

First report of *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae), biotypes B and Q, in soybean *Glycine max* (L.) Merrill (Fabales: Fabaceae) in Paraguay

Primer reporte de *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae), biotipos B y Q, en soja *Glycine max* (L.) Merrill (Fabales: Fabaceae) en Paraguay

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Abstract. Whiteflies (Hemiptera: Aleyrodidae) are significant agricultural pests worldwide, primarily because they act as vectors for various plant viruses, including geminiviruses. Additionally, whitefly activity fosters the development of fungal colonies, such as sooty mold (*Capnodium* spp.), which grows on the sugary secretions (honeydew) produced by these insects. The presence of sooty mold reduces the plant's photosynthetic capacity, further worsening the negative impact on its growth and development. Together, these factors make whitefly control a challenge for farmers, who must implement integrated pest management strategies to mitigate both the direct damage and the associated diseases that whiteflies can spread. Although approximately 1,200 species of whiteflies have been described, fewer than 40 are considered primary pests, with *Bemisia tabaci* (Gennadius, 1889) and *Trialeurodes vaporariorum* (Westwood, 1889) responsible for the greatest agricultural losses worldwide. The emergence of *B. tabaci* biotypes has prompted significant research, as these biotypes have been linked to economic damage to crops in several nations of the Americas, including Argentina and Brazil. In Paraguay, there is a need to identify local biotypes of *B. tabaci* led to the collection of whitefly samples from soybean plots in Capitán Miranda and Natalio, Itapúa, Paraguay. DNA was extracted using the Wizard genomic purification kit, followed by PCR amplification of the mitochondrial cytochrome oxidase I (mtCOI) region using specific primers for *B. tabaci* and *T. vaporariorum*. Agarose gel electrophoresis confirmed the presence of B and Q biotypes of *B. tabaci* in both Paraguayan locations, with no detection of *T. vaporariorum*. Sequencing of the PCR products revealed a 100% match with known sequences for *B. tabaci* and its biotypes, as confirmed by GenBank BLAST analysis. This research highlights the critical importance of continuously monitoring the population dynamics and geographical distribution of different biotypes. While the expansion of a polyphagous and dynamic pest like the whitefly cannot always be prevented, constant monitoring enables early detection of its spread and allows for the adjustment of management strategies tailored to each region. These measures will not eliminate the pest but will help mitigate damage and manage the problem sustainably and efficiently. The study also emphasizes the need to evaluate

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the effectiveness of insecticides, as resistance has been reported in other countries—an issue not yet explored in Paraguay.

Key words: Agriculture; insect vectors; pests; plant viruses; whiteflies.

Resumen. Las moscas blancas (Hemiptera: Aleyrodidae) son plagas agrícolas importantes en todo el mundo, debido principalmente a su papel como vectores de diversos virus que afectan a las plantas, entre ellos los geminivirus. Además, la actividad de las moscas blancas favorece el desarrollo de colonias de hongos, como la fumagina (*Capnodium spp.*), que se desarrolla sobre las secreciones azucaradas (melaza) que estos insectos producen. La presencia de fumagina reduce la capacidad fotosintética de las plantas, agravando aún más el impacto negativo en su crecimiento y desarrollo. En conjunto, estos factores hacen que el control de las moscas blancas sea un desafío para los productores agrícolas, quienes deben implementar estrategias integradas de manejo de plagas para mitigar tanto el daño directo como las enfermedades asociadas que este insecto puede propagar. Aunque se han descrito aproximadamente 1.200 especies de moscas blancas, menos de 40 se consideran plagas primarias, siendo *Bemisia tabaci* (Gennadius, 1889) y *Trialeurodes vaporariorum* (Westwood, 1889) las causantes de las mayores pérdidas agrícolas a nivel mundial. La aparición de biotipos de *B. tabaci* ha impulsado importantes investigaciones, ya que estos biotipos han sido implicados en la generación de daños económicos a los cultivos en varias naciones de América, incluidas Argentina y Brasil. En Paraguay, la necesidad de identificar biotipos locales de *B. tabaci* condujo a la recolección de muestras de moscas blancas en parcelas de soja en Capitán Miranda y Natalio, Departamento de Itapúa, Paraguay. El ADN obtenido se extrajo utilizando el kit de purificación genómico Wizard, seguido de la amplificación por PCR de la región del citocromo oxidasa I mitocondrial (mtCOI) utilizando cebadores específicos para *B. tabaci* y *T. vaporariorum*. La electroforesis en gel de agarosa confirmó la presencia de los biotipos B y Q de *B. tabaci* en ambas localidades de Paraguay, sin detección de *T. vaporariorum*. La secuenciación de los productos de PCR reveló una coincidencia del 100% con las secuencias conocidas para *B. tabaci* y sus biotipos, como lo confirmó el análisis GenBank BLAST. Esta investigación resalta la importancia crítica de monitorear de manera continua la dinámica poblacional y la distribución geográfica de los diferentes biotipos, aunque la expansión territorial de una plaga polífaga y dinámica como lo es la mosca blanca no siempre se puede prevenir, el monitoreo constante permite detectar su propagación de manera temprana y ajustar las estrategias de manejo adaptadas a cada región. Estas medidas no eliminarán la plaga, pero ayudarán a mitigar los daños y a gestionar el problema de manera sostenible y eficiente. También destaca la necesidad de evaluar la efectividad de los insecticidas, ya que se ha informado resistencia en otros países, una preocupación aún no explorada en Paraguay.

