Acorbat Revista de Tecnología y Ciencia

Biocontrol potential of the *Cryphonectria hypovirus* 1 (CHV1) against *Fusarium oxysporum* f. sp. *cubense* race 1

Laura Arango-Palacio[1], María Dolores García-Pedrajas[2], Carmen Cañizares-Nolasco[2], Carlos Alberto Peláez Jaramillo[3], Andrés Mauricio Pinzón-Núñez[3*]

[1]Politecnico Colombiano Jaime Isaza Cadavid, Medellín-Antioquia 050022, Colombia, [2]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga, Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Estación Experimental "La Mayora", Avenida Dr. Wienberg s/n, 29750 Algarrobo-Costa, Málaga, España, [3]Universidad de Antioquia (UdeA), Facultad de Ciencias Exactas y Naturales, Grupo de Investigación Agrobitotecnología, Ciudad Universitaria, Medellín-Antioquia 050022, Colombia

***Autor de correspondencia:**
Andrés Mauricio Pinzón-Núñez
andresmpinzonn@gmail.com

Contribución:
Artículo Científico

Sección:
Fitosanidad

Recibido:
15 diciembre, 2025
Publicado:
30 abril, 2026

Cita:
Arango-Palacio L, García-Pedrajas MD, Cañizares-Nolasco C, Peláez Jaramillo CA, Pinzón-Núñez AM. 2026. Biocontrol potential of the *Cryphonectria hypovirus* 1 (CHV1) against *Fusarium oxysporum* f. sp. *cubense* race 1. *Acorbat Revista de Tecnología y Ciencia* 3(1): 116 https://doi.org/10.62498/ARTC.26116

ABSTRACT

The incursion of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) in Latin America threatens global banana production, demanding innovative disease management tools. Mycoviruses have emerged as promising agents for sustainable fungal biocontrol. We assessed the hypovirulence effect of *Cryphonectria hypovirus 1* (CHV1) on *F. oxysporum* f. sp. *cubense* Race 1 (Foc R1) as a model. CHV1-infected strains showed reduced growth, altered pigmentation, and diminished virulence on 'Gros Michel' bananas compared to virus-free controls. This marks the first successful CHV1 introduction into Foc, highlighting mycoviruses as a powerful, eco-friendly approach with the potential to revolutionize the management of this devastating pathogen.

Keywords: *Fusarium oxysporum* f. sp. *cubense*, Mycovirus, Banana, CHV1, *Cryphonectria hypovirus* 1

RESUMEN

La incursión de *Fusarium oxysporum* f. sp. *cubense* Raza Tropical 4 (Foc R4T) en América Latina amenaza la producción mundial de banano, lo que exige herramientas innovadoras para el manejo de la enfermedad. Los micovirus han surgido como agentes prometedores para el biocontrol sostenible de hongos. Evaluamos el efecto de hipovirulencia de *Cryphonectria hypovirus* 1 (CHV1) sobre *F. oxysporum* f. sp. *cubense* Raza 1 (Foc R1) como modelo. Las cepas infectadas con CHV1 mostraron menor crecimiento, alteraciones en la pigmentación y reducida virulencia en bananos 'Gros Michel' frente a controles libres de virus. Este es el primer registro exitoso de CHV1 en Foc, destacando a los micovirus como una estrategia ecológica con potencial para revolucionar el manejo de este patógeno devastador.

Palabras clave: *Fusarium oxysporum* f. sp. *cubense*, Micovirus, Banano, CHV1, *Cryphonectria hypovirus* 1

Copyright: © 2026 por los autores. Licencia Acorbat Internacional. Este artículo es de acceso libre y distribuido bajo los términos de Acorbat Internacional

