

Oliveira-Garcia et al. (2022)

Supplementary Materials:

Table S1. PCR primers used in this study.

Nourse1pNR1-fw	5'-TGACCGGTGCCTGGATCTTCCTATAGAATC-3'
Nourse2pNR1-rv	5'-GGTCGGCATCTACTCTATTCCCTTGCCCTC-3'
CgPrho4-fw	5'-GCCAGTGGAATTCTGATTGC-3'
CgPrho4_5'-flank-rv	5'-GAATGCGGCTCTAGAGCGGCGGCAGACTCTAAGTGGTGTG-3'
CgTrho4_3'-flank-fw	5'-CTTAATGATGATTACTAACAGATATCAAGCTTAACCTCAGTCACCGCTCCTC-3'
CgTrho4-rv	5'-ACCCAATGCTGCTACAGGAG-3'
CgPrho4nest-fw	5'-GATGGAAGCGTTCCTTCTTG-3'
CgTrho4nest-rv	5'-CCCGAGTGTCTAGGTATGGTACG-3'
CgRho4probe-fw	5'-TTTACACTTGCCCTCCTTGC-3'
CgRho4probe-rv	5'-CGGCAGACTCTAAGTGGTGTG-3'
CgRho4-RT-PCR-fw	5'-GGCGACGGTGGTTGCG-3'
CgRho4-RT-PCR-rv	5'-CCAGACGGCGGGTGTGT-3'
Cg_ITS2-F1.1	5'-CGTCGTAGGCCCTTAAAGGTAG-3'
Cg_ITS2-R1	5'-TTACGGCAAGAGTCCCTC-3'

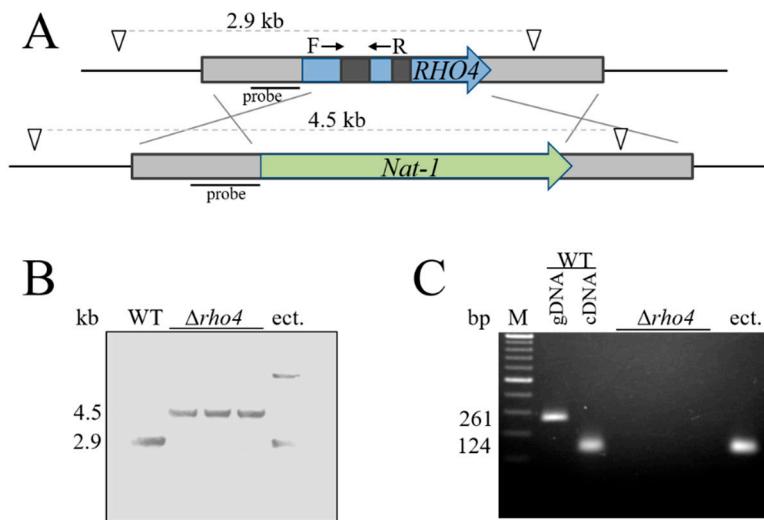


Figure S1. Deletion of *RHO4* of *C. graminicola* and identification of transformants with homologous and ectopic integration of the deletion cassette. **(A)** Deletion strategy of *RHO4* of *C. graminicola*. The gene *RHO4* is given in blue, introns are given in dark grey, 5'- and 3'-flanking regions in light grey. *Nat-1*, nourseothricin N-acetyl transferase gene. White arrowheads indicate *XhoI* restriction sites, black arrows labelled with F and R indicate positions of forward and reverse PCR primers. Black line below bars indicates position of the probe used in Southern blot analysis. **(B)** Southern blot analysis of the WT strain, the $\Delta\rho4$ strains, and ectopic strains. **(C)** RT-PCR confirming the presence of *RHO4* transcripts in the WT strain and the ectopic strain, as well as the absence of *RHO4* transcripts in three randomly chosen $\Delta\rho4$ strains. cDNA, complementary DNA; $\Delta\rho4$, deletion mutants; ect., ectopic strain; gDNA, genomic DNA; M, DNA size standard; WT, wildtype strain.

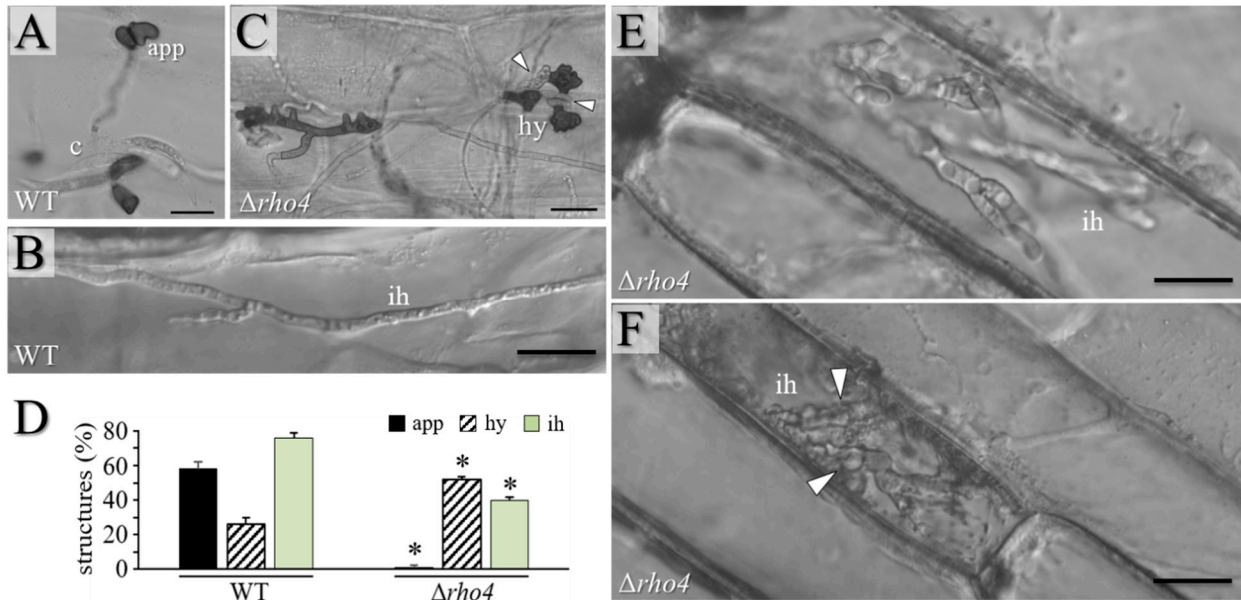


Figure. S2. $\Delta\rho\text{ho}4$ mutants differentiate defective infection structures on the alternative host, *Allium cepa* cv. Shakespeare. **(A)** Conidia (c) of the *C. graminicola* WT strain germinate and differentiate in appressoria (app) and hyphopodia (D) on onion epidermal cells. **(B)** WT infection hypha (ih) formed after invading the onion epidermis. **(C)** The $\Delta\rho\text{ho}4$ mutant differentiates hyphopodia (hy), which show lateral germination (arrowheads). **(D)** Appressorium, hyphopodium, and infection hypha differentiation by WT and $\Delta\rho\text{ho}4$ strains. Error bars are standard deviations; asterisk indicates statistically significant differences of corresponding structures (T-test, $P \leq 0.05$). **(E and F)** In planta differentiated infection hyphae (ih) of the $\Delta\rho\text{ho}4$ strain. $\Delta\rho\text{ho}4$ infection hyphae densely colonize the host cell, with swellings indicative of cell wall defects (ih, arrowheads). Microscopy was done at 72 hpi. Size bars in (A – C, E and F) are 20 μm .