Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Phytophthora root and collar rots in fruit orchards in the Czech Republic

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OPEN ACCESS

Citation: Grígel J., Černý K., Mrázková M., Havrdová L., Zahradník D., Jílková B., Hrabětová M. (2019) *Phytophthora* root and collar rots in fruit orchards in the Czech Republic. *Phytopathologia Mediterranea* 58(2): 261-275. doi: 10.14601/Phytopathol_Mediter-10614

Accepted: March 25, 2019

Published: September 14, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Epaminondas Paplomatas, Agricultural University of Athens, Greece.

Summary. A recent outbreak of *Phytophthora* diseases in fruit orchards was identified in the Czech Republic. The diseased trees showed characteristic symptoms including yellowing, wilting and sparse foliage, decreased yields, root and collar rot, and withering and dying of trees. In some orchards up to 10–15, and rarely up to 55%, of trees died. In total, 387 symptomatic trees of nine species from 44 fruit orchards, 16 samples of irrigation water from four orchards and 35 samples of nursery stock, were surveyed in 2012–2018. Oomycetes were recovered from 50.6 % of sampled trees in orchards, from 71.4 % of shipments of ex vitro-produced nursery plants, and from 93.8 % irrigation water samples. Seventeen *Phytophthora* species and 13 *Pythium* sensu lato species were recovered. The most frequent species in orchards were *Phytophthora cactorum*, *Phytophthora plurivora* and *Phytopythium vexans*. The most frequent species in nursery stock were *P. cactorum*, *Phy. vexans* and *Globisporangium intermedium*, and *Pythium helicoides*, *Phytophthora lacustris* and *Pythium litorale* were the most frequently found in irrigation water. The most frequent oomycete species recovered from nursery stock were also frequently isolated from orchards and planting material. The pathogenicity of 11 *Phytophthora* species was assessed *in vitro* in two frequently used rootstocks – ´St. Julien´ (plum) and ´M26´ (apple). This revealed that the less known and relatively rarely isolated species (*P. gregata*, *P. chlamydospora* × *amnicola*, *P. inundata*, *P. lacustris* and *P. sansomeana*) were more virulent than the more frequently occurring species *P. cactorum* and *P. plurivora*, and could pose potential threats to fruit orchards in the future. The increase of *Phytophthora* in fruit orchards is probably related to the trading and planting of infected nursery stock, to climate change and water stress, and to dismissal of the problem of *Phytophthora* diseases by stakeholders and inappropriate management. Future investigation should focus on the development of effective disease management, including assessment and selection of rootstocks for resistance.

Keywords. *Phytophthora*, *Malus*, *Prunus*, fruit orchards, rootstock.

INTRODUCTION

Phytophthora species are well known pathogens of many fruit tree species including apple, plum and pear (Mircetich and Browne, 1987; Erwin and Ribeiro, 1996; Sutton *et al*., 2014). These pathogens have been frequent-

ly reported to cause serious and increasing problems, especially in the Mediterranean and European countries (Smith *et al*., 1988; Laviola *et al*., 1990; Harris, 1991; Thomidis, 2003; Pane *et al*., 2009; Nakova, 2010a). Although research on the diversity and epidemiology of *Phytophthora* pathogens in woody plants is already a tradition in the Czech Republic (Černý *et al*., 2011; Mrázková *et al*., 2011; 2013), and a total of 26 *Phytophthora* species have been isolated from many forest and ornamental woody plants (CCPO, 2018), with many able to parasitize fruit trees (including *P. cactorum* (Lebert & Cohn) J. Schröt., *P. cambivora* (Petri) Buisman, *P. cinnamomi* Rands, *P. citrophthora (*R.E. Sm. & E.H. Sm.) Leonian, *P. cryptogea* Pethybr. & Laff., *P. plurivora* T. Jung & T.I. Burgess, *P. syringae* (Kleb.) Kleb. (Erwin and Ribeiro, 1996), investigation of these pathogens in fruit trees has not been carried out in the country.