INTRODUCTION

Bananas are considered one of the most important and influential tropical fruits worldwide, due to their high consumption, contribution to the economies of producing, exporting, and consuming countries, and fundamental in food security (FAO, 2024). However, one of the principal limitations of the crop is Fusarium wilt (FWB) the most devastating disease of the banana industry worldwide, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*), which colonizes and obstructs the vascular bundles, preventing the absorption of water and nutrients, generating wilting and consequently the death of the plant (Dita *et al*., 2018). Viruses have been described with the capacity to infect phytopathogenic fungi, in which the viral infection has been associated with the alteration of phenotypic characters, the most relevant being the reduction of virulence (hypovirulence) (Nuss 2005). Hypovirulence was first successfully described in populations of *Cryphonectria parasitica*, the causal agent of chestnut blight (Choi and Nuss, 1992). *Chryphonectria hypovirus* 1 (CHV1) is a member of the virus family *Hypoviridae* and was the first mycovirus reported to cause hypovirulence in its host and is characterized by reduced growth, sexual and asexual reproductive ability, and virulence of the infected fungus (Choi and Nuss, 1992). According to Ye *et al*. (2023), disclosed the presence of 20 novel mycoviruses in the plant pathogenic fungus *Fusarium oxysporum* f. sp. *cubense*, using a metatranscriptome-based strategy. However, no data have been reported on the possible effects of these mitoviruses on the phenotype of their host. Recently, Lin and collaborators (2024) published the first introduction of a novel hypovirulence-associated Hadaka virus 1 in *Foc*. In this study, we showed that the transformation process by protoplasts allowed expand hypovirus host range and studying hypovirus-mediated alterations in new fungal hosts, in this case, to one of the fungal phytopathogenic species of greatest economic importance in the world. This will generate knowledge for developing new biotechnological strategies for use within integrated disease management.

MATHERIALS AND METHODS

Fungal Strains, Plasmid, and Plant Materials

Fungal strains were isolated from symptomatic banana plant tissue samples collected in the Urabá region of Antioquia, Colombia. All isolates were morphologically characterized and identified using PCR with specific primers for *Fusarium oxysporum* f. sp. *cubense* race 1 (*Foc* R1), as described by Li *et al.* (2013). Among all identified isolates, the one that exhibited the highest degree of severity was selected based on the results of pathogenicity tests conducted on "Gros Michel" banana

plants. This strain was then used to introduce the CHV1 mycovirus via protoplast transfection, using the plasmid pXH9, which contains a full-length cDNA copy of *Cryphonectria hypovirus* 1 (CHV1). Also, *F. verticillioides* (ID7856) and *F. solani* (ID0897) were included in the analysis as infected reference strains. All plants used for pathogenicity assays were grown in a climate-controlled plant growth room maintained at a temperature of 27 °C ± 2 °C.

Fungal Protoplast Isolation and Virus Transfection

Protoplasts of the *Foc* R1 strain were prepared by treating mycelia grown on PDB (Potato Dextrose Broth, Difco Laboratories, Detroit, MI, U.S.A.). The mycelia were inoculated at a concentration of 1 x 108 conidia/mL and incubated for 11-12 hours at 28 °C with shaking at 200 rpm. After incubation, the mycelia were filtered through a Miracloth membrane to collect fresh mycelium. Next, the collected mycelia were mixed with 20 mL of OM Buffer (1.2 M MgSO4 and 10 mM Na2HPO4) containing 50 mg of Driselase and 1 g of Extralyse (InterSpex Products). This mixture was incubated for 3 hours at 28 °C with shaking at 50 rpm. Once protoplast formation was observed, the mixture was filtered through a 40 µm nylon filter and washed with STC solution (10 mM Tris-HCl pH 7.5, 50 mM CaCl2, 0.8 M Sorbitol) to collect the protoplasts. The collected protoplasts were then centrifuged for 15 minutes at 3000 rpm at 4 °C. The resulting pellet was resuspended in 300-400 µL of STC solution. Protoplasts were counted at a 1:100 dilution and then centrifuged again for 5 minutes at 3000 rpm at 4 °C. Finally, the pellet was resuspended in an appropriate volume of STC to adjust the concentration to 2-3 x 108 protoplasts/mL. Protoplast transfection was carried out using a modified version of previously described methods (Choi and Nuss, 1992). Each transformed organism was then individually transferred to PDA containing 50 µL of hygromycin, followed by colony purification.

