Botryosphaeria spp. isolated from olive (*Olea europaea*) sampled for detection of *Verticillium dahliae*

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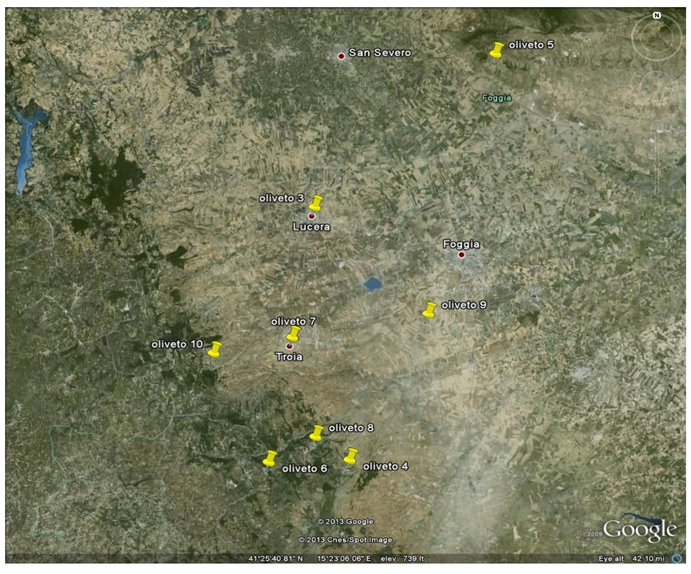
Introduction

Verticillium dahliae is a major cause of olive wilt in many European regions where crops susceptible to *V. dahliae,* particularly cotton, have been grown before. To improve the detection and control of *V. dahliae* in soils and in plants from these soils throughout a number of European countries, a three year EU funded project, VERTIGEEN (www.vertigeen.eu), was started recently.
Development of an optimal sampling strategy and efficient sample processing methods for innovative PCR-based detection of *Verticillium dahliae* (Vd) in diseased olive trees were important aspects of this project(see posters by Hiemstra *et al.* and Keykha Saber *et al.*).
Therefore samples were collected from diseased olive trees in different Mediterranean countries and examined for presence of *V. dahliae*.

Samples from Foggia (Italy)



One group of samples was collected from diseased old olive trees at different locations around the city of Foggia in Italy (Figure 1). These trees were suspected to suffer from Verticillium wilt because of the symptoms of dried leaves and shoot dieback (apoplexy syndrome (1)).

Figure 1. Olive samples were collected from different locations around the city of Foggia in Italy

Molecular detection and identification

However, when tested with a Vd-specific SyberGreen Q-PCR only very few samples were positive for Vd (Table 1 and Figure 2).
This result was supported by a standard agar-plating assay. Almost no growth of Vd was observed, whereas several other fungi were isolated frequently. Sequence analysis of the ITS regions of the most commonly isolated types (BLAST search) resulted in the most probable identity of these fungi being *Aspergillus niger, Fusarium* spp. and several *Botryosphaeria spp*. with *B. obtusa* and *B. stevensii* being most frequent (Table 2).

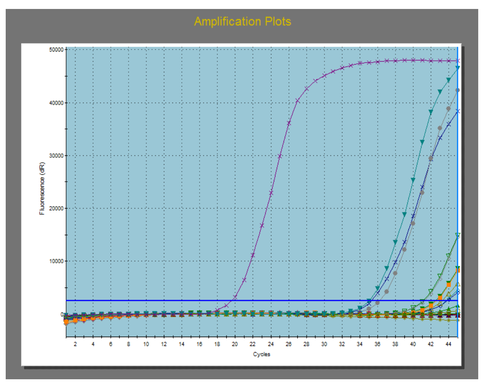


Figure 2. Results of *Verticillium dahliae* Q-PCR testing on samples from Foggia

Tree	Cultivar	Location	*V.Dahliae* %
oliveto 1	Coratina	Troia	12.5
oliveto 2	Coratina	Troia	25
oliveto 3	Leccina	Lucera	0
oliveto 4	Frantoiana	Deliceto	37.5
oliveto 5	Coratina	Rignano Gerganico	37.5
oliveto 6	Leccine	Panni	37.5
oliveto 7	Coratina	Troia	12.5
oliveto 8	Peranzane	Bovino	28.6
oliveto 9	Leccine	Segezia	25
oliveto 10	Peranzane	Castelluccio Valmaggiore	0

Table 1. PCR results (percentage positive) for detection of *Verticilium dahliae* on olive samples at different locations around the city of Foggia in Italy (8 samples analysed per tree)

Several species of the *Botryosphaeriaceae* are known as common pathogens causing fruit rots or dieback in many woody plants (2) and *B. dothidea* and *B. obtusa* have been reported causing fruit rot and branch canker or dieback in olive (3, 4).

