Session C: Resistance breeding and new phytopathogens

CPL 1: New, emerging and re-emerging fungal diseases on medicinal and aromatic plants in European domain

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Abstract

Plant diseases cause agricultural and economic loss and impact negatively on human and animal health through mycotoxins and allergens produced by them. They also have consequences for biodiversity conservation. The pathogens could be classified in five categories: new - detected within the last five years; emerging - have always been present in an area but have grown in importance over the years; re-emerging - have been previously controlled but are once more a major problem associated with chemical resistance or changes in management or cultivars; threatening - not reported or limited in distribution in Europe and chronic-spreading – known for longer than 20 years and causing increased concern. Diseases emerge or re-emerge due to changes in farming practices, development of new strains of the pathogen, climate change, introduction of the pathogen to new geographical locations, or introduction of more efficient pathogen vectors. During the last years emerging infectious diseases (EIDs) are of special concern to researchers. Among all pathogens fungi are responsible for the greatest damage to plants in both agricultural and natural ecosystems. They represent over 70 % of all plant pathogens and over 30 % of plant EIDs. Surveys on fungal diseases of medicinal and aromatic plants have been carried out in the framework of several research projects between Germany, Bulgaria, Lithuania and Poland in the last two decades. EIDs have been reported, either as novel pathogens or as familiar pathogens affecting new host species. The importance of the problem could be illustrated by such examples as some phytopathogenic fungi on Apiaceae and Lamiaceae hosts discussed in the present work.

Keywords: Apiaceae, Lamiaceae, fungal pathogens, asexual morphs

Introduction

Medicinal and aromatic plants (MAPs) are considered minor crops generally grown on limited area. There was a view that they had no serious diseases. The sources of information related to the diseases of MAPs were mostly limited to the areas in which their cultivation reached appreciable levels. During the last decades, mainly Europe and America have experienced an increase trend towards healthy diet and natural products, which led to a growing demand for MAPs, partly satisfied by collections of wild-growing plants, but to an enhanced extent by cultivation. The increased interest in the use of MAPs is also recognizable regarding the bigger diversity of genera processed in Europe. A negative consequence of the growing concentration in cultivation is an increase of pathogen occurrence (Gabler, 2002). The control techniques take a great relevance inside the recommended growing protocols for MAPs because the diseases can cause economically considerable decrease in yield but also in the quality of production (Carruba et al., 2015). The pathogens could be classified in five categories: new - detected within the last five years; emerging - have always been present in an area but have grown in importance over the years; re-emerging - have been previously controlled but are once more a major problem associated with chemical resistance or changes in management or cultivars; threatening - not reported or limited in distribution in Europe and chronic-spreading – known for longer than 20 years and causing increased concern (Damsteegt, 1999). Despite the fact that MAPs produce secondary metabolites with antimicrobial, including antifungal action, they are attacked by a number of pathogens including fungi. The information about the most commonly widespread fungal pathogens affecting MAPs is summarized by Carruba et al. (2015). During the last years emerging infectious diseases (EIDs) are of special concern to researchers (Andersen et al., 2004; Fletcher et al., 2010; Fisher et al., 2012).The purpose of present work is to share long-years' experience and knowledge about new, emerging and re-emerging pathogens mainly on representatives belonging to Apiaceae (caraway, coriander, dill, fennel,) and Lamiaceae (oregano, sage, summer savory), which are known as medicinal, spice and essential oil crops.