Increases of the characteristic symptoms of *Phytophthora* diseases (root and collar rots, yellowing of foliage, withering of trees), tree death and yield reductions have been registered in many fruit orchards in different regions of the Czech Republic. The country is a regionally important producer of fruit in Central Europe. The most important fruits grown in the Czech Republic are apples (nearly 8,000 ha of orchards), plums (2,056 ha), sour cherries (1,425 ha), apricots (925 ha), cherries (901 ha), pears (745 ha) and peaches (407 ha; Buchtová, 2015). If *Phytophthora* pathogens are responsible for these diseases, they pose important and long-term risks to fruit producers. The aim of the present study was to verify whether *Phytophthora* pathogens were responsible for the recently increasing losses in Czech Republic fruit orchards, to evaluate pathogen diversity and virulence.

MATERIALS AND METHODS

Sampling and isolation

Sampling for the detection and isolation of *Phytophthora* pathogens was carried out from May to November during the fruit tree growing seasons of 2012 to 2018. Trees with sparse, yellowing or wilting foliage and declining trees were identified in the surveyed orchards. If collar rot or rot of the main roots were detected (based on the presence of of necroses, exudates, cracks in the bark, or bark discoloration), the bark of each tree was carefully removed, and the proximal transition zone between necrotised and healthy tissues was detected. Freshly colonised conductive tissues with characteristic reddish, brownish or honey colours were found, aseptically sampled (ca. 20–50 cm^2 of tissue) and stored in sterile plastic bags. In the case of root rots, samples of soil with damaged feeding roots of each tree were collected from three sites at depths of 10–20 cm in the root zone. A mixed sample of approx. 1–2 L was then created from each sampled tree. The samples were individually packaged in plastic bags and transferred to the laboratory for immediate processing.

Collected necrotised conductive tissues of collar or main roots were rinsed under tap water in the laboratory, and segments (3 × 3 mm) from the edge of each active lesion were excised. The pieces were then surface-disinfected by immersion in 95 % ethanol for 10 sec, rinsed in deionized sterile water and dried with sterile filter paper. The tissue pieces were then placed onto selective PARPNH agar medium (V8-juice 200 mL, pimaricin 10 ppm, ampicillin 200 ppm, rifampicin 10 ppm, quintozene 25 ppm, nystatin 50 ppm, hymexazole 50 ppm, agar 15 g, and $CaCO_3$ 3 g**;** Jung *et al*., 1996) in 9 cm diam. Petri dishes, and incubated at 20°C in the dark. After 2 to 5 d, the PARPNH plates were inspected for the presence of characteristic coenocytic mycelium, and hyphal tips of each isolate were transferred onto plates containing V8A medium (V8-juice 200 mL, agar 15 g, and $CaCO_3$ 3 g; Erwin and Ribeiro, 1996) for isolation of oomycetes. Separate tissue pieces were also placed in plates containing malt extract agar (Becton, Dickinson and Co.) for isolation of fungi. Pure isolates were transferred onto oatmeal agar (HiMedia Ltd) slants in tubes. Cultures of oomycetes were stored at 12°C in the Czech Collection of Phytopathogenic Oomycetes (CCPO, 2018).

Soil samples were processed using a baiting method adopted from that of Werres and Junker (2015). Approximately 150 cm^3 of soil containing damaged roots was placed in a deep plastic bowl, deionized water was added to 2–3 cm above the sample, and baits of young, healthy, previously tap water-rinsed leaves of the susceptible *Rhododendron yakushimanum* ′Silberwolke′ were added onto the water surface. These bait cultures were then incubated at room temperature in natural day/night conditions. The water was changed to prevent bacterial upsurge. When characteristic lesions on the baits appeared, the segments with necrotised tissues were excised, surface sterilized and incubated on PARPNH agar medium (described above).

Irrigation water samples were tested for the presence of oomycetes in August and September 2017 using the method of Werres and Junker (2015). Sources of irrigation water were investigated in six localities in southern Moravia and eastern Bohemia in the same fruit orchards investigated for the presence of *Phytophthora* diseases. Four washed and healthy leaves of different cultivars of susceptible *R. yakushimanum* Nakai (′Polaris′, ′Silberwolke′, ′Sonatine′) and *R.* ×*hybridum* Ker Gawl. (′Cun-

nigham White´) were placed into sterilised bags made from sparse fabric. Four bags containing leaf baits were put into the tested sources of irrigation water (small ponds, brooks and streams). In each case, the bags were left to float close under the water surface, and after 1 week, the samples were collected, transported to the laboratory and processed using leaf baits (described above).

