

Pathogenicity in *Verticillium* on strawberry plants

P. Schubert¹, J. Gollmack², H. Schwärzel², P. Lentzsch¹

Abstract

In the most common strawberry cv. 'Elsanta', Verticillium infection can lead to rapid wilt and even death of plants. It is known, that a dead plant can be located directly beside vital ones. In a survey of 8 fields in Brandenburg, Mecklenburg-Vorpommern, Sachsen-Anhalt and Sachsen, 432 genotypes of Verticillium dahliae Kleb. were isolated from wilted and even vital plants from 8 fields and classified by PCR-fingerprints. For strawberries, the genotypes can be classified as apathogenic, weakly and highly pathogenic according to the results of climate chamber experiments on strawberry transplants.

At landscape scale, similarity analysis of the PCR fingerprints of 432 genotypes resulted in 13 genetic subtypes. Several of these subtypes occurred at all fields, whereas 1 subtype was found in one location only. At field scale, 2 to 11 different subtypes per field were observed. Vital plants were colonised by up to 9 subtypes, wilted plants by up to 11 subtypes. Population structure of Verticillium subtypes is different between vital and wilted plants, the same subtypes can occur in either plant group. In our plot experiments, wilt symptoms could be reduced by changing the Verticillium population structure in the plant. Inoculation of plants with a mixture of three Verticillium genotypes sustained plant vitality over a period of 15 months (WO 2007/051654).

Keywords: *Verticillium dahliae*, wilt, control

Introduction

Verticillium wilt is an important disease in strawberries. Most of the main cultivars (e.g. 'Elsanta') are highly susceptible to *Verticillium* wilt (Tahmatsidou, Paroussi and Voyiatzis, 2002). Recommendations for farmers only include the avoiding of infested fields. Incidence of microsclerotia in the soil is used for prediction of the wilt potential (Termorshuizen et al, 1998). Management practises like crop rotation are only of weak consequence in relation to *Verticillium* wilt because of the wide range of hosts and the long persistence of microsclerotia in the soil. Control of wilt symptoms by using commercially available bacteria as antagonists was inconsistent and insufficient in our studies (data unpublished). Distribution of fungi in the field is often described as clonal (Morgan et al, 2002). Such pattern was also observed as hot spots of colonisation in the rhizosphere of plants (Gollmack et al, 2004). The distance between hot spots is correlated with the genotype of the fungus. The observed pattern of wilted plants beside vital ones seems to be a result of clonal distribution of the pathogen. Thus we examined, whether the level of wilt symptoms in strawberries is a result of local differences in *Verticillium* population structure or of different pathogenicity of *Verticillium* genotypes. Therefore we compared plants with different wilt symptoms by analysing *Verticillium* population structure and pathogenicity of *Verticillium* genotypes at 8 locations along a transect of about 400 km length, including 4 counties.

¹ Leibniz-Zentrum für Agrarlandschaftsforschung (ZALF), Eberswalder Str. 84, 15374 Müncheberg, Germany

² Landesamt für Verbraucherschutz, Landwirtschaft und Flurneuordnung (LVLFG), FG Obstbau, Baumschule, Technik, Eberswalder Str. 84, 15374 Müncheberg, Germany
peter.schubert@lvf.brandenburg.de

Material and Methods

Fields and Isolates

For the field trials, 8 strawberry producing farms with *Verticillium* wilt problems were chosen. They are located in the north-east of Germany (counties Mecklenburg-Vorpommern, Brandenburg, Sachsen-Anhalt, Sachsen). Climate conditions varied from marine (Rostock nearby the Baltic sea) to slightly continental (Müncheberg).

Up to 5 fields per farm (strawberry cv. 'Elsanta') were analysed for incidence of *Verticillium* wilt. *Verticillium* symptoms were classified according to a rating scale from 1 to 9 (Büttner, 1985) with strong (rank 1) to no symptoms (rank 9) on plants, respectively. Samples were taken from each of the 5 fields: 5 plants with strong symptoms, 5 plants with medium symptoms and 5 vital plants per field. Samples consisted of the whole plant and about 2 litre of soil containing roots. Samples were stored at 0 °C in plastic bags.

To obtain isolates of *Verticillium*, each sample was divided into plant and soil. Further work was done under sterile conditions. 4 petioles per plant were surface sterilized and used for isolation. Near the stipule, 3 chips of 1 cm length were taken from every petiole and transferred to 3 selective cultivation media (Capek-Dox, vegetable and prune agar; Kabir, Bhat and Subbarao, 2004). After incubation at 26 °C for 3 days, followed by rating and visual selection, the isolates were transferred to fresh media and further cultivated.