Materials and Methods

The investigations on fungal diseases of MAPs were conducted in the framework of several research projects between Germany, Bulgaria, Lithuania and Poland in the last two decades. Observations for disease incidence were made on plants in commercial fields and private gardens. Samples of diseased plants were taken for laboratory studies. The presence of fungi was established on the basis of etiological symptoms occurring on the infected above-ground and underground plant parts and mycological analysis. A complex approach was applied in pathogen diagnostics and their characterization including phytopathological, mycological, phytochemical, immunological, molecular and statistical methods. Pathogenicity of the isolates was proved using whole plants or detached plant parts. Toxigenic properties of fungal isolates were investigated by extraction and purification of secondary metabolites and application of a leaf puncture bioassay for the rapid determination of phytotoxic activity of the fractions and of pure compounds (Evidente et al., 2011). The investigation on molecular identity and similarity of the fungal isolates was carried out by blast sequencing the internal transcribed spacer (ITS) region of rDNA. Total genomic DNA was extracted from pure cultures. The generated DNA sequences of ITS region were compared with other fungal sequences from the National Center for Biotechnology Information (NCBI, Bethesda, USA) database and our own accessions isolated from different host plants using BLASTn (Basic Local Alignment Search Tool) (Kačergius et al., 2011). The phylogenetic analysis was performed applying the Neighbor-Joining method by MEGA5 (Tamura et al., 2011). Searching for resistance to some fungal diseases was performed in field experiments including Bulgarian and German cultivars of fennel, dill and caraway. The evaluation of the cultivar reaction was made on the basis of disease incidence (% diseased plants), disease severity (disease index, DI) and disease progress (area under the disease progress curve, AUDPC).

Results

Apiaceae hosts

Besides the chronic-spreading and well known fungal pathogens on Apiaceae hosts, several asexual morphs of *Diaporthe* species have been reported as the causal agents of new diseases. *Phomopsis diachenii* Sacc. occurred on caraway (*Carum carvi* L.) as an emerging disease in Czech Republic and caused considerable yield losses (more than 50 %) (Ondřej, 1997). Several years later the first detection of the pathogen in Germany was made (Gabler und Ehrig, 2000). *P. diachenii* showed very high aggressiveness in pathogenicity tests. Its temperature optimum was between 25 ^{0}C and 30 ^{0}C. There was a positive correlation between the disease progress in the field and some climate data, above all the air temperature and rainfall, which could be a possible explanation for high disease occurrence in hot showery weather (Gabler, 2002). Later, this caraway disease has been reported in Bulgaria (RODEVA and GABLER, 2004), Poland (MACHOWICZ-STEFANIAK, 2009), Hungary (NAGY, 2009) and Lithuania (MAČKINAITĖ, 2012). At the end of the 1970s, an outbreak of new disease (umbel browning and stem necrosis) occurred on fennel (*Foeniculum vulgare* Mill.) in France and was described as a new species *P. foeniculi* Du Man. et Vegh (DU MANOIR and VEGH, 1981). In 1990 the presence of the pathogen was detected for the first time in Italy (MUGNAI and ANZIDEI, 1991). The fungus regarded as highly aggressive caused considerable losses in yield. In Germany *P. foeniculi* was detected for the first time in 1991 (PLESCHER, 1992). Lately, this fennel disease has been found in Bulgaria for the first time (RODEVA and GABLER, 2011). *Phomopsis* sp. was established as the causal agent of the same disease of dill, too (RODEVA and GABLER, 2006). Pathogenicity tests and cross-inoculations were performed with *P. diachenii*, *P. foeniculi* and *Phomopsis* sp. isolated from caraway, fennel and dill, respectively. On the basis of disease development and plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA) using a polyclonal *Phomopsis*-genus-specific antiserum (IgG59/III) it was found that all isolates provoked the typical *Phomopsis* symptoms on all three host plants; however, the disease scores and ELISA values varied (RODEVA and GABLER, 2006; 2011). The pathogens were reisolated from inoculated plants. The sexual counterpart of *Phomopsis* spp. isolated from caraway, fennel and dill was not observed under field conditions. Its development was provoked on diseased stem fragments incubated in moist chamber or in vitro mainly on oatmeal and malt yeast extract agar and was assigned to *Diaporthe angelicae* (Berk.) D.F. Farr & Castl. (RODEVA and GABLER, 2004; 2011). Umbel browning and stem necrosis were also found as typical symptoms on wild-growing Apiaceae species and *Phomopsis* spp. were isolated as causal agents (RODEVA et al., 2006), which deserved great interest because these hosts could serve as a source of inoculum for cultivated ones. On the other hand, the negative impact of *Phomopsis* diseases on biodiversity might reduce the potential for the discovery of new pharmaceuticals or new crops. Morphological and molecular investigation was performed on 46 isolates obtained from cultivated and wild-growing Apiaceae hosts in Bulgaria, Lithuania and Germany. The phylogenetic analysis revealed a close relationship of *D. angelicae* and its asexual morphs like *P. diachenii*, *P. foeniculi* and some newly isolated *Phomopsis* spp. in Bulgaria (from coriander and parsley) and Lithuania (from coriander and dill) (KAČERGIUS et al., 2011). The diseases caused by *Phomopsis* spp. were highly devastating for studied umbelliferous plants and led to premature drying up of umbels and stems and even the plant death. Full destruction of affected umbels was observed thereby preventing seed set. The highest plant susceptibility was observed during flowering. It could be possible these new emerged warm-temperature diseases may become more important in the future years. The possibility of limiting the growth and development of *P. diachenii* was studied applying in vitro two biotechnical preparations (Biosept Active and Beta-chikol) and 12 fungicides from different chemical groups. The most promising compound in reducing the growth and development of *P. diachenii* was mancozeb (ZALEWSKA et al., 2013). The resistance evaluation of fennel, dill and caraway cultivars made on the basis of disease incidence, disease severity and disease progress showed that all cultivars under study were attacked but differences in their susceptibility were established, which is very promising in searching sources of resistance and their use in breeding (RODEVA and GABLER, 2007). Disease resistance is an important character of cultivars, especially MAPs as the application of pesticides is hardly accepted.

