Detection of *Verticillium dahliae* in soil as a basis for disease risk prediction - 15 years soil analysis in Germany.

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Verticillium dahliae causes in Germany severe economic losses in production of strawberry and trees. Reduction of soil inoculum by crop rotation has little effect, because of the wide host range of the fungus and the long viability of microsclerotia. The use of synthetic soil fumigants is restricted and therefore the disease is generally preventive controlled by determining the amount of inoculum in soil with a pre-planting soil analysis as basis for a disease risk assessment of potential planting sites. 15 years ago a soil test was established in practice, which is now widely used by growers in Germany and in neighbouring countries.

The detection method

Microsclerotia in soil are detected with a wet-sieving and plating method according to Harris et al. (1993) and modified by Neubauer and Heitmann (2011) using a semiselective polypectate medium. The method with a detection limit of 0.4 colony forming units/g dried soil is described in Fig. 1.

The pectate medium allows a safe discrimination between *V. dahliae* and *V. tricorpus* (Fig. 2). The procedure is strongly standardized because small modifications are influencing the detection percentage. Using these standardized method three laboratories from the plant protection services Lower Saxony, Oldenburg, and North Rhine Westphalia, Bonn, as well as from the University of Applied Sciences, Osnabrück, analyzing more than 800 soil samples per annum (Fig. 3).

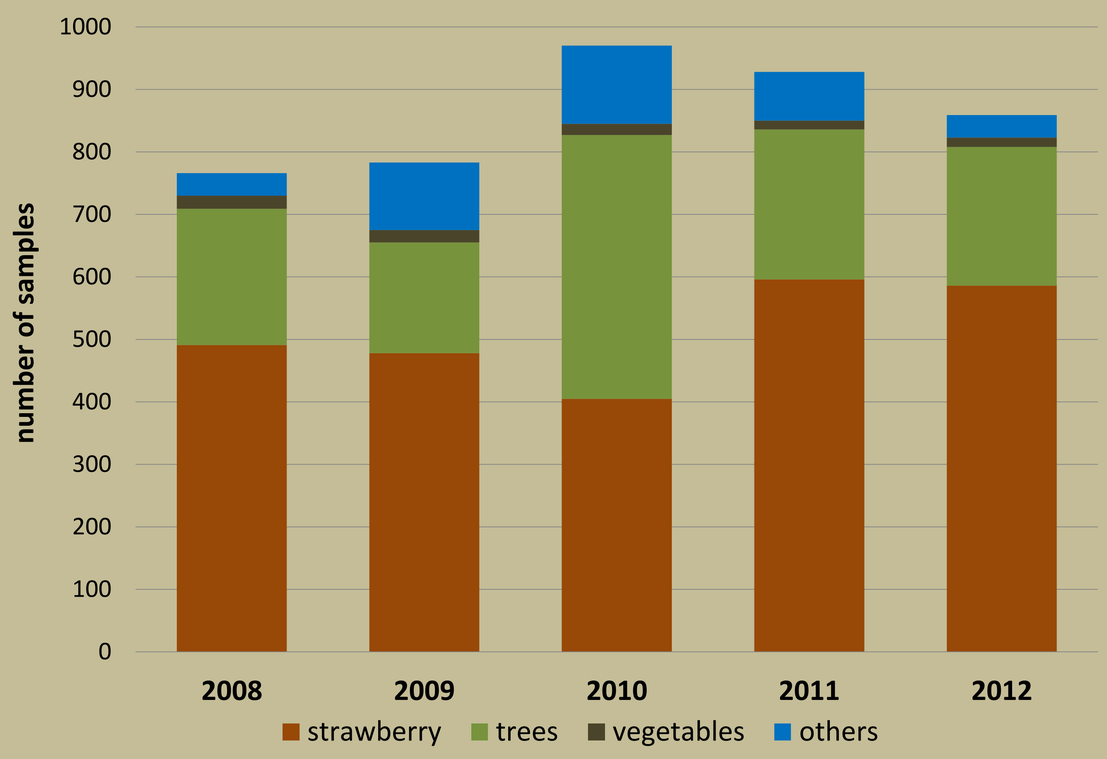
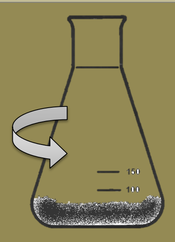


Fig. 3: Number of soil samples tested in Bonn, Oldenburg and Osnabrück for *Verticillium*.