Detection of dsRNAs in *Foc* R1

Infection of fungal isolates with CHV1 was tested by performing a RT-PCR on denatured dsRNAs using specific primers CHV1F2 and CHV1R3 proposed by Shapira *et al*., (1991). Approximately 3.5 g of ground fungal tissue from each isolate was used to purify dsRNAs by chromatography on cellulose, following the methodology described by Valverde *et al*. (1990). Nucleic acids were electrophoretically fractionated on a 1.0% agarose gel.

Analysis of Fungal Vegetative Growth

To assess the growth rate, 10 µL of conidia suspensions containing 1×10^5 conidia/mL from each strain were inoculated into the center of PDA Petri dishes. These

dishes were then incubated in the dark at 26 °C for 7 days. The radial growth was measured daily to determine the growth rate. In another experiment, 10 µL of PDB was inoculated with 10 µL of each transformant strain's inoculum, which also had a concentration of 1×10^5 conidia/mL. This mixture was shaken at 200 rpm for 7 days at a temperature of 28 °C. After this incubation period, serial dilutions were performed at a ratio of 1:10, down to 1×10^{-2}. From the 10^{-2} dilution, 10 µL was taken to count the conidia using a Neubauer chamber, and the desired concentration was then adjusted accordingly. To evaluate pigmentation, 10 mL of PDB were inoculated with three mycelium discs from each transformant strain preserved in 60% glycerol. These were then shaken for 7 days at 200 rpm at a temperature of 28 °C.

Phytopathogenicity Assay

The virulence of infected and mycovirus-free strains towards "Gros Michel" banana seedlings (*Musa* spp.) was assessed in a greenhouse under controlled conditions. To conduct this experiment, agar discs of each transformant were placed onto PDA medium and incubated for 7 days at 28 °C. After incubation, 10 mL of ADE was added to each PDA Petri dish. Using a Digralsky loop, the mycelium was completely dissolved, and the resulting solution was poured into 300 g of previously hydrated rice that had been sterilized in an autoclave at 121 °C for 20 minutes. After the required colonization time, the rice was washed with ADE, filtered, and the resulting solution was collected in a beaker. A 1000 mL solution was ultimately prepared, achieving a 1×10^7 conidia/mL concentration. Following the preparation of the inoculant suspensions, 20 "Gros Michel" banana seedlings were taken for each strain, with two biological replicates consisting of 10 repetitions each. Each plant was inoculated via drenching with 100 mL of inoculum at the concentration of 1×10^7 conidia/mL. After four weeks, the first symptoms of the disease began to appear, and the severity was evaluated using the CORBANA diagrammatic scale.

RESULTS AND DISCUSSION

Effect of CHV1 on colony morphology, mycelial growth, and pigmentation

We successfully obtained fifteen hygromycin B-resistant transformants by transforming fungal protoplasts with the plasmid PXH9. The insertion of the CHV1 viral genome into a virus-free recipient *Foc* strain was confirmed by amplifying denatured dsRNAs using specific primers. Consequently, we identified ten distinct morphotypes, (data not shown), however, we selected four morphotypes that exhibited different levels of viral accumulation, to establish the assays (Figure 1A). The phenotypic changes observed in the virus-infected *Foc* strains (CHV1_*Foc*) were

significantly different compared to the virus-free strain (Figure 1B). Briefly, on the PDA plates, the average growth rates of the transformants were 5.1 mm/day, while those of the virus-free strains were 9 mm/day. On the other hand, the CHV1_*Foc*In morphotype refers to a transformant in which the CHV1 viral genome was not completely transferred, and it was used as a control. The recipient strains of *Foc* exhibited reduced growth rates and increased pigmentation compared to the virus-free strains (Figures 1B and D). When we compared the growth rates of conidia from freshly harvested serially diluted (10-2) samples, we observed a noticeable difference in growth rates between the virus-free strain and CHV1_*Foc*In (Figure 1C).