The tests for the presence of oomycetes in nursery stocks from commercial trade were performed in the winter and spring of 2017 and 2018. In total, 33 shipments of 19 fruit tree types of *ex vitro* (produced by layering) and *in vitro* (produced from micropropagation) nursery stock of pome and stone fruits were investigated. From plants delivered from diverse national sources or from other EU countries (France, Italy, Poland, the Netherlands), at least 15 individual plants from each shipment showing damage of roots were selected and further processed. The whole root systems of plants (the material was sent as bare roots with partially cut and washed roots) were cut off and put into plastic bowls. Deionized water was added to each bowl, samples were incubated, and the isolates acquired using the baiting method (described above).

Morphological identification of isolates

Isolates were identified by comparing their colony growth patterns and morphological features with the species descriptions reported in the literature (Erwin and Ribeiro, 1996; Brasier *et al*., 2003; Gallegly and Hong, 2008; Hansen *et al*., 2009; Nechwatal *et al*., 2013; Nagel *et al*., 2013). Colony morphology was observed for 3- and 7-d-old cultures grown on corn meal agar (CMA, Sigma Aldrich), malt extract agar (MEA, Sigma Aldrich), carrot agar (CA, HiMedia Ltd), potato dextrose agar (PDA, HiMedia Ltd) and V8 juice agar (V8A), in 90 mm diam. Petri dishes at 20°C in darkness (two replications per medium). The characteristics of organism structures (oogonia, antheridia, chlamydospores and hyphal swellings) were measured after 5–10 d of incubation on V8A. Sporangia were produced after 24–48 incubation at 20°C by placement of a disk of mycelium from a 10-d-old culture grown on V8A in soil extract, prepared according to Jung *et al.* (1996). Measurements were performed with a light microscope under 400× or 1000× magnification, and 20 measurements were made for each evaluated characteristics.

Molecular characterization of isolates

The total genomic DNA was extracted from isolates using a DNeasy UltraClean Microbial Kit (QIAGEN, Germany) according to the manufacturer's instructions. For molecular identification, either the ITS region with ITS1/ITS4 primers (White *et al*., 1990) or the cytochrome *c* oxidase subunit I (COI) region was amplified with OomCoxILevup/Fm85mod primers (Robideau *et al*., 2011). All PCR reactions were performed in a Mastercycler Nexus Gradient GSX 1 thermal cycler (Eppendorf) and visualised using agarose gel electrophoresis in 1% TBE buffer along with a 100-bp DNA ladder (New England Biolabs) as a size marker. PCR products were purified and sequenced in both directions by Macrogen Inc. (KR) using the same primers applied for PCR amplification. The sequences obtained were edited and aligned in BIOEDIT v. 7.0.9.0 (Hall, 1999) and compared with the sequences present in the GenBank database using a NCBI BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/) to identify the closest related sequences. Only published sequences were considered.

Pathogenicity tests

Thirteen *Phytophthora* isolates obtained during orchard and nursery stock monitoring were selected to represent the extent of orchards of pome and stone fruit and the diversity of the isolated pathogens and used in the assay. The isolates were: *P. cactorum* (isolate no. 809), *P. cambivora* (819), *P. chlamydospora* Brasier & E.M. Hansen × *P. amnicola* T.I. Burgess & T. Jung (801), *P. cryptogea* (812), *P. gonapodyides* (H.E. Petersen) Buisman (864), *P. gregata* T. Jung, Stukely & T.I. Burgess (865), *P. sansomeana* E.M. Hansen & Reeser (868) and *P. syringae* (945), which all originated from apple trees, and *P. cactorum* (795) was from cherry, *P. cambivora* (815), was from cherry, *P. inundata* Brasier, Sánch. Hern. & S.A. Kirk (924) was from wild plum, *P. lacustris* Brasier, Cacciola, Nechw., T. Jung & Bakonyi (791) from apricot, and *P. plurivora* (789) was from cherry. Fresh cultures of isolates were obtained by transferring agar plugs to Petri plates containing V8A agar and incubating at 20°C in the dark for 7 d. The isolates were each incubated in 250 mL beakers with 25 mL CMA medium amended with antibiotics (pimaricin, 10 mg; ampicillin, 250 mg; and rifampicin, 10 mg) for 7 d at 20°C. Virulence of the isolates was tested on broadly used 1-y-old apple rootstocks of ´M26´ and plum rootstocks of ´St. Julien´ using the methods of Jeffers *et al.* (1981) and Thomidis *et al.* (2008). The dormant shoots of tested rootstocks were divided into segments (length 10 cm and width 0.5–0.8 cm), using a flamed sharp knife, and were each trimmed to a slant at the base. Four segments were aseptically put into medium covered by a pathogen colony and incubated for 10 d at 20°C in the dark. After incubation, the

