Detection of Microsclerotia of *Verticillium dahliae* in Soil Samples and Prospects to Reduce the Inoculum Potential of the Fungus in the Soil

Robert STEFFEK [1(✉)]
Andreas SPORNBERGER [2]
Josef ALTENBURGER [1]

Summary

Verticillium dahliae causes a wilting disease in strawberries and is very destructive on the light, sandy soils prevailing in many Austrian strawberry regions. Our work aims at the testing and providing different strategies to control *Verticillium* in the field: 1) a soil test to check the presence of microsclerotia before planting; 2) testing new, *Verticillium* tolerant early season varieties as alternative to 'Elsanta' and 3) testing measures to reduce the microsclerotia content in the soil. The paper describes a field monitoring to study the factors that influence the relationship between inoculum concentration in the soil and the disease incidence in the field, and on-farm biofumigation trials aiming at testing the applicability of the method to reduce the microsclerotia of *Verticillium dahliae* in the soil.

Key words

soil-borne pathogens, wilt, strawberry, *Brassica juncea*, biofumigation

[1] AGES, Austrian Agency for Health and Food Safety; Institute for Plant Health, Department Fruit Production and Viticulture, A-1220 Vienna, Spargelfeldstrasse 191, Austria
✉ e-mail: robert.steffek@ages.at
[2] University of Natural Resources and Applied Life Sciences, Vienna, Department of Applied Plant Sciences and Plant Biotechnology, Institute of Horticulture and Viticulture, A-1180 Vienna, Gregor-Mendel-Straße 33, Austria

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Introduction

Verticillium dahliae causes a vascular wilt in strawberries and several other cultivated plant species. The strain infecting strawberries has a very wide host range: beside other fruit crops it attacks solanaceous plants (potatoes, tomatoes, pepper…), cucurbitaceous plants (cucumbers, courgettes and marrows), legumes (pea, clover, alfalfa…), sugar beet, linseed …

During dry-stress periods, symptoms appear as a more or less rapid wilting and as dying of the outer leaves while the newly developing leaves remain stunted until the plant dies (Fig. 1). A typical symptom can be seen with a section through the crown: as a result of the activity of the fungus in the xylem the vascular bundle appears brown (Fig. 2, Fig. 3). The disease causes plant and crop losses and is very destructive on the light, sandy soils prevailing in many Austrian strawberry regions. *Verticillium dahliae* forms resting microsclerotia in the soil, which allow the fungus to survive even though conditions are unfavourable. Due to their longevity the loss of potential strawberry production area is a problem wherever cultivable land is limited. Currently no suitable chemical control measures are available in Austria. Husbandry measures applied to control *Phytophthora* diseases on poorly drained soils (such as cultivation on ridges, compost amendments …) are inappropriate to reduce the inoculum of *Verticillium* in the soils. Glucosinolate-containing *Brassica* sp. releases volatile isothiocyanates which are toxic to different pathogens (Sarwar & Kirkegaard, 1998).

Far-reaching aim of our work is the testing and providing of different strategies to control *Verticillium* in the field:

1. Implementation of a reliable, traceable detection method in order to offer the strawberry growers a soil test to check the presence of microsclerotia before planting.
2. Collecting data on early season varieties, especially concerning their growth on *Verticillium* infested sites (see paper in this volume)
3. Testing measures to reduce the microsclerotia content in the soil.

This paper describes results from one year period, concerning:

a. A field monitoring to study factors that influence the relationship between inoculum concentration in the soil and the wilt incidence in the field.
b. On-farm biofumigation trials aiming at testing the applicability of the method to reduce the microsclerotia of *Verticillium dahliae* in the soil.

