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Correspondence:
dario.gaudioso@unifi.it
stefania.tegli@unifi.it

Volatile Organic Compounds as Diagnostic Biomarkers for Seed-Borne Pathogens: a Sustainable Approach to Legume Crop Health Management

D. Gaudioso[1], L. Calamai[1], C. Pastacaldi[1], C. Beltrami[1], L. Boatto[1], S. Tegli[1]

[1]Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Molecular Plant Pathology Lab, Via della Lastruccia, 10, IT-59100 Sesto Fiorentino, Firenze, Italy.

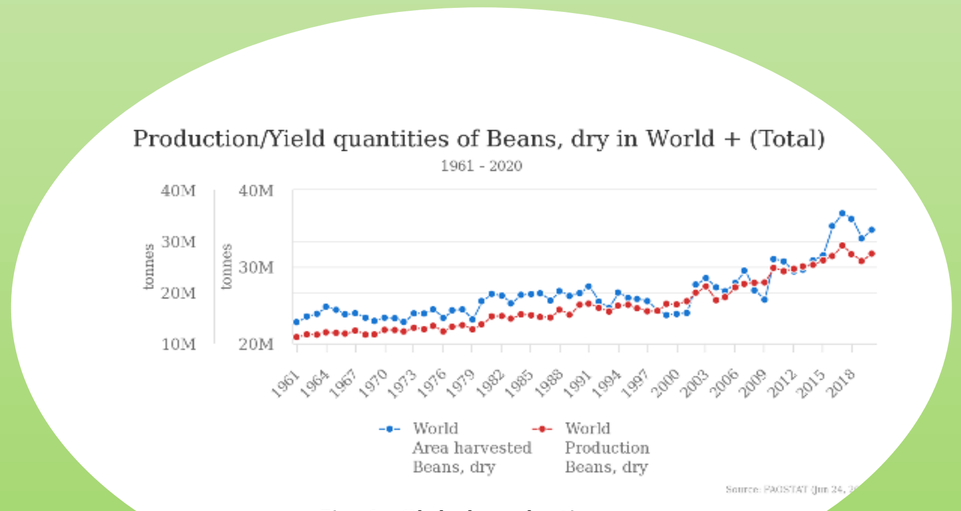

***Fig. 1* - Global production of bean (FAOSTAT, 2022)**

Introduction

Leguminous crops represent a cornerstone of global agriculture due to their high protein content, nitrogen-fixing ability, and essential role in sustainable food systems. As demand for legumes continues to grow worldwide (*Fig. 1*), so does the risk associated with the dissemination of seed-borne pathogens, notably *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*), a Gram-positive bacterium responsible for bacterial wilt of common beans (*Fig. 2*). Beyond their nutritional value as an affordable, high-quality protein source, legumes contribute to biodiversity conservation and provide vital ecosystem services. Their role is especially significant in developing countries, where they can supply over 60% of daily protein intake. Additionally, their gluten-free nature and capacity to enhance soil fertility make them a key component of both sustainable agriculture and human nutrition. However, the increasing scale of legume cultivation and global trade has amplified the risk of pathogen spread. In particular, the latent infections and xylem-limited colonization characteristic of *Cff* hinder early detection, underscoring the urgent need for innovative and reliable diagnostic tools.

***Fig. 2* – *Cff* symptoms on Cannellino bean leaves**

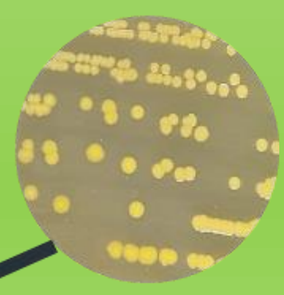

***Fig. 3* – Bean seeds infected by differently pigmented *Cff* strains**

Materials and Methods

Here an *in vitro* model based on the use of legume flour media was developed, hereafter defined as "naturalized media". VOCs production was estimated both *in vitro,* on synthetic and naturalized media, as well as on *Cff* infected bean seeds (*Fig. 3*).
HS-SPME-GC-MS analyses were performed in vials containing 3 mL of culture medium at 48, 72 h post-inoculation with several representative *Cff* strains**,** by using an Agilent 5977 mass spectrometer and Qualitative Analysis B.06.00 and MS Quantitative Analysis software for data analysis.