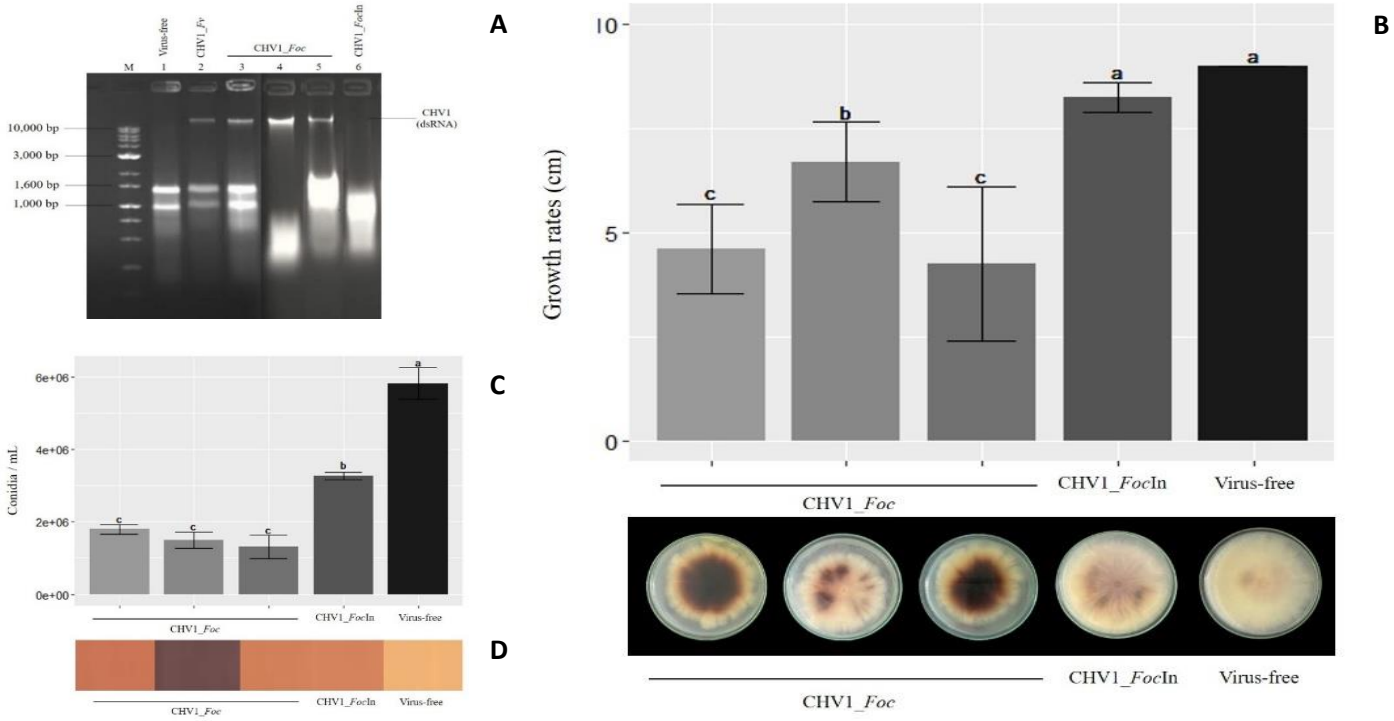


Figure 1. Effect of CHV1 on colony morphology, mycelial growth, and pigmentation in *Foc* A. Hygromycin B-resistant transformants were obtained by transforming the fungal protoplasts with the plasmid PXH9. RT-PCR analysis of dsRNA in fungal strains. Lane M, 1-kb ladder DNA size marker; Lane 1, virus-free strain; Lane 2, *Fusarium verticillioides* (reference strain) that is infected with CHV1; Lane 3, 4, and 5 virus-infected strains; Lane 6, virus not completely transferred B. Growth rates and colony morphology of *Foc* as affected by transmission of dsRNA C. CHV1 infection on conidia of *Foc* D. Changes in pigmentation of *Foc* colonies. Different letters denote significant between treatments according to *Kruskal-Wallis* non-parametric tests at 5% probability.