Figure 1.
Wilting symptoms in strawberries infested by *Verticillium dahliae*; wilting of the older, outer leaves; the young leaves remain stunted

Figure 2.
Section through the crown of a strawberry plant infested by *Verticillium dahliae*: as a result of the activity of the fungus in the xylem, the vascular bundle appears brown

Figure 3.
Discoloration in an advanced stage

Materials and methods

Detection of microsclerotia of *Verticillium* in soil samples

For estimation of the microsclerotia in soil samples we used the slightly modified wet sieving method described by Harris *et al.*, 1993: the soil is air dried and sieved (2mm), the samples are agitated in water on a shaker at 240 rpm. The suspension is sieved twice: 160

µm and 25 µm; the material remaining on the 25 µm sieve is recovered, resuspended in water and an aliquot part transferred to a semiselective medium. Instead of the described soil extract medium we used a medium containing Polygalacturon acid (PGA) (Kabir *et al.*, 2004). After 3 weeks incubation at 22°C in the dark, the soil particles were removed from the plates and the plates were scanned under a dissection microscope for the presence of microsclerotial colonies growing radial around the primary sclerotia.

Field survey of factors influencing the relationship between inoculum concentration in the soil and the wilt incidence in the field

In 2005 soil samples from 21 strawberry fields in eastern Austria were collected and analyzed for the presence of microsclerotia of *V. dahliae* (as described above). The farmers received the results characterizing the particular soil before the planting of strawberries (cold stored plants, cultivar 'Elsanta') in these fields. Horticultural management was according to farm practice. Precipitation-irrigation data were recorded during the growing season. In late summer the occurrence of wilt symptoms in the field was evaluated.

Biofumigation trials

The trials were conducted on three farms. Different glucosinolat-containing *Brassica* sp., the varieties: 'ISCI-99' (received from ISCI-Bologna), 'Vitasso', both *B. juncea* and 'Petranova' (*B. napus*) were sown in spring 2005. At full bloom the intercrop was chopped and in one work step incorporated into the soil. The devices used were in accordance with farm practice: mower or chopper and rotary cultivator or grubber. After incorporation the fields were irrigated to saturation (15-20 mm). The efficacy of the measure was determined by examining the reduction of a standard inoculum of *Verticillium dahliae* which was done according to the protocol of Hawke and Lazarovits, 1994 and buried to the depth of 10 cm immediately after incorporation of the *Brassica* intercrop. Two weeks later the bags were removed and the number of viable microsclerotia was determined. In addition soil samples were taken in order to test the effect on soil microbiology by counting the Colony forming units on dilution plates (Gams *et a.l*, 1998). At two sites strawberries (cv. Elsanta) were planted. Their growth as well as the emergence of weeds was recorded in late summer.

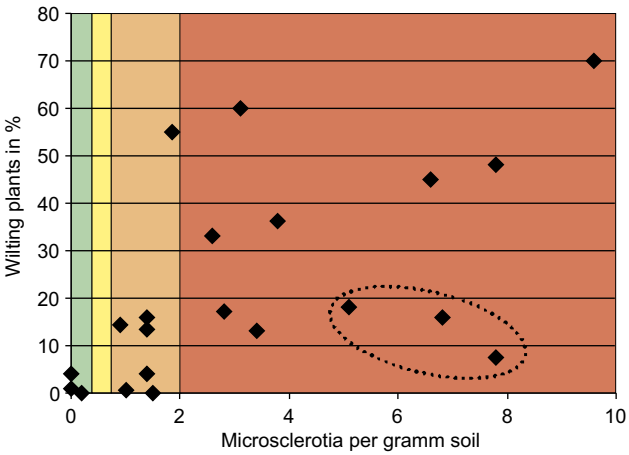

Figure 4.
Occurrence of wilt (cv. Elsanta) as a function of microsclerotia content in the soil

Results and discussion

Field survey of factors influencing the relationship between inoculum concentration in the soil and the wilt incidence in the field

