Wolbachia Infection in the Walnut-Husk Fly *Rhagoletis completa* Cresson 1929 (Diptera: Tephritidae)

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Zusammenfassung Wir berichten über eine *Wolbachia*-Infektion in einer österreichischen Population der Walnussfruchtfliege *Rhagoletis completa*. Erste Untersuchungen mit konventioneller PCR zeigten keine sichtbaren Banden. In Folge wurden zwei Techniken zum Nachweis von Niedrigtiter-Infektionen - Southern Blot und nested-PCR – angewandt, mit denen *Wolbachia* gefunden werden konnte. Die Sequenzierung von Klonen des *wsp* Amplikons zeigte die Präsenz von sehr unterschiedlichen *Wolbachia* Stämmen in der Walnussfruchtfliege.

Key Words: *Rhagoletis completa*, *Wolbachia*, invasive species

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Introduction

The Walnut Husk Fly, *Rhagoletis completa* (Diptera: Tephritidae), is a pest in *Juglans* spp.. Native to Midwestern United States, it was introduced to Europe in the early 1990s (Merz 1991). Since then the spread of this species was quite rapid and today *R. completa* has been described in several countries in Central Europe (Aluja & al. 2011). Unlike other *Rhagoletis* species, *R. completa* oviposits a batch of eggs into the walnut mesocarp. Larvae feed in the walnut husk tissue causing shell staining and darkened stones (Boyce 1934).

Wolbachia is an endosymbiotic bacterium present in up to 65% of insects. It manipulates the host's reproduction by causing male-killing, parthenogenesis, feminization and cytoplasmic incompatibility (CI) (Werren & al. 2008).

Several *Rhagoletis*-species like *R. cerasi* (Riegler & Stauffer 2002, Arthofer & al. 2009b), *R. cingulata* (Schuler & al. 2009) and *R. pomonella* (Schuler & al. 2011) are described to be infected by *Wolbachia*. *Wolbachia*-infections in low-titer, not detectable by conventional PCRs, are supposed to be more prevalent than assumed so far (Arthofer & al. 2009a,b, Hughes & al. 2011). Although *R. completa* is considered to be *Wolbachia* free (Drosopoulou & al. 2010), this study was designed to detect *Wolbachia* in low-titer using two independent techniques: Southern blot and nested PCR.

Materials & Methods

In 2008, larvae of *R. completa* were collected in the Institute's garden in Vienna and stored in ethanol at -20 °C. DNA was extracted and PCR using the general *wsp* primers for *Wolbachia* detection (Braig & al. 1998) was applied. Subsequently a Southern hybridization of the PCR amplicons (Arthofer & al. 2009b) and a nested PCR with internal *wspif* and *wspir* primers (Arthofer & al. 2009a) were performed. An aliquot of the nested PCR amplicon was ligated into a pTZ57R/T vector (Fermentas) and transformed into competent JM109 *Escherichia coli* cells. Twelve white colonies were picked and Sanger sequencing was performed using M13 primers.

Results & Discussion

PCR with general *wsp* primers resulted in no visible amplicons on the agarose gel. Both Southern hybridization and nested PCR revealed positive *Wolbachia* bands (Fig. 1), indicating that *R. completa* is infected with *Wolbachia*. Fourteen plasmid sequences from a total of three individuals revealed four different sequence types. In a BLAST search and a phylogenetic analysis (data not shown), one sequence grouped in the *w*Mel clade together with *w*Cer1 and *w*Cer2 of *R. cerasi*, and a second sequence is identical to that of the B group strain *w*Cer5 of *R. cerasi*. The other two sequences had no exact matches in Genbank. The short 317bp *wsp* amplicon hinders a more detailed strain characterization.

Low-titer *Wolbachia* are difficult to characterize genetically and phenotypically and are considered to not induce reproductive phenotypes like CI (Ikeda & al. 2003). Phenotypic effects of such cryptic strains are not known in *R. completa*, and a study including more individuals from different European and American populations would be necessary to obtain deeper knowledge on the genetic characteristics and the phenotype of the diverse strains.

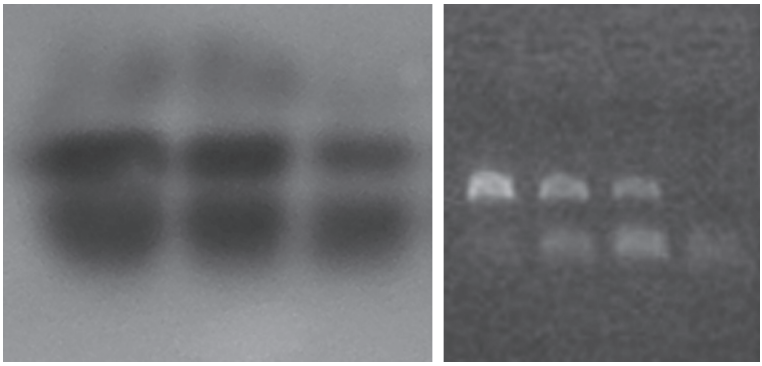

Fig. 1: Three individuals of *R. completa* showed positive bands after Southern hybridization (left) and after nested PCR (right).

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