It is known that some *Phomopsis* species, including *P. foeniculi* produce in liquid culture phytotoxic metabolites, which could be involved in pathogenesis (EVIDENTE et al., 1994; CORSARO et al., 1998). Considering the economical importance of fennel fruits, the increased disease occurrence in Bulgaria and the fact that the causal agent *P. foeniculi* is a toxigenic fungus, research was undertaken to isolate and characterize the phytotoxins produced by Bulgarian strains. Using a bioassay guided isolation and purification procedure, different metabolites were isolated from the fungal culture filtrates. They were identified by spectroscopic methods as nectriapyrone, a pentaketide monoterpenoid, and altersolanols A and J and macrosporin, three octaketides anthracenones (EVIDENTE et al., 2011). These metabolites differed from foeniculoxin, that is a geranylkhydroquinone (EVIDENTE et al., 1994) and obviously from the exopolysaccharides (CORSARO et al., 1998) isolated from an Italian strain of the same fungus. Plant growth and development of

MAPs as well as the nature of secondary metabolites are affected by the physical environment, including light, temperature, rainfall, and soil properties (MÁTHÉ, 2010). The same circumstances could play a role in the secondary metabolite production of their pathogens illustrated by the fact that different isolates of same pathogen and host have produced different phytotoxic metabolites under ecological conditions in Italy and Bulgaria.

The ascomycetous fungus *Septoria carvi* Syd.was found as a dangerous pathogen of caraway in Poland (ZALEWSKA and MACHOWICZ-STEFANIAK, 2003). The fungus caused many small necrotic spots on petioles, leaves, stems and umbels. Infected plants could prematurely die. The pathogen was aggressive in pathogenicity tests. The disease occurred epidemically during warm and humid growing seasons. *S. carvi* was recorded in Czech Republic (ODSTRČILOVÁ et al., 2002), Germany (GABLER and MACHOWICZ-STEFANIAK, 2004), Austria (BEDLAN, 2005) and Lithuania (MAČKINAITĖ, 2012). In Bulgaria this pathogen has not been found yet (RODEVA and GABLER, 2009).