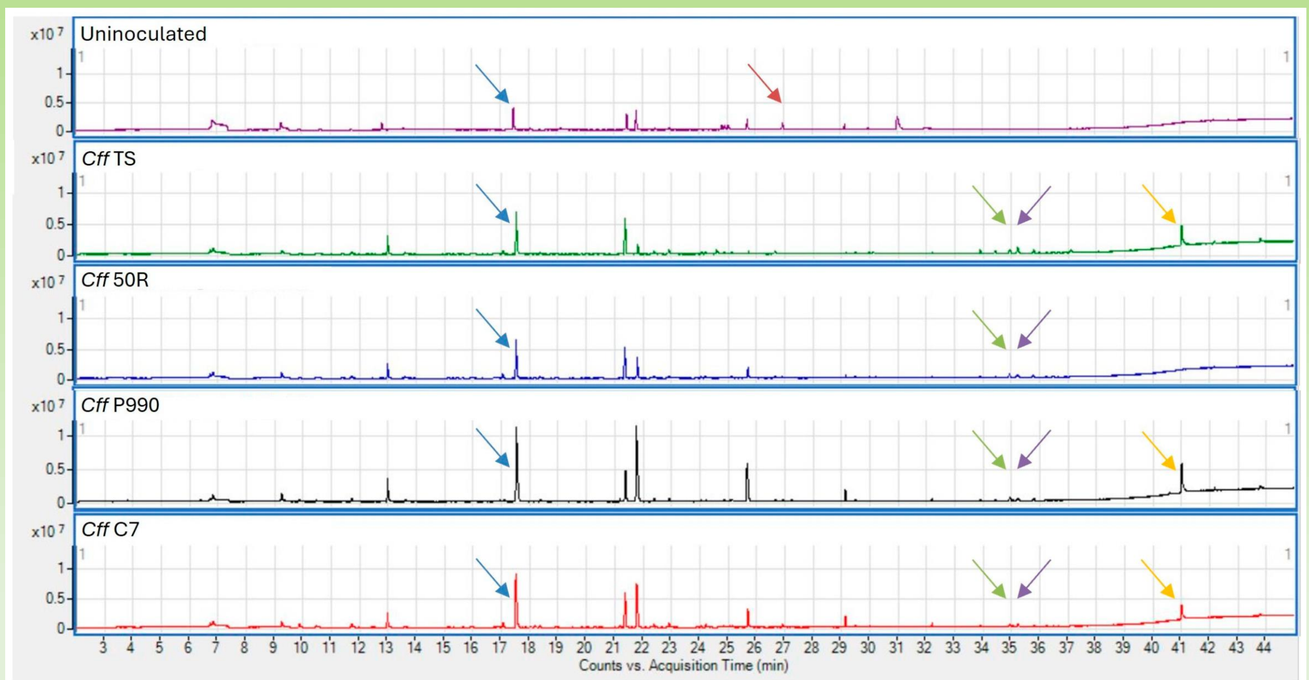

***Fig. 4* – Chromatograms representing the VOCs emitted in the headspace of the vials containing a Cannellino naturalized medium uninoculated or inoculated with *Cff* TS, *Cff* 50R, *Cff* P990, *Cff* C7. Arrows point to the most significant VOCs, in color code mode: (blue) 2-methyl-1-butanol + 3-methyl-1-butanol; (red) benzaldehyde; (green) phenylmethanol; (violet) 6,10-dimethyl-5,9-undecadien-2-one; (orange) 2-methoxy-4-vinylphenol.**
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Results

Among the volatile organic compounds (VOCs) identified in this study, benzyl alcohol (*Fig. 4-5*) emerged as a particularly promising candidate for use as a diagnostic marker. Furthermore, strain-specific compounds were detected in the *Cff* isolates tested, which may hold significant epidemiological value for tracking the distribution, spread, and evolution of this quarantine plant pathogen. Subsequent *in vivo* validation using artificially *Cff*-infected Cannellino beans confirmed the diagnostic potential of phenylmethanol and 2-methoxy-4-vinylphenol. These compounds were able to reliably distinguish *Cff* from other bacterial pathogens such as *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*) and *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) (*Fig. 5*).