Hypovirulence of CHV1 on *Foc* R1

Assessing the pathogenicity of CHV1_*Foc* infected strains and virus-free strains on "Gros Michel" banana seedlings indicated that inoculation with virus-free strains induced larger lesions than virus-infected strains (Figure 2B). Notable differences were found in both internal and external symptoms compared to the virus-free strain (Figures 2A, and B). After 35 days post-inoculation (dpi), plants inoculated with the CHV1_*Foc* strain exhibited milder symptoms, compared to those inoculated with the virus. The latter displayed yellowing of the leaves and necrosis of the stem and rhizome, which are typical symptoms of the disease. The severity of wilt disease in leaves and bulbs was classified into fifteen and five grades, respectively, where grade 0 indicated no symptoms and fifteen and five grades indicated severe symptoms. This strongly indicates that CHV1 reduces the virulence of *Foc*.

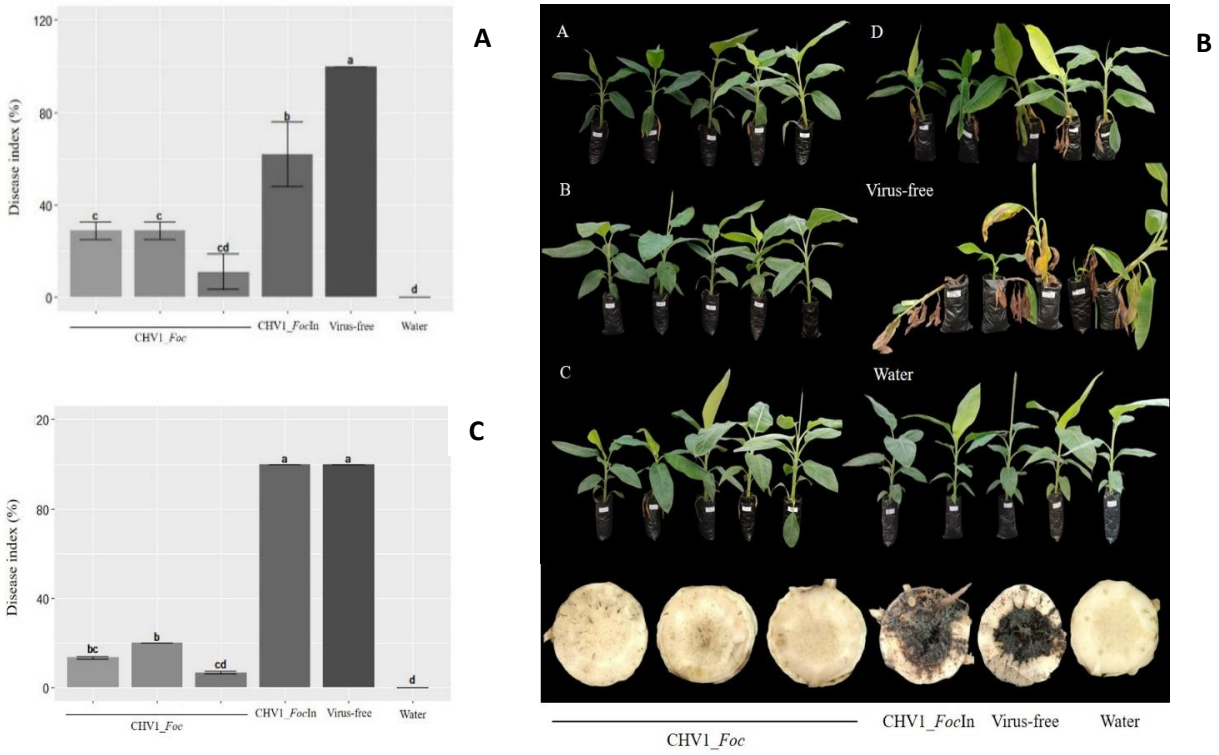


Figure 2. Pathogenicity assessment of the indicated strains in "Gros Michel" banana seedlings A. Index of external symptoms of Fusarium wilt based on disease severity grades B. Leaf and bulb wilt symptoms observed in susceptible banana plants inoculated with the CHV1_*Foc* and Virus-free strain for 35 days C. Index of internal symptoms of Fusarium wilt based on disease severity grades. Different letters denote significant between treatments according to *Kruskal-Wallis* non-parametric tests at 5% probability.