Mycocentrospora acerina (R. Hartig) Deighton, not previously recorded on caraway in Poland, has been found only recently (ZALEWSKA et al., 2015). Anthracnose caused by *M. acerina* has been considered as one of the major problems in caraway production in Netherlands for a long time (EVENHUIS et al., 1995). In Bulgaria *M.acerina* has been reported on wild growing caraway (Vanev 1988) but the anthracnose occurrence has never been observed on cultivated one (RODEVA and GABLER, 2009).

Two new diseases were found on coriander in Bulgaria caused by ascomycetous fungi. Wilting and root rot incited by *Macrophomina phaseolina* (Tassi) Goidanich led to the death of whole plants (RODEVA et al., 2010). The same fungus was also isolated from premature dead dill and caraway plants in the field (Rodeva, personal communication). In the laboratory experiments, the asexual morphs of the fungus were found either of microsclerotia or pycnidia but in the field only sclerotial form was observed. The microsclerotia served as units of long-term survival in soil. Their accumulation lead to an increase of the primary inoculum for root infection of coriander and many other host plants (more than 500 species) of the pathogen. The disease could be a threat for coriander and other Apiaceae hosts under conditions favorable for its development especially water deficit. Association of coriander with the host range of *M. phaseolina* has to be taken into consideration in crop rotations.

Phoma glomerata (Corda) Wollenweber and Hochapfel causing stem rot of coriander was found for the first time in Bulgaria and elsewhere (RODEVA et al., 2013). The identification was confirmed by PCR amplification, based on internal transcribed spacers and the 5.8 rDNA (ITS1-5.8-ITS2). The amplicon was sequenced and analyzed using BLASTn and showed a homology of 100 % with a corresponding sequence of *P. glomerata* (accession number DQ093699). The same pathogen was recorded as causal agent or crown rot diseases of fennel in Southern Italy (LAHOZ et al., 2007).

The basidiomycetous fungus *Itersonilia perplexans* Derx has been reported to be the causal agent of leaf blight of dill (*Anethum graveolens* L.) in several European countries, as Italy (MATTA and GARIBALDI, 1968), Germany (GEßNER, 1988; KUSTERER et al., 2002), Austria (BEDLAN, 1988), Swiss (USOLTSEVA and DAHL, 2006). Lately, it has been found in Bulgaria (RODEVA et al., 2009), UK (LAMBOURNE, 2011) and Cyprus (KANETIS et al., 2015). Serious infections by the pathogen were observed on different herbs including dill, coriander and parsley in Germany (Baden-Württemberg) in 2014 and 2015 (HINRICHS-BERGER, 2015). Since *I. perplexans* grows best at high relative humidity (>70 %.) and temperatures of 10-15 °C the way to diminish the disease appearance and development is to keep the dill plants under as dry conditions as possible. The initial symptoms of *I. perplexans* resembled those caused by lack of nutrient substances or water. For this reason it is very important to clear up the cause as the first symptoms appear and to take control measures. The infected plants have to be destroyed and do not put in the compost.

Lamiaceae hosts

Phomopsis sclarea Sarwar was isolated from sage (*Salvia officinalis* L.) in Poland for the first time in 2010 and recognized as the main cause of the necrotic spots on stem, peeling off and breaking the bark (Zimowska, 2010). In Germany, this pathogen was isolated from *S. officinalis* also for the first time in 2010. Additionally, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *C. dematium* (Pers.) Grove were found but these pathogens were not predominant. The most serious damages were due to infections by the ascomycetous fungus *Boeremia exigua* (Desm.) Aveskamp, Gruyter & Verkley (syn. *Phoma exigua* var. *exigua* Desm.) and the downy mildew fungus *Peronospora salviae-officinalis* Y.J. Choi, Thines & H.D. Shin. Downy mildew fungi spread increasingly and are a considerable risk factor for many crops worldwide (Gabler, personal communication).