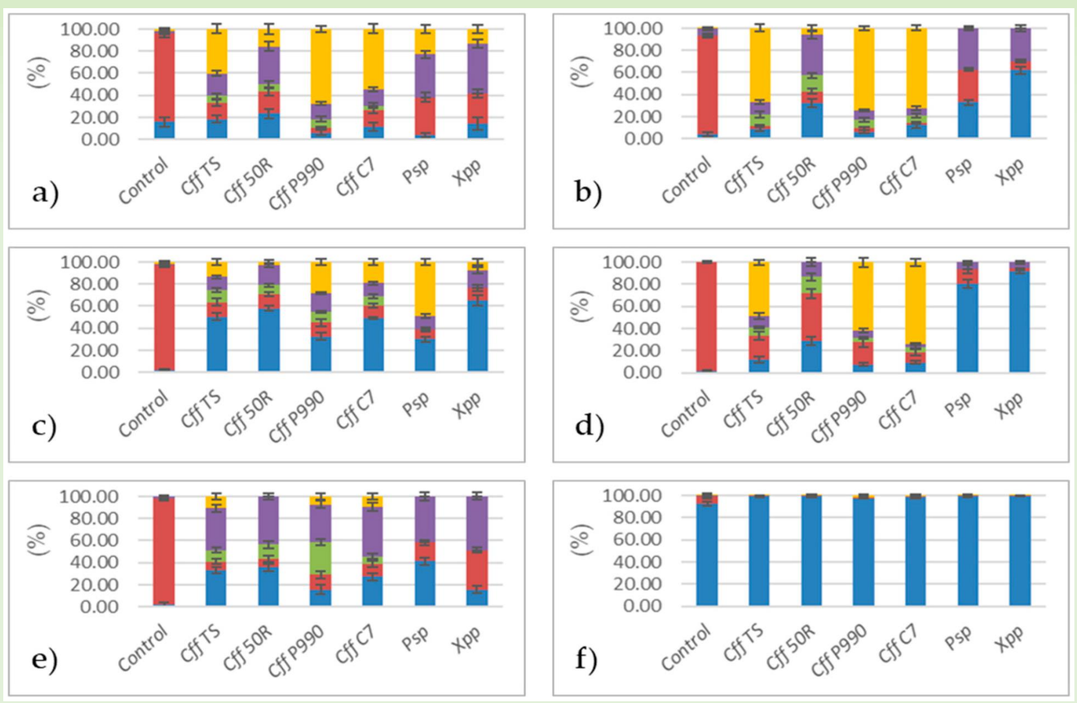

***Fig. 5* – Percentage of single VOC out of the total sum of the most abundant VOCs produced in vitro by *Cff* strains (TS, 50R, P990, C7), *Psp* and *Xpp*, on naturalized media made with flours of (a) *G. max*; (b) *V. unguiculata*; (c) *P. sativum*; (d) *P. coccineus*; (e) *L. culinaris*; (f) *T. durum*. Compounds: (blue) 2-methyl-1-butanol + 3-methyl-1-butanol; (red) benzaldehyde; (green) phenylmethanol; (violet) 6,10-dimethyl-5,9-undecadien-2-one; (orange) 2-methoxy-4-vinylphenol. Values are the mean of nine replicates ± percentage uncertainty.**

Conclusions

Accordingly, VOC fingerprinting emerges as a promising tool for the rapid and reliable screening of asymptomatic seeds, offering a sustainable approach to seed health management. Given the increasing globalization of seed trade, improving phytosanitary control is mandatory to prevent the introduction of seed-borne pathogens into new regions. The development of portable VOC-detection technologies could enhance pathogen surveillance at critical points in the seed trade chain, mitigating risks of disease outbreaks and supporting sustainable legume production in the face of growing global challenges.

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