The content of microsclerotia per g of soil ranged between 0 and 9.6 (Fig. 4). On 18 of the 21 soils we expected a high to very high risk for *Verticillium* to cause economic losses (Harris & Yang, 1996). The field evaluation showed that the inoculum concentration in the soil has a major impact on the appearance of wilt symptoms, although in 2005 due to cool, rainy summer the severity of symptoms was mild and plant losses were low. This indicates the importance of irrigation during stress periods, especially in years with dry and hot summers and at sites heavily infested (Fig.4 dotted circle). The occurrence of wilt in the field is also influenced by other factors:

- the pathogenicity of the isolate
- the time of planting and the vigour of the plants
- certain soil features (structure, sand content, humus content, microbiology …)

Biofumigation trials

Depending on the site and the *Brassica* sp. used, the reduction of microsclerotia ranged between 0 and 30 %. A significant decrease was determined only at one field in the case of the intercrop 'Vitasso'. The low efficacy of biofumigation on one field was probably due to the use of a mower, where the crop was not macerated before incorporation. This emphasizes the importance of fine chopping to macerate the plants (Mathiesen *et al.*, 2004). The microsclerotia of *Verticillium dahliae* are more resistant to biofumigation than other pathogens (Harding, 2001; Manici, 2000). The strongly weed-suppressing effect was remarkable. After incorporation, the germination of common chickweed (*Stellaria media),* shepherd's purse *(Capsella bursa-pastoris)* and deadnet-

Figure 5.
Weed suppressing effect of biofumigation: the picture was taken six weeks after the biofumigation of the plot and four weeks after the planting of 'Elsanta': the germination of weeds was completely suppressed in the biofumigated plots (left part of the picture). In the control plots *common chickweed* was occurring area wide (right part of the picture)

tle (*Lamium amplexicante)* was completely suppressed in the *Brassica* plots, while in the control plots chickweed was growing widely (Fig. 5). The interval of 14 days between incorporation and planting did not lead to phytotoxic reactions of the strawberries.

Conclusions

The establishment of the wet-sieving method at our institute opens prospects for Austrian strawberry growers to pre-planting soil analysis and selection of the best suitable field for the strawberry crop. In many cases the soil test indicates a high infestation of the soil, emphasizing the necessity of taking measures available to reduce the inoculum in the soil.

Biofumigation would be well accepted by the farmers: sowing machine, chopper and rotary cultivator are usually available on strawberry farms and the work expended is relatively small. To recommend biofumigation as a measure against *Verticillium* wilt, the efficacy must be improved. This item will be part of a follow-up project in 2006 and 2007.

References

Gams W., Hoekstra E. S. and Aptroot A. (1998): CBS course of mycology, 4th edition. ISBN 90-70351-36-6. 165pp

Harding R., 2001: *In vitro* suppression of Potato pathogens by volatiles released from Brassica residues. Horticulture Biofumigation update, No. 14, November 2001

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

Harris D. C., & Yang J. R. 1996. The relationships between the amount of *Verticillium dahliae* in soil and the incidence of strawberry wilt as a basis for disease risk prediction. Plant Pathol. 45:106-114.

Hawke M. A. & Lazarovits G. 1994: Production and manipulation of individual microsclerotia of *Verticillium dahliae* for use in studies of survival. Phytopahtology, Vol. 84, No. 9, 1994.

Kabir Z., Bhat R. G. & Subbarao K. V. 2004: Comparison of Media for Recovery of *Verticillium dahliae* from Soil. Plant-Disease, 1, 2004 49-55

Manici L. (2000): Soilborne pathogen control. Horticulture Biofumigation update, No 12. November 2000

Mathiesen J., Warton B. & Stackleton M. (2004): The importance of plant maceration and water addition in achieving high *Brassica* derived isothiocyanate levels in soil. Proceedings of the first international biofumigation symposium. Agroindustria Vol.3: 277-280

Sarwar M. & Kirkegaard J. 1998: Biofumigation potential of Brassicas, Part I-III. Plant and Soil 201, 71-112

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