogon virginicus* (Broomsedge blue stem) *Digitaria sanguinalis* (Crab grass), *Panicum dichotomiflorum* (Fall panicum), *Paspalum urvillei* (Vasey grass), and *Oenothera laciniata* (Evening Primrose).

Brome mosaic virus (BMV) Testing

The Agdia® DAS ELISA system for BMV was used to test for infection of wheat and weed leaves. The leaves from wheat and weed plants were ground using a motorized leaf squeezer with the addition of 2 ml of extraction buffer. The extraction buffer consisted of sodium sulfite (1.3 g), polyvinylpyrrolidone (20.0 g), powdered egg chicken albumin (2.0 g) Tween-20 (20.0 g) in a final volume of 1 liter PBST (Phosphate Buffered Saline, Tween). All subsequent steps were according to the manufacturer's instructions. The substrate reaction was read using a microtiter plate reader at 405 nm (Spectrophotometer). Reactions were allowed to develop for 120 minutes at room temperature. A positive reaction for BMV was an absorbance value greater than the mean plus three times the standard deviation of three healthy wheat samples. If no known healthy wheat samples were taken, five values from field samples which reflect negative value were selected and the same method was followed.

Inoculation Tests

Triticum aestivum seeds (McCormick and Jackson varieties) were germinated in Dillen® pots (8-1/2″ x 5-3/4″). The soilless potting medium Pro Mix (Premier peat, Riviére-du-Loup, Québec, Canada) was used as a growing medium for the plants. These plants were grown and maintained in a greenhouse at the Plant Science Greenhouse Complex on the campus of Auburn University, AL. To prepare inoculum, BMV infected leaves from wheat fields were ground with a pestle and mortar with 1 ml 0.1 M potassium phosphate buffer (pH 6.0). Fifteen-day-old wheat seedlings grown in the

greenhouse were inoculated by rub inoculation using inoculum-saturated cheese cloth. The plants were dusted with carborundum prior to inoculation. The inoculated wheat plants were tested for BMV infection by ELISA after 15 days. The Oklahoma BMV isolate (obtained from Dr Richard Nelson, Samuel Roberts Noble Foundation, Ardmore, OK) was inoculated on 15-day-old McCormick and Jackson wheat plants and *Nicotiana benthamiana* plants in the greenhouse. The Oklahoma BMV isolate and Alabama BMV isolate were maintained in the greenhouse throughout the year by mechanical passage every two months onto newly germinated wheat plants.

Soil Sampling

A total of 32 soil samples were collected biweekly at EVSRC from pre-plant in October to harvest in May. Each soil sample was collected with a soil probe of 2.5 cm in diameter and 20 cm in depth and placed in a plastic bag and sealed. The adjacent fields not planted in wheat were also sampled. Soil samples were collected once a month in the wheat fields at GCREC from October to May. In all other wheat producing counties, grower fields and variety trials at other research centers, samples were collected at least twice during the growing season in 2004 and 2005. Soil samples were placed in plastic bags and were stored in a refrigerator at 4°C in the laboratory until processed.

Nematode Extraction

Nematodes were extracted from each soil sample by mixing of 250 cc of soil with large amounts of water in a 1 gallon bucket. The soil solution was mixed by hand and

allowed to settle for 20 -30 s to allow soil particles to sink to the bottom of the bucket. The mixture was passed through nested sieves of 250-µm pore sieve and 25-µm pore sieve. Debris trapped in the 250-µm sieve was discarded and the nematodes were collected from the 25-µm sieve by washing into a beaker. The nematodes were then processed by the Sugar-Flotation method (32). The extracted nematodes were poured into separate test tubes, centrifuged for 4 min at 3000 rpm and later were pelleted and the supernatant discarded. The pellet was resuspended in a 1 M sucrose solution and centrifuged for 3 min at 3000 rpm. The supernatant, which contained the nematodes, was collected on a 25-µm pore sieve. The nematodes were stored in sealed jars at room temperature until identified. They were observed with a Nikon SMZ800 dissecting microscope and identified to genus.