Palabras clave: Agricultura; insectos vectores; moscas blancas; plagas; virus de plantas.

Whiteflies (Hemiptera: Aleyrodidae) are insects that comprise several species and biotypes and are considered major global pests because they are vectors of plant viruses such as geminiviruses and because they promote fungal colony growth (Brown 1998; Faria and Wraight 2001). Although around 1,200 species of whiteflies are described, fewer than 40 species are considered pests (Frohlich 1999), with two species, *Bemisia tabaci* (Gennadius, 1889) and *Trialeurodes vaporariorum* (Westwood, 1889), causing the most severe losses (Byrne *et al.* 1990). The emergence of *B. tabaci* biotypes globally has driven resources toward the study of these insects (Martin *et al.* 2000). Thirteen species of whitefly have been reported in Argentina and of these species, only *Aleurothrixus aepim* (Goeldi, 1886), *Aleurothrixus floccosus* (Maskell, 1896), *B. tabaci*, *Siphoninus phillyreae* (Cohic, 1968) and *T. vaporariorum* are known to cause economic damage to crops and trees (Viscarret 2000). Furthermore, the presence of *B. tabaci* biotype B has been reported in Argentina, as well as biotypes B and Q in Brazil, in soybean cultivation.

The need to identify biotypes present in Paraguay led to whitefly collection in productive soybean plots in the towns of Capitán Miranda (CM) and Natalio (NA), Department of Itapúa, during January and February 2022. The trifoliate leaves of soybean plants were collected in jars with screw caps, identified by location, and transported to the IPTA Entomology laboratory in Capitán Miranda, where the whitefly adults were transported in 2 ml vials with 70% alcohol and refrigerated at -20 C. DNA was extracted from a pool of individuals from each population using the Wizard Genomic DNA Purification Kit (Promega), ten (NA) and four (CM) individuals previously frozen. Subsequently, each pool was added to a 1.5 mL microcentrifuge tube containing 600 µL of Nuclei Lysis Solution. The sample was homogenized for 10 seconds using a vortex mixer and a rod. Subsequently, the steps of the manufacturer's protocol for isolating genomic DNA from animal tissue were continued. PCR was performed using primers that amplify the mitochondrial cytochrome oxidase I (mtCOI) region; general primers for *B. tabaci*: Bt Cox 1-628F (5'- GATCGAAATTAAATAGATCTTTATGATCC-3') / Bt Cox 2-1629R (5'- TGTCTATTGTAAGACTAGCACTATTTG-3') (Andreasen *et al.* 2017), specific primers for biotype B: BioB-F (5'-CTAGGGTTATTGTTGAGGTCATCATATATTTC-3') / BioB-R (5'-AATATCGA CGAGGCATTCCCC-3') and biotype Q: BioQ-F (5'-CTTGGTAA CTCT TCTGTAGATGTGTGTT-3') / BioQ-R (5'-CCTTCCCGCAGAAGAAATTGTTGTC-3') (Shatters *et al.* 2009), and specific primers for *T. vaporariorum*: TvapF (5'-TGTCAITTAATCCCCTACTTC-3') / TvapR (5'-ACAAAAACTGGGAAAGAAGAAG-3') (Andreasen *et al.* 2017). Agarose gel electrophoresis confirmed the presence of *B. tabaci* biotypes B and Q, as well as the absence of *T. vaporariorum*, within the populations from both the CM and NA localities. Sequenced PCR products (Macrogen Korea) obtained from the localities of Capitán Miranda and Natalio, Itapúa for *B. tabaci* general (OR450017, OR513104), Biotype B (OR525705, OR525706) and Biotype Q (OR608921, OR608922) were aligned using the GenBank BLAST tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). A 100% match was found with the following sequences: *B. tabaci* (KR559508, OP779682.1), Biotype B (OR342410, OR342409) and Biotype Q (MK249373.1, MK249373.1), confirming the presence of both biotypes of *B. tabaci* in Paraguay.



