

# FAST AND RELIABLE QUANTIFICATION OF *VERTICILLIUM DAHLIAE* MICROSCLEROTIA IN SOIL

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## OLD AND NEW TECHNIQUES

Knowledge of the degree of *Verticillium dahliae* contamination in their fields is important for growers. Wet-sieving of soil samples, followed by plating and microscopic analysis (WSP), is still the most commonly used technique to quantify *V. dahliae* microsclerotia in soil. However, the method is restricted to small soil samples, does not allow easy differentiation between *Verticillium* species, and takes at least 4 weeks to complete. To counteract some of these disadvantages, two alternative techniques were developed: the density flotation (DF) method and a new semi-automated zonal centrifugation (ZC) method (Figure 1).

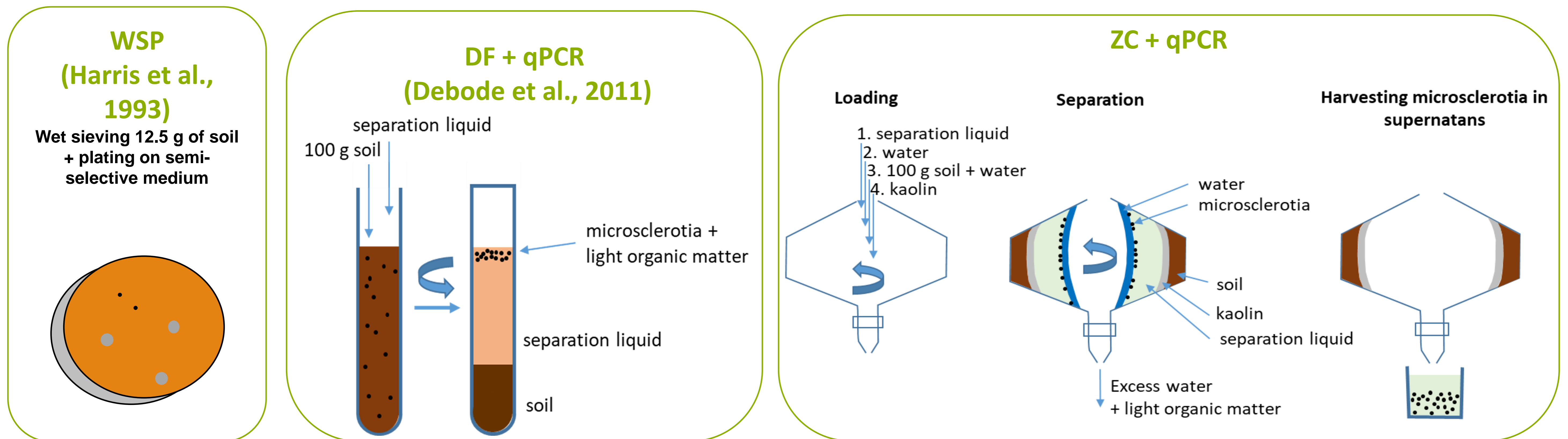


Figure 1. Three techniques to quantify *Verticillium dahliae* microsclerotia in soil

## OPTIMIZATION OF THE ZC METHOD & COMPARISON OF THE 3 TECHNIQUES

In the current study, we tested and optimized the ZC method (Figure 1), allowing a less labour-intensive analysis of a larger number of samples in a single day. Major steps are: the automatic stepwise addition of materials to the centrifuge rotor (Figure 1), recovery of the microsclerotia from the suspension onto a 20 micron filter, and identification and quantification of the microsclerotia using DNA extraction and qPCR. To optimize the ZC method, three separation liquids were tested at various concentrations (Figure 2, left). Subsequently, the ability of the ZC to extract *V. dahliae* microsclerotia from soil was compared with DF using serial dilutions (1:1, 1:5, 1:25, 1:125) of naturally-infested soil (Figure 2, middle). Finally, WSP was compared with DF results by analysing naturally contaminated soil samples with both techniques (Figure 2, right). The highest recovery with the ZC method was obtained using 40% CaCl<sub>2</sub> as separation liquid. DF and ZC results were well correlated and at similar levels. WSP results were correlated with DF results ( $r = 0.92$ ) but yielded different numbers of microsclerotia.

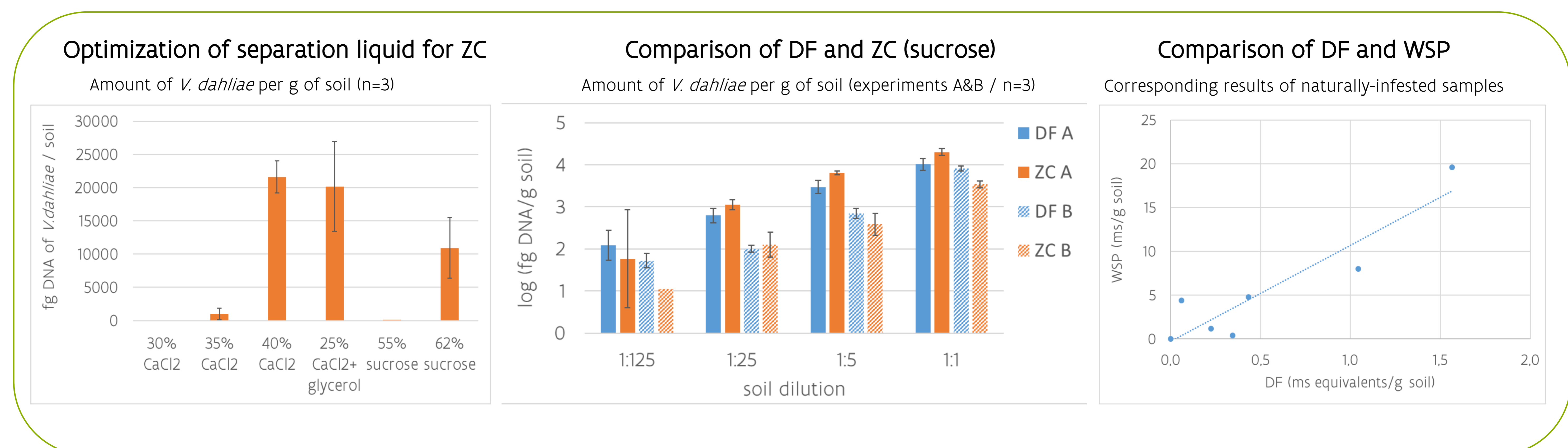


Figure 2. results of the optimization and validation process. Left: comparison of separation liquids for ZC, Middle: comparison of DF and ZC, Right: comparison of ZC and WSP

**BOTH DF AND ZC ARE RELIABLE METHODS FOR *V. DAHLIAE* QUANTIFICATION IN SOIL AND ARE CONSIDERABLY LESS LABOUR-INTENSIVE THAN THE WSP METHOD, WITH ZC ALLOWING SEMI-AUTOMATED AND THUS LEAST LABOUR-INTENSIVE SAMPLE ANALYSIS.**

## REFERENCES:

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