Boeremia strasseri (Moesz) Aveskamp, Gruyter & Verkley (syn. *Phoma strasseri* Moesz) was commonly isolated from the stems and the rhizomes of peppermint (*Mentha piperita* L.) showing symptoms of necrosis and tissue disintegration. The disease caused by the pathogen is called black stem and rhizome rot of peppermint (Zimowska, 2012). *Colletotrichum fuscum* Laubert, Gartenwelt has never been recorded on oregano (*Origanum vulgare* L.) anywhere in the world until recently. In Poland it was found as a new disease inciter causing characteristic symptoms on oregano leaves in the form of necrotic, rounded, concentrically zoned spots. This species, like other *Colletotrichum* species, had high thermal requirements and the optimum was 28 °C. Severe occurrence of anthracnose of oregano caused by *C. fuscum* must be therefore taken into account during the vegetation periods when the temperature is high (Zimowska, 2015). A severe outbreak of the black stem disease on *O. vulgare* subsp. *hirtum* (Link) Ietsw. (syn. *O. heracleoticum* Benth.) caused by *B. exigua* was reported in Germany (Saxonia-Anhalt) for the first time in 2002. The disease caused serious damages. The development of the pathogen (temperature optimum 20 °C) was favored by extreme cool and wet weather conditions in spring and early summer and by the high susceptibility of the predominantly cultivated cultivar (Gabler, 2004). A resistance screening method was developed for breeding of new cultivars with improved resistance (Gabler, 2006). *O. vulgare* subsp. *hirtum* was found as very susceptible host of *Puccinia menthae* Pers.in Bulgaria (Rodeva, personal communication).

A downy mildew disease of summer savory (*Satureja hortensis* L.) with a high damaging potential was observed in Germany for the first time in 2004. *Peronospora saturejae-hortensis* Osipyan was identified as causal agent with the help of molecular sequence analyses. The results demonstrated that the causal agent of this downy mildew was not *P. lamii* A. Braun as was often assumed (Gabler et al., 2012). The pathogen required for optimal development special weather conditions*:* leaf wet duration >6 h, air temperature <15 °C and relative air humidity >85 % at the same time. Such circumstances resulting in serious damages were given at the experimental site (Quedlinburg) only once (2009) within five years (2007-2011) (Gabler, 2013).

Conclusions

This paper included a non-exclusive list of new, emerging and re-emerging pathogens and was only an attempt to summarize some results obtained from an international research group bringing together the efforts of scientists from Bulgaria, Germany, Poland and Lithuania. The distribution and relative importance of the recorded pathogens differed significantly between years and localities depending on the specific geographical and environmental conditions. The pathogens considered as chronic-spreading and well known in one country could be new or emerging in another. Some changes in the pathogen populations affecting MAPs were observed in favor of the development of thermophilic pathogens (*Colletotrichum* spp., *Phomopsis* spp., *M. phaseolina*), which could be due to global climate warming. Because of selection pressure it is possible some newly emerged warm-temperature pathogens may become more important. Some other pathogens preferring cool and wet weather were more prevalent when such conditions occurred (*B. exigua*, *I. perplexans*, *P. saturejae-hortensis*). An integrated disease management should be applied to control the new, emerging and re-emerging diseases. There are not many chemicals registered for use on MAPs. Having in mind that MAPs are a source of natural therapeutic substances the use of fungicides must be limited to a minimum number of treatments and dosage selecting highly effective, low-toxicity and low-residue products, which for organic cultivation are even prohibited. Monitoring of health and early detection of diseases in plants is critical issue. It is very important to focus on accurate diagnosis of new disease problems. Changes in disease occurrence impact disease management practices and also suggest a consideration of using resistant varieties to prevent these new diseases from becoming established on a large scale. To reduce the pathogen population a crop rotation is strongly advised avoiding usage of plant species susceptible to the same pathogens.

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