Insect collection and identification

Insects were collected with an aspirator from wheat and weed plants at EVSRC and GCREC during April and May of 2005. Insects were also collected at wheat fields in Dekalb and Limestone counties during the wheat growing season. The most prevalent insects were flea beetles (*Altica* spp.) found on wheat and *O. laciniata* (evening primrose), a weed commonly found around the wheat fields in Alabama. After collecting, the flea beetles were maintained on wheat (McCormick and Jackson varieties) in a 0.6 m^3 insect cage in the greenhouse. The beetles were identified with the assistance of Dr. Wayne Clark, Department of Entomology and Plant Pathology, Auburn University, using insect keys. The beetles were sent to the Systematic Entomology Laboratory at Maryland

for the further confirmation of the genus and species. Other insects, in addition to flea beetles, were also collected.

Insect Virus Transmission Study

BMV (Oklahoma) infected wheat plants in one pot and uninfected wheat plants in the three others pots were placed in a 0.6 m^3 insect cage at the greenhouse. The flea beetles, which were collected from Fairhope, AL, were released into the cage and were allowed to feed on all the plants simultaneously for 7 days. The beetles were removed by hand and wheat leaves were collected and tested for BMV by ELISA. In a second experiment several uninfected wheat plants in four different pots were placed in an insect cage and the beetles collected from Fairhope were allowed to feed for one week. The leaves were then tested for BMV infection using ELISA.

Some of the beetles collected from GCREC were tested by DAS ELISA. Sub-sets of beetle samples (300) were stored at -80° C. Pooled samples of five beetles were tested for BMV by ELISA as described previously. Beetles were homogenized in 100 µl extraction buffer in an eppendorf tube using a teflon homogenizer. A positive reaction for BMV was an absorbance value greater than the mean plus three times the standard deviation of 10 pooled beetle samples having the least ELISA values (the values ranged from 0.0990 to 0.1150). All the samples having the absorbance value greater than 0.130 were considered positive.

RESULTS AND DISCUSSION

BMV was identified from all the counties with varied levels of infection from field to field. Based on the pattern of infected plants, it was clear that the virus was distributed randomly throughout the fields. Although, BMV was not found in each field sampled, it was detected from wheat samples from Escambia, Mobile, Elmore, Autauga, Dallas, Henry, Macon, Baldwin, Dekalb and Limestone counties (Fig. 1). Wheat fields in Escambia County had the highest incidence of BMV (13.29 %). The infection levels ranged from 2.04 % to 13.29 % depending upon the county. The virus infection levels for samples collected from different fields in Alabama are illustrated in Table 1. Some of the wheat leaves which did not show any symptoms tested positive by ELISA suggesting that BMV may be present in plants which seemed to be healthy. Although, wheat leaves were sampled from February to May in 2004 and 2005, BMV was detected only from late March. The Alabama isolate of BMV was successfully inoculated onto uninfected wheat plants in the greenhouse indicating that the virus could be transferred mechanically. Several weeds in and around the wheat fields were tested for the presence of BMV but only *O. laciniata* tested positive for the virus. This is the first report of BMV in *O. laciniata*.

Several plant parasitic nematodes were identified from the soil samples after extraction by the sugar-centrifugation method. None of the soil samples contained *Xiphinema* spp. nematodes, a putative vector for BMV under laboratory conditions (47). However, *Xiphinema* sp. was found associated with other fields which were not planted

in wheat, such as a peanut field at EVSRC. None of the nematodes identified were known to be parasitic on wheat. However, the nematodes were indicative of the crops previously grown in those fields, e.g., *Rotylenchus reniformis* in fields used previously for cotton. The plant parasitic nematodes which were found in wheat fields in various counties of Alabama are listed in Table 2. Viruses that are spread by nematodes generally cause infection in discrete patches because of their uneven distribution (53). The *Xiphinema* spp. are generally reported to be vectors for viruses which infect perennials, such as fruit trees (43). *X. americanum* transmits *Tomato ringspot virus* which causes apple union necrosis and decline with M106 rootstock on Red Delicious variety (51).

In the feeding experiment with field collected flea beetles, two plants in one pot tested positive for BMV suggesting that the beetles transferred the virus from the infected source plants to uninfected plants. When flea beetles collected from the field were placed directly on uninfected wheat plants in the cage, no virus was detected. Some of the flea beetles collected from Fairhope, AL were tested by ELISA and shown to contain BMV. Out of 50 sets of beetles (5 in each set) tested by ELISA, 23 samples were positive for BMV with seven of the samples being highly prominent with respect to the threshold.

