

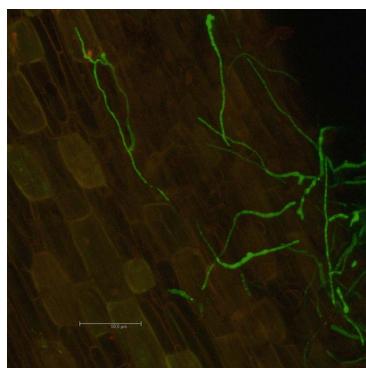
Time course of early and long-term colonization events of olive (*Olea europaea* L.) roots by *Trichoderma harzianum*

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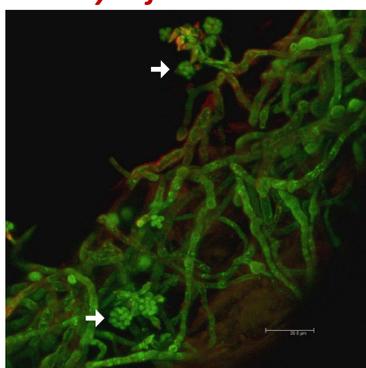
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Trichoderma harzianum CECT2413 has been demonstrated to be an effective biocontrol agent (BCA) of Verticillium wilt (*Verticillium dahliae* Kleb.) of olive (VWO) under control conditions. Previous works also showed *in vitro* mycoparasitism of *V. dahliae* by strain CECT2413 and its ability to promote olive growth and external/internal colonization in tobacco plants. Bioformulations of this BCA, alone or in combination with other *Trichoderma* spp. strains, have been recently patented and licensed. The present study aimed to evaluate the colonization capability of olive roots (var. Picual) by CECT2413 during a prolonged period of time (>100 days). A gfp-labelled derivative of CECT2413 and confocal laser scanning microscopy (CLSM) were used. CLSM imagery showed that CECT2413-gfp profusely colonized olive roots during the first three days after inoculation (DAI). Mycelium and abundant asexual reproductive structures (conidiophore-like) were clearly visible over the root surface. From this time point, fluorescent fungal structures resembling chlamydo spores were observed, while a progressive loss of mycelium became apparent. The majority of CECT2413-gfp biomass during this period (from 7-14 DAI) corresponded to conidia and chlamydo spores found over the epidermis and root hairs. Colonization preference of specific root sites was not evident and conidia, chlamydo spores and mycelium were found all over the examined roots. Long-term observations revealed the presence of chlamydo spores and conidia on the rhizoplane but lack of mycelium.

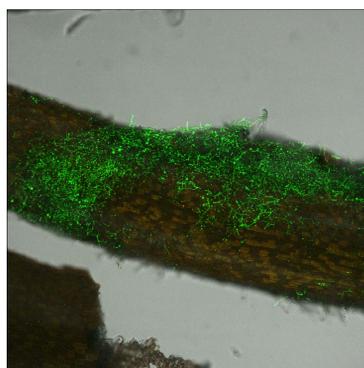
1-3 Days after inoculation



Conidia germinated and mycelium

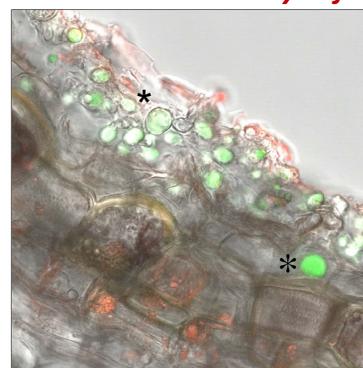


Conidiophores (white arrows) and mycelium

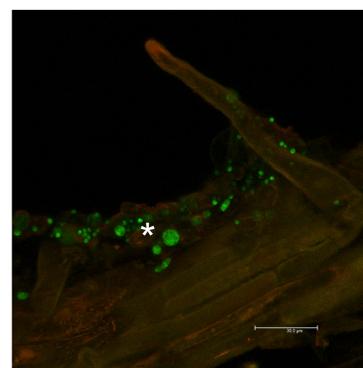


Mycelium layer

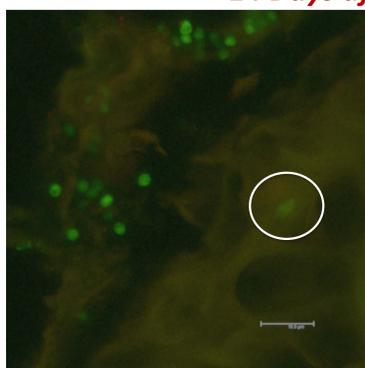
7 Days after inoculation



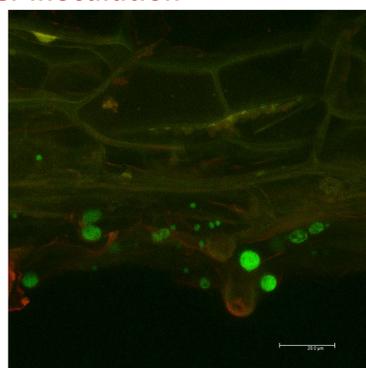
Chlamydo spores (black/white asterisks)