Table 2. Identity of *Botryosphaeria spp.* on olive samples from Foggia, based on ITS sequence analysis

Tree	Identity (BLAST search analysis)
oliveto 2	*Diplodia seriata; teleomorph: Botryosphaeria obtusa*
oliveto 2	*Diplodia mutila; teleomorph: Botryosphaeria stevensii*
oliveto 3	*Diplodia seriata; teleomorph: Botryosphaeria obtusa*
oliveto 3	*Diplodia mutila; teleomorph: Botryosphaeria stevensii*
oliveto 4	*Botryosphaeria dothidea*
oliveto 4	*Diplodia seriata; teleomorph: Botryosphaeria obtusa*
oliveto 5	*Diplodia seriata; teleomorph: Botryosphaeria obtusa*
oliveto 7	*Diplodia mutila; teleomorph: Botryosphaeria stevensii*
oliveto 8	*Diplodia spp. (Tiarosporella spp.)*
oliveto 8	*Diplodia spp. (Tiarosporella spp.)*
oliveto 9	*Botryosphaeria sarmentorum*
oliveto 9	*Diplodia seriata; teleomorph: Botryosphaeria obtusa*
oliveto 9	*Diplodia spp. (Tiarosporella spp.)*
oliveto 10	*Diplodia mutila; teleomorph: Botryosphaeria stevensii*
oliveto 10	*Diplodia spp.*

Therefore we used generic primers for *Botryosphaeria* species (5) to test all samples once again. This resulted in 66 per cent of the branches from the above mentioned Italian samples being positive for *Botryosphaeria* (Figure 3) in contrast to the samples from other countries that all tested negative.

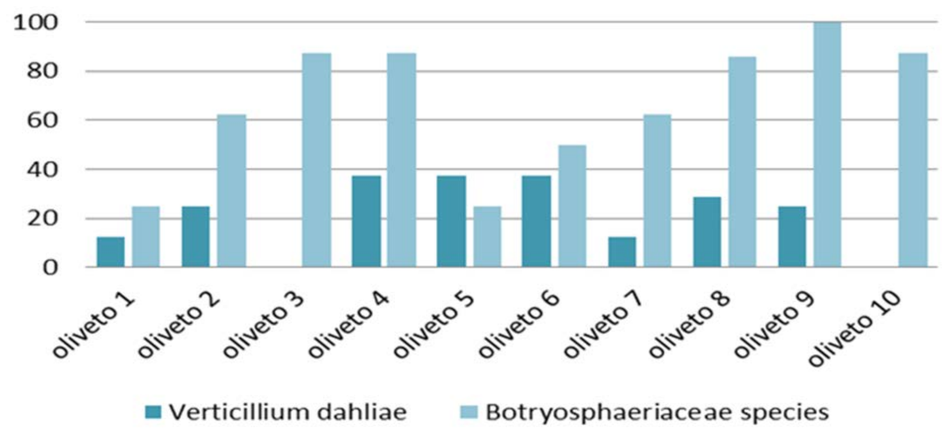


Figure 3. Comparison of PCR results (percentage positive) for detection of *Verticilium dahliae* and *Botryosphaeria spp*. on olive samples at different locations around the city of Foggia in Italy (8 samples analysed per tree)

Conclusion

These results indicate that at least locally fungi other *than V. dahliae* may contribute to dieback and death of olive branches which should be taken into account when assessing *Verticillium* wilt incidence. A specific on site test for *V. dahliae* would help to discriminate infection by these fungi from infection by *V. dahliae* as the symptoms might be misleading.

References:
1. Jiménez-Díaz R.M., Cirulli M., Bubici G., del MarJiménez-Díaz M., Antoniou P.P. & Tjamos E.C., 2012. Verticillium wilt, a major threat to olive production: current status and future prospects for its management. Plant Disease 96(3): 304-329.
2. Moral J., Luque F. & Trapero A. 2008a. First Report of *Diplodia seriata*, the Anamorph of "*Botryosphaeria obtusa*, Causing Fruit Rot of Olive in Spain. Plant Dis. 92: 311.
3. Moral J., Muñoz-díez, C., González N., Trapero A. & Michailides T. J. 2008b. Characterization and pathogenicity of *Botryosphaeriaceae* species collected from olive and other hosts in Spain and California. Phytopathology 100: 1340-51.
4. Sinclair W. A., Lyon H. H. & Johnson W. T. 1987. Diseases of trees and shrubs. Cornell University Press.
5. Spagnolo A., Marchi G., Peduto F., Phillips A. J. L. & Surico G. 2011. Detection of *Botryosphaeriaceae* species within grapevine woody tissues by nested PCR, with particular emphasis on the *Neofusicoccum parvum / N. ribis* complex. Eur. J. Plant Pathol. 129: 485-500, 2011.

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