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The Publication Commission of the IOBC-WPRS:

Dr. Ute Koch
Schillerstrasse 13
D-69509 Moerlenbach (Germany)
Tel +49-6209-1079
e-mail: u.koch_moerlenbach@t-online.de

Dr. Annette Herz
Julius Kühn-Institute (JKI)
Federal Research Center for Cultivated Plants
Institute for Biological Control
Heinrichstr. 243
D-64287 Darmstadt (Germany)
Tel +49 6151 407-236, Fax +49 6151 407-290
e-mail: Annette.Herz@julius-kuehn.de

Address General Secretariat:

Dr. Gerben Messelink
Wageningen UR Greenhouse Horticulture
Violierenweg 1
P.O. Box 20
NL-2665 ZG Bleiswijk, The Netherlands
Tel.: +31 (0) 317-485649
e-mail: Gerben.Messelink@wur.nl

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IOBC Working Group Integrated Control in Protected Crops Temperate Climate

Convenor: Bruno Gobin, PCS Ornamental Plant Research, Belgium

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Hypovirulent *Cryphonectria parasitica* strains for biocontrol of chestnut blight in nurseries

Mirna Ćurković-Perica¹, Ljiljana Krstin², Zorana Katanić², Marin Ježić¹, Lucija Nuskern¹, Igor Poljak³, Marilena Idžojtić³

¹*University of Zagreb, Faculty of Science, Department of Biology, Marulićev trg 9a, 10000 Zagreb, Croatia;* ²*University of J. J. Strossmayer in Osijek, Department of Biology, Ulica cara Hadrijana 8/A, 31000 Osijek, Croatia;* ³*University of Zagreb, Faculty of Forestry, Department of Forest Genetics, Dendrology and Botany, Svetošimunska cesta 25, 10000 Zagreb, Croatia*

Abstract: *Cryphonectria parasitica* is an aggressive ascomycete responsible for chestnut blight disease of American and European sweet chestnut. It infects the host via air-borne spores which contact wounds in the bark. The fungus grows through and under the bark into the cambium, causing cankers that progressively enlarge, girdle and kill branches and trunks of infected seedlings and trees. Because of the widespread infection of all chestnut forests in America and Europe by this pathogen, traditional grafting methods became impossible, because every graft is infected by the fungus. Grafting today is performed in specialized nurseries and greenhouses to minimize the possibility of infection. Fungicides are used as preventive treatments, and surfaces disinfected before grafting. In an attempt to preserve highly valuable chestnut genotypes, scions from plantations several hundred years old were grafted in a nursery. In such cases fungicide treatments and surface disinfection are often ineffective because *C. parasitica* might already be present inside the scions.

An alternative approach involves treatment with *Cryphonectria hypovirus 1* (CHV1)-infected, hypovirulent *C. parasitica* strains. CHV1 is unencapsidated double-stranded RNA virus of the family *Hypoviridae* that causes persistent infection of its fungal host. It reduces fungal growth, virulence, sexual and asexual reproductive ability by deregulating a lot of host genes. The virus is transferred from infected, hypovirulent *C. parasitica* strains to virulent ones by hyphal anastomosis. It is also transferred by conidia. CHV1-infected *C. parasitica* strains enable healing of cankers and recovery of chestnut seedlings and trees.

In this research twelve hypovirulent *C. parasitica* strains were tested for their biocontrol potential against chestnut blight on three chestnut genotypes in controlled greenhouse conditions. Each treatment was performed in three replicates. Four virulent *C. parasitica* strains belonging to different vegetative compatibility (vc) types were transfected with three virus isolates that belong to two distinct subtypes of CHV1: Italian subtype I and French subtype F1. The French virus isolate was chosen for this experiment as it was believed to have a stronger effect on the pathogenic fungus than isolates of the Italian subtype.

After measuring the canker area on chestnut stems four weeks after inoculation of hypovirulent *C. parasitica* strains, results revealed that the debilitating effect of one of the Italian subtype CHV1 isolates was comparable to that observed for the French isolate, while the other Italian subtype isolate had a weaker effect on the majority of fungal strains tested. However, the same virus/fungus combinations did not have equal efficacy in different chestnut genotypes. Therefore chestnut susceptibility and recovery are influenced by the response of chestnut genotypes to particular virulent and hypovirulent *C. parasitica* strains, respectively, and chestnut-fungus-virus interactions affect the success of this biocontrol strategy.

For successful biocontrol of this disease in general and especially on grafted seedlings in nurseries, the biocontrol agent, i.e. hypovirulent *C. parasitica* strain(s) harboring chosen CHV1 isolates, should be applied taking into account not only the fungal strain(s) that caused the infection, but also chestnut genotype. Although *C. parasitica* is the only known natural host of CHV1, transfection of several pathogenic fungi with CHV1 dsRNA, reduced the virulence of the recipient mycelia as well, emphasizing the potential of this virus in biocontrol of other phytopathogenic fungi. Furthermore, other hypovirulence inducing mycoviruses have been documented in several plant pathogen systems making the application of virus-mediated biocontrol increasingly more feasible, especially in protected crops.

Key words: chestnut blight, virus-mediated biocontrol, genotype susceptibility, grafting