The success of biological control depends on integrating multiple factors. Among the factors influencing the success of biological treatment, we can mention in first place vegetative incompatibility which reduces the horizontal transmission, which is considered as the most important parameter in models of pathogen invasion. This biological control method is very effective when the population structure of the *vic* genes of the parasitic fungus is known, and the compatible hypovirulent strains are applied (Rigling *et al*., 1994).

Mycoviruses, ubiquitous in fungi, have become more readily discoverable with the advent of next-generation sequencing technology. However, the majority of mycoviruses establish latent infections, and only a small fraction exhibits noticeable symptoms, such as diminished virulence, slow growth rates, and impaired sporulation in their fungal hosts. Identifying hypovirulent properties in these mycoviruses is a time-consuming and labor-intensive process. In this study, we showed that the transformation process by protoplasts allowed expand hypovirus host range and studying hypovirus-mediated alterations in new fungal hosts, in this case, to one of the fungal phytopathogenic species of greatest economic importance in the world. This will generate knowledge for developing new biotechnological strategies for use within integrated disease management.

CONCLUSIONS

Strains of *F. oxysporum* f. sp. *cubense* Race 1 infected by *Chryphonectria hypovirus* 1 showed reduced growth, altered pigmentation, and diminished virulence on ‘Gros Michel’ bananas compared to virus-free controls. This marks the first successful CHV1 introduction into Foc, highlighting mycoviruses as a powerful, eco-friendly approach with the potential to revolutionize the management of this devastating pathogen.

REFERENCES

Dita M, Barquero M, Heck D, Mizubuti ESG, and amp; Staver CP. 2018. Fusarium Wilt of Banana: Current Knowledge on Epidemiology and Research Needs Toward Sustainable Disease Management. Frontiers in Plant Science, 9. https://doi.org/10.3389/fpls.2018.01468

Choi GH, Nuss DL. 1992. A viral gene confers hypovirulence-associated traits to the chestnut blight fungus. EMBO J 11, 473–477, https://doi:10.1002/j.1460-2075.1992.tb05077.x

FAO. 2024. Organización de las Naciones Unidas para la Alimentación y la Agricultura. Foro Mundial Bananero. Disponible en: https://www.fao.org/world-banana-forum/about-the-forum/fourth-conference/en/

Lin Y, Pan G, Qi Y, Wang B, Jin C, Fang W. 2024. A novel hypovirulence-associated Hadaka virus 1 (HadV1-LA6) in *Fusarium oxysporum* f. sp. *cubense*. mSphere 0:e00428-24. https://doi.org/10.1128/msphere.00428-24

Nuss, D. 2005. Hypovirulence: Mycoviruses at the fungal–plant interface. Nat Rev Microbiol 3, 632–642. https://doi.org/10.1038/nrmicro1206

Shapira R, Choi GH, amp; Nuss DL. 1991. Virus-like genetic organization and expression strategy for a double-stranded RNA genetic element associated with biological control of chestnut blight. The EMBO Journal, 10(4), 731–739. https://doi.org/10.1002/j.1460-2075.1991.tb08004.x

Rigling D, Heiniger U, Hohl HR. 1994. Reduction of laccase activity in dsRNA-containing hypovirulent strains of *Cryphonectria* (Endothia) parasitica. Phytopathology. 79:219–223. https://doi.org/10.1094/Phyto-79-219.

Valverde RA, Nameth ST, Jordan RL. 1990. Analysis of doublestranded—RNA for plant-virus diagnosis. Plant Disease 74, 255–258, https://doi.org/10.1094/PD-74-0255.

Ye L, Shi X, He Y, Chen J, Xu Q, Shafik K, Fu L, Yin Y, Kotta-Loizou I, Xu W. 2023. A novel botybirnavirus with a unique satellite dsRNA causes latent infection in *Didymella theifolia* isolated from tea plants. Microbiology Spectr 11:e00033-23. https://doi.org/10.1128/spectrum.00033-23