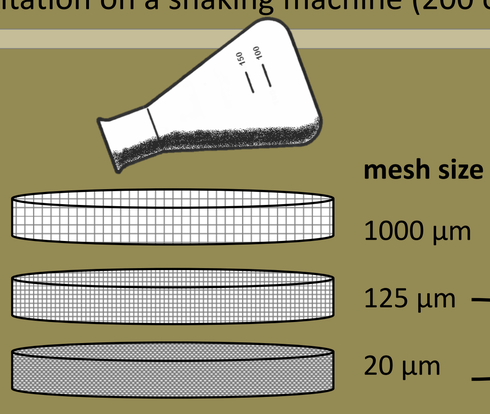
➢ Suspending soil sample

- Soil is air dried and sieved (2 mm)
- 50 g are weighed into an elenmeyer flasks and filled up to 100 ml with distilled water
- Suspension broken down by agitation on a shaking machine (200 osc./min.)



➢ Wet sieving

sieving time: 2 min.
sieving interval: 10 sec.
oscillation amplitude: 3 mm



Recovering soil fraction 20 µm to 125 µm

- Fraction is washed into an elenmeyer flask and filled up to 100 ml with distilled water

Plating on a pectat-medium

- Aliquot: 500 µl/plate (10 plates/sample); Incubation: 14 days, 20 °C, darkness

Scanning of plates for colonies

- Soil particles are washed away with tap water
- Scanning for colonies of *Verticillium* using a binokular microscope
- Each `colony forming unit´ is assessed as one microsclerotia.

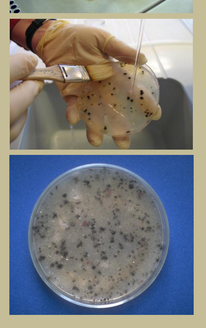


Fig. 1: Standardized wet-sieving and plating method used in German labs for quantitative detection of *Verticillium* in soil.

Fig. 2: Colonies of *V. tricorpus* and *V. dahliae* on the pectat medium

Accuracy of the method

Previously studies showed significant correlations between inoculum densities assessed by the presented plating method and observed disease incidence in strawberries. Therefore a system with 5 classes of infestation levels was established as a basis for the disease risk prediction (Table 1). In further studies the accuracy of the method was evaluated. The non-random distribution of microsclerotia in soil causes a large variability of results in replicated analysis especially in soils with low inoculum densities near the detection limit. The accuracy of the method is increasing with increasing infestation levels. But relating to the classification of the samples and sites the method shows a satisfactory performance and reproducibility. Using this detection method it is not possible to discriminate between *V. dahliae* and *V. longisporum*. But on the basis of information about the cropping history of a site the species of the determined population can be estimated.

Class	Microsclerotia/g dried soil	Level of infestation	Disease risk for susceptible host plant
1	< 0,4	not detectable	low
2	0,4-2,0	low	low
3	2,1-5,0	medium	moderate
4	5,1-15,0	high	high
5	> 15,0	very high	high

Table 1: Classification of planting sites relating to infestation level and diseaese risk on basis of detected *Verticillium* population.

References:

Harris, D. C., Yang, Y. R., Ridout, M. S., 1993: The detection and estimation of *Verticillium dahliae* in naturally infested soil. Plant Pathology 42, 238-250.

Neubauer, C., Heitmann, B., 2011: Quantitative detection of *Verticillium dahliae* in soil as a basis for selection of planting sites in horticulture. Journal für Kulturpflanzen, 63 (1), 1-8.