Genotyping of *Verticillium*

Fungal isolates from plants of all levels of symptoms (1-9) were identified as *Verticillium* spp. by morphological and genetic methods. Fungal isolates with at least one of the following morphological characteristics were selected for genotyping: production of microsclerotia, position of phialides, presence of melanised hyphae. PCR analysis was conducted to identify *Verticillium* species. Consensus sequence was analysed from available sequences of the ITS1-5.8S r RNA region in EMBL/NCBI genebanks, primers selected by primer3 software. *Verticillium tricorpus* – DNA resulted in a PCR-fragment of 270bp, restricted by enzyme BstUI in two fragments of 220bp and 50bp. *V. dahliae* and *V. albo-atrum* produced a PCR-fragment length between 260 and 270 bp, restricted in two fragments of 177bp and 85 to 92bp. To distinguish these two species, a *V. dahliae* specific reverse primer (VDP2; Aspromougkos, 2002) was used resulting in a 370bp fragment for *V. dahliae* only. Reference strains were also identified by this method. 421 Isolates could be identified as *V. dahliae*. Genetic similarity was calculated from the RAPD-PCR-fingerprints of *V. dahliae* isolates (method in: Lentzsch and Gollmack, 2006). 42 isolates were selected, representing the complete genetic distance within the sampled population of the 8 fields.

Plot Experiment

A field trial was conducted in Müncheberg (county Brandenburg) during the 2006 and 2007 growing period. Bare-root transplants were taken from reproduction beds in December 2005 and stored at 0 °C until establishment in May 2006. Plants were established on single-row beds with 1.00 m space between rows and 0.30 m in-row planting space. Cultural practice followed standard recommendations, including sprinkler irrigation. The planting site was known to be highly infected with *Verticillium* microsclerotia. Losses due to *Verticillium* wilt were about 50 % in previous trials with cv. 'Elsanta'. *Verticillium* genotypes were cultivated on sterilized wheat straw.

The straw was fully overgrown with mycelium after 10 days. 3 g of straw inoculums was placed in each planting hole before planting. Trial consisted of 7 treatments and control: genotypes A (isolate 337), B (isolate 806), C (isolate 784), combinations of A+B, B+C, A+C and mixture A+B+C.

Results and Discussion

With a sensitive genetic screening we differentiated genetic subtypes of *V. dahliae*. First, the isolates were tested for *V. dahliae*. Cultivar-specific differences in the ITS-region of ribosomal DNA were used to distinguish *V. dahliae*, *V. tricorpus* und *V. albo-atrum*. Other cultivars of *Verticillium* did not produce any PCR-reaction. *V. dahliae* isolates were further differentiated by RAPD-PCR and were grouped according to the similarity of their fingerprints into 13 subtypes.

On 6 sites we found a very similar population structure of *Verticillium* (fig. 1). Low diversity with only 4 or 5 subtypes was recorded at Abbläß and Schirgiswalde. Abbläß was the only site with hill culture covered with plastic mulch, whereas Schirgiswalde was unique in its location on a slope above gravel layers. The subtype 6 was exclusively found at Schmergow, the remaining subtypes were commonly distributed.

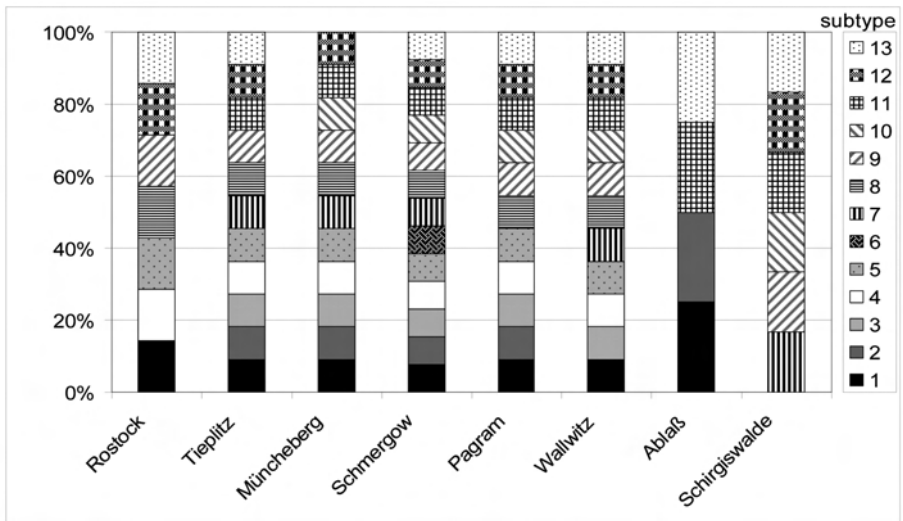


Figure 1: Distribution of *Verticillium dahliae* subtypes at 8 sites in relative abundance per site

Strawberry plants were colonised with *Verticillium dahliae* genotypes regardless of the presence of symptoms. At all field sites, the shift from vitality to wilt symptoms was related to changes in population structure (fig. 2, example: site Müncheberg). On all sites, wilted plants were colonised by up to 4 additional subtypes.

Verticillium genotypes representing genetic subtypes were tested for their pathogenicity in climate chamber trials. Inoculation with different *Verticillium* genotypes resulted in different plant reactions. Within a single treatment, there was little variation in the plant reaction to inoculation with the respective *Verticillium* genotype.