Figure 1. *Bemisia tabaci* adults on the underside of soybean leaves. Natalio, Department of Itapúa, Paraguay. / **Figura 1.** Adultos de *Bemisia tabaci* en el envés de las hojas de soja. Natalio, Departamento de Itapúa, Paraguay.

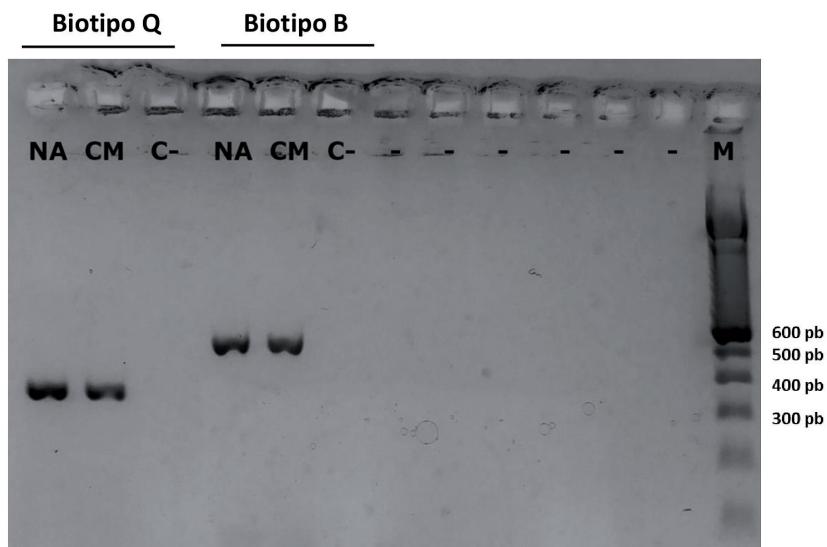


Figure 2. Agarose gel using a specific primer confirms the presence of *Bemisia tabaci* biotypes B and Q in the localities of Capitán Miranda (CM) and Natalio (NA). / **Figura 2.** Gel de agarosa utilizando un cebador específico que confirma la presencia de *Bemisia tabaci* biotipos B y Q en las localidades de Capitán Miranda (CM) y Natalio (N).

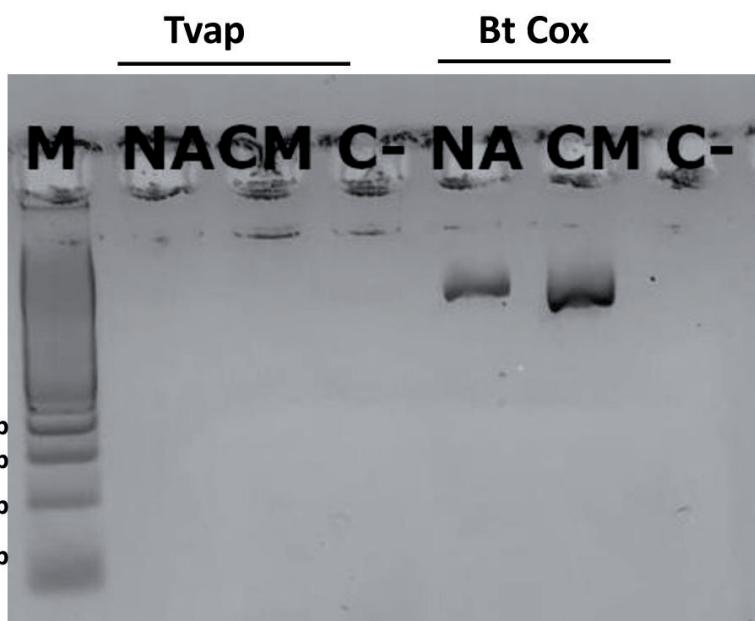


Figure 3. Agarose gel with a general primer (Bt Cox) for *Bemisia tabaci* and a specific primer (Tvpap) for *Trialeurodes vaporariorum*. / **Figura 3.** Gel de agarosa con primer general (Bt Cox) para *Bemisia tabaci* y primer específico (Tvpap) para *Trialeurodes vaporariorum*.

This research underscores the importance of monitoring whitefly population dynamics and biotype regionalization in agricultural production areas. It is essential to avoid the geographical expansion of whiteflies and associated crop damage. The research also highlights the need for assessing insecticide effectiveness, as resistance has been reported in several neighboring countries, a concern not yet explored in Paraguay.

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Author Contributions

NNE-M: Writing of the manuscript. **MAF-G:** Support in writing the manuscript. **LNT-S:** Analysis of DNA sequences. **CNB-C:** DNA extraction, PCR amplification. **FG:** Collection of specimens. **CC:** Correction of the manuscript. **AS-V:** Reviewing the text.

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