14 Days after inoculation

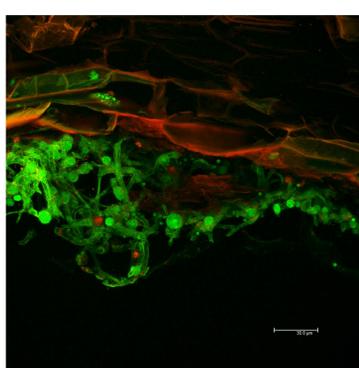


Occasional observation of mycelium in the mesodermis

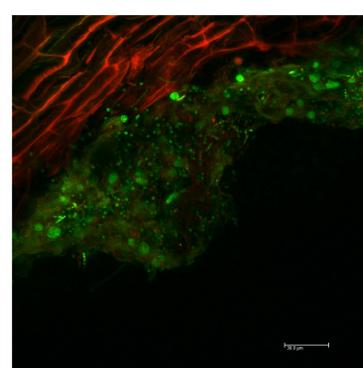
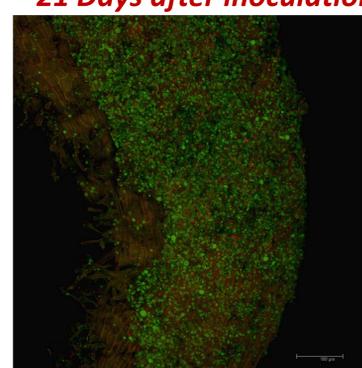


Conidia and chlamydo spores surrounding root hairs

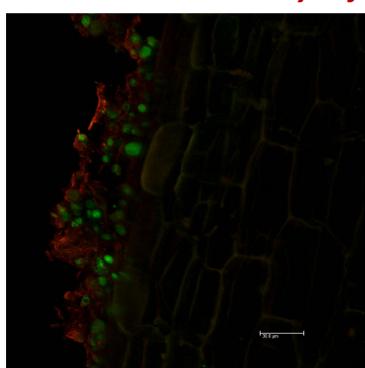
21 Days after inoculation



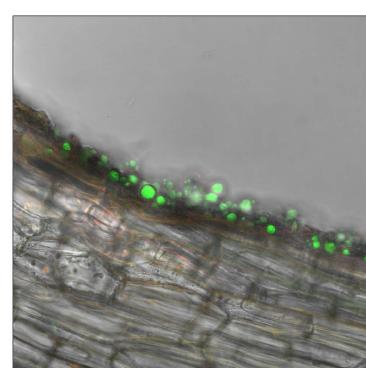
Mycelium, chlamydo spores and conidia in the rhizoplane



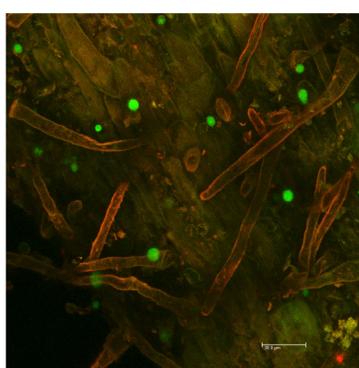
29 Days after inoculation



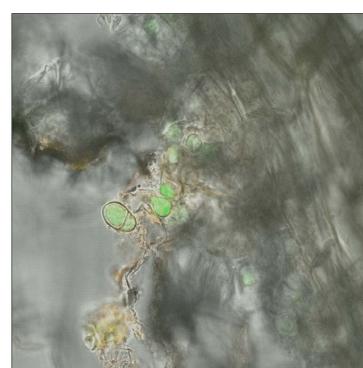
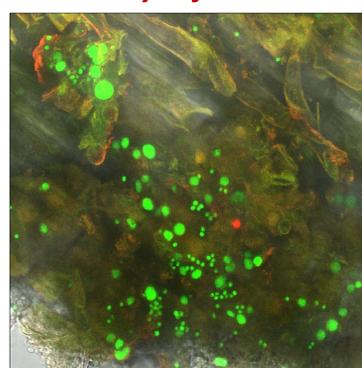
Prevalence of chlamydo spores and conidia



90-109 Days after inoculation



Chlamydo spores and conidia in the rhizoplane without mycelium



CONCLUSION

Strain CECT2413 is an excellent colonizer of olive roots which helps to understand its effectiveness as an efficient BCA against VWO

Supported by ERDF-cofinanced grants from the Ministry of Economy and Competitiveness (MINECO, project BIO2012-33904) and the Junta de Andalucía (project AGR-6038) and NUTESCA SL. (Jaén, Spain)]