As a result of the climate chamber trials, genotypes could be classified as apathogenic, weakly pathogenic and highly pathogenic. Pathogenicity of the 3 tested genotypes per subtype was very similar, thus it was possible to assign every genetic subtype to one of the pathogenicity groups. As shown in figure 2, the majority of plants was colonised by apathogenic subtypes. A change in population structure in form of the occurrence of 2 additional apathogenic subtypes was related to wilt incidence. At all sites, the presence or absence of symptoms could not be related to the presence or absence of a single *Verticillium* subtype. The development of wilt symptoms was rather due to the *Verticillium* population structure and differed between microsites. This indicates that the incidence of wilt symptoms is regulated by *Verticillium* itself. A possible explanation could be an interaction of *Verticillium* subtypes within the plant.

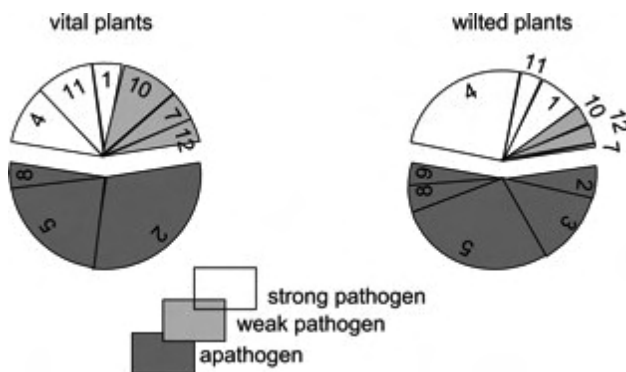


Figure 2: Abundance of *Verticillium dahliae* subtypes in vital and wilted plants at site Müncheberg

In a plot experiment we tried to induce wilt symptoms by simultaneous inoculation of cv. 'Elsanta' with two pathogenic genotypes. Reduced symptoms occurred in the treatment of combined inoculums in comparison to inoculation with a single genotype (data not shown). In a further plot experiment we used 3 genotypes in a fully combined and replicated design (fig. 3).

Isolates 337 (apathogenic), 784 and 806 (temperature-dependent weakly pathogenic, Schubert et al., submitted) and the combinations of these strains were tested to evaluate wilt control potential of the mixture of *Verticillium* isolates. In July of 2006, 2 month after planting, minor differences in vitality occurred.

In October 2006, inoculation with isolates 784 and 806 and the mixture of the three genotypes resulted in vital plants. In the mixture treatment, 2% of the plants showed minor wilt symptoms of rank 6, the remaining plants were vital with 27% of rank 7 and 71% of rank 8-9.

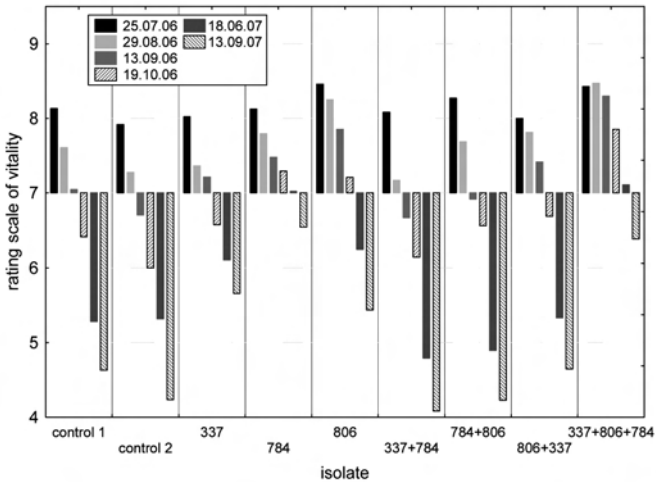


Figure 3: Dynamic of wilting of plants (cv. 'Elsanta') inoculated with 3 genotypes and their combinations in a plot experiment

In June of the second year, 20% of the control plants were dead, 40% showed symptoms and 40% were vital. In contrast, most of the plants of treatment 784 (74%) and mixture treatment (80%) were vital in June.

In September 2007, vitality general declined, but 71% of the plants in treatment 784 and 68% in the mixture treatment were vital. Vitality of control plants further decreased: 30% of the plants were vital and 30% were dead.

Conclusions

At landscape scale, the *Verticillium* subtypes were commonly distributed at 6 sites, without any indication for local specification. Apparently, pathogenicity of a genotype was only expressed if there was no other genotype present in the plant. Development of symptoms was a result of the *Verticillium* population structure at a microsite and of the unknown interaction within the plant. Such a system could be changed by inoculation with a mixture of 3 *Verticillium* genotypes to sustain the vitality of the plant. Further research is needed to analyse the underlying mechanisms of colonisation and interaction of fungal genotypes in plants. In future studies, additional sites of different climate regions and different soil conditions should be included to confirm the control potential of the mixture of three *Verticillium* genotypes under these conditions.

Acknowledgements

This project was granted by Deutsche Bundesstiftung Umwelt (DBU) (Az 18940).

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