The majority of the flea beetles collected were mostly associated with *O. laciniata*. The flea beetles collected from *O. laciniata* and wheat were identified as *Altica foliaceae* by insect keys and confirmed by the Systematic Entomology Laboratory. Based on these results, the flea beetle (*Altica foliaceae*) appears to be a vector for BMV in Alabama.

O. laciniata, the weed which tested positive for BMV, was found all around the

wheat fields. This weed might provide a continuous source of virus which can easily infect wheat during every growing season. The flea beetles which were proved to be vectors were also found in large numbers around the wheat fields and on weed plants. They might transfer the virus from the *O. laciniata* to the wheat fields. The flea beetles may transfer the virus to other commercial crops like corn, oats etc. leading to the rapid spread of the virus in the state.

They were no reports of heavy losses caused by this virus to wheat and, hence, is not considered as a major disease in Alabama at this time. However, the disease severity caused by this virus on wheat under synergistic conditions is not known. A synergistic disease response would be more severe than the additive effect of each of the individual viruses. BMV along with other viruses might lead to an intense disease condition causing severe losses to wheat yields. There were reports of multiple virus infections on winter wheat in Alabama (5).

III. SUMMARY

Wheat plants from different counties of Alabama were surveyed for the presence of *Brome mosaic virus* (BMV) infected plants. The plants having virus like symptoms were identified and approximately 50 samples were collected per acre. Leaves were also collected from plants which seemed to be healthy without any viral symptoms. BMV was identified using direct double antibody sandwich ELISA according to the manufacturer's instructions. Infected wheat plants were identified in Autauga, Baldwin, Dallas, Dekalb, Elmore, Escambia, Henry, Limestone, Mobile and Macon counties suggesting that it was widespread throughout the wheat growing areas of Alabama. The sap extracted from the leaves testing positive for BMV was mechanically inoculated onto uninfected wheat plants grown in the greenhouse. The leaves which were inoculated are later tested by ELISA for the presence of the virus. The leaves tested positive for BMV, indicating that the virus could be transmitted mechanically.

Leaves from weeds growing in the vicinity of the wheat fields were collected at E.V. Smith Research Center (EVSRC) in Shorter, AL and the Gulf Coast Research and Extension Center (GCREC) in Fairhope, AL. The leaves were processed and tested by ELISA for the presence of BMV. The weeds which were tested by ELISA were *Cynodon dactylon* (Bermuda grass), *Andropogon virginicus* (Broomsedge) *Digitaria sanguinalis*

(Crab grass), *Panicum dichotomiflorum* (Fall panicum), *Paspalum urvillei* (Vasey grass), and *Oenothera laciniata* (Evening Primrose). *O. laciniata* was the only weed species that tested positive for BMV.

In the wheat variety trials at the EVSRC in Central AL, 32 soil samples were collected from plots twice a month from pre-plant in October to harvest in May. The adjacent fields not planted in wheat were also sampled. In the wheat variety trials at the GCREC in South AL, soil samples were collected monthly during the growing season. In all other wheat producing counties, grower fields and variety trials at other research centers, samples were collected at least twice during the growing season in 2004 and 2005. The nematodes were extracted from soil samples by the sugar centrifugation method and were identified. Plant parasitic nematodes were detected in all samples but no *Xiphinema* spp. was found in relation to any wheat field. However, *Xiphinema* sp. was found in adjacent fields associated with other crops in some locations.

Flea beetles collected from wheat fields, were able to transmit the virus from BMV infected wheat plants to uninfected plants maintained in the same cage indicating the vector behavior of the insects. Flea beetles collected from Fairhope also tested positive by ELISA for BMV. The flea beetles were identified as *Altica foliaceae* by the Systematic Entomology Laboratory.

BMV was first found in Henry County in 2002. It is currently detected in more than ten counties in Alabama indicating that it is being spread throughout the state. There were no reported losses to wheat production as the incidence levels were low but seeing the distribution of the virus in the last two years clearly indicate that the virus has the

potential to cause large damage to wheat fields in coming years. Moreover, increased disease severity caused by this virus on wheat under synergistic conditions is not known.

The outcome of this research will help growers understand the potential impact of this new virus in Alabama.

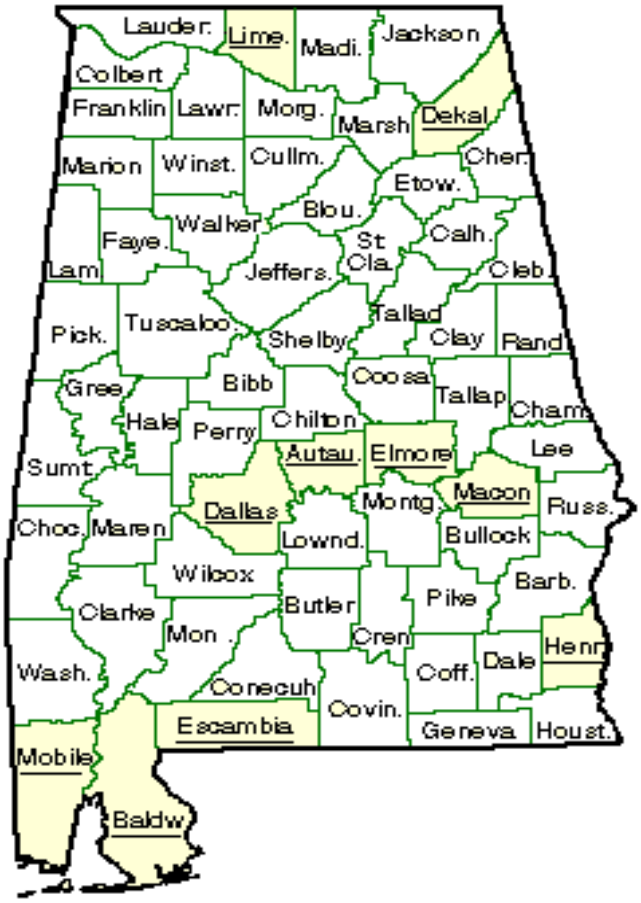

Fig. 1. Map showing the counties (shaded in yellow) where *Brome mosaic virus* infected wheat fields were present.

Fig. 2. *Brome mosaic virus* infected plant (left) and an uninfected wheat plant (right).

Table 1. *Brome mosaic virus* incidence for samples collected from different counties in Alabama.

Counties	**Fields**	**# samples[a]**	**# samples infected**	**% infection**
Escambia	Total	564 (1756)	75	13.29
	Field 1	124 (429)	28	22.58
	Field 2	159 (460)	19	11.94
	Field 3	65 (164)	0	0.00
	Field 4	138 (425)	23	16.66
	Field 5	78 (278)	5	6.66
Mobile	Total	493 (1460)	30	6.08
	Field 1	224 (739)	12	5.35
	Field 2	173 (471)	14	8.09
	Field 3	96 (250)	4	4.16
Elmore	Total	88 (350)	10	11.36
	Field 1	88 (350)	10	11.36
Autauga	Total	61 (244)	2	3.27
	Field 1	61 (244)	2	3.27
Dallas	Total	53 (214)	4	7.54
	Field 1	53 (214)	4	7.54
Henry	Total	74 (296)	2	2.70
	Field 1	74 (296)	2	2.70
Macon	Total	324 (938)	12	3.70

	Variety trial	324 (938)	12	3.70
Baldwin	Total	463 (1340)	44	9.50
	Field 1	202 (619)	26	12.87
	Field 2	158 (474)	7	4.43
	Field 3	103 (247)	11	10.67
Dekalb	Total	173 (692)	8	4.62
	Field 1	60 (240)	4	6.66
	Field 2	113 (452)	4	3.53
Limestone	Total	98 (392)	2	2.04
	Field 1	59 (243)	2	3.38
	Field 2	39 (149)	0	0.00

[a] Number of samples represent the number processed with the total number collected presented in parentheses. Samples collected from the field were pooled for processing for ELISA as groups of either two or four

Table 2. List of plant parasitic nematodes found in different counties of Alabama in fields planted in wheat.

Counties	Lance	Stubby root	Spiral	Ring	Lesion	RootKnot	Reniform
Macon	+	-	-	-	+	+	-
Escambia	-	+	+	-	+	+	+
Mobile	-	+	+	+	+	-	-
Baldwin	-	+	+	+	+	+	+
Limestone	+	-	-	+	+	-	+

\+ Presence of nematode
\- Absence of nematode

Lance nematode: *Hoplolaimus* sp.
Stubby nematode: *Paratrichodorus* sp
Spiral nematode: *Helicotylenchus* spp.
Ring nematode: *Mesocriconema sp*.
Lesion nematode: *Pratylenchus* spp.
RootKnot nematode: *Meloidogyne* spp.
Reniform nematode: *Rotylenchus* sp.

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