epidermis of each segment was carefully removed, and the length of necrosis (from the agar surface) was measured on two opposite sides. The virulence of each strain was assessed in five replicates, i.e., 40 measurements were made and evaluated for each pathogen × rootstock combination. This experiment was carried out during February 2018.

Statistical analyses

One-way analysis of variance with Welch correction (Sheskin, 2011) was used to test the hypothesis that the size of necrosis was the same for all isolates and for both rootstocks. Welch correction was used because different variances were measured. A multiple comparison procedure using Welch tests with Holm correction (Sheskin, 2011) was used to evaluate the differences between individual isolates. Statistical analyses were performed using the R statistical program (R Core Team, 2017).

RESULTS

Occurrence of Phytophthora *and other root and collar pathogens*

During this investigation thousands of trees were found with characteristic *Phytophthora* disease symptoms. These included: rot of feeding and main roots; collar rot; presence of tarry and rusty spots on bark; lesions of bark and conductive tissues; sparse, yellowing and wilting foliage; premature leaf fall; and withering and death of trees (Figures 1 and 2). Diseased trees were identified in nurseries and in young and established 10–30-y-old orchards. Symptomatic trees were individually present in orchards but were usually grouped in disease "hot spots", with each containing dying or dead trees in the middle and with less-damaged trees exhibiting yellowing foliage around the margin. Sometimes, diseased transplants were identified in highly affected parts of orchards (Figure 2). The number of diseased trees varied greatly in the investigated orchards, from a few individuals to many hundreds of trees (up to ca. 15% or more of trees in orchards).

A total of 387 symptomatic fruit trees (65.1 % were pome fruit and 34.9 % were stone fruit trees) of nine different plant species, from 44 locations, were sampled from 12 of 15 regions of the Czech Republic, during 2012 to 2018 (Figure 3). In total, Oomycete species were isolated from 196 tree samples (50.6% of those sampled), and 13 *Phytophthora* species were identified. The most frequent pathogens were *P. cactorum* (58% of *Phytophthora* isolates), which was isolated from apple (*Malus domestica* Borkh.), apricot (*Prunus armeniaca* L.), cherry (*Prunus cerasus* L.), peach (*Prunus persica* (L.) Batsch), pear (*Pyrus communis* L.), plum (*Prunus domestica* L.), quince (*Cydonia oblonga* Mill.) and wild cherry (*Prunus avium* L.), and *P. plurivora* (16%), which was isolated from apple, cherry, myrobalan (*Prunus cerasifera* Ehrh.), pear and plum (Figure 4). Other species with lesser isolation frequencies were *P. syringae*. *P. chlamydospora×amnicola*, *P. cambivora* and others (Table 1, Figure 4). The greatest diversity of *Phytophthora* pathogens was identified in the Hradec Králové region (eight species), the South Moravian region (7) and South Bohemian region (6; Figure 3). *Phytophthora chlamydospora* × *amnicola* (GenBank Accession No. MK012378), *P. inundata* (MK012377) and *P. sansomeana* (MK012376) were identified as new taxa for the Czech Republic. Further, many *Pythium* s. l. isolates were obtained. In total, isolates of 13 *Pythium* s. l. species were obtained from 13.5% of sampled trees of seven host species. Among the most frequent pathogenic species were *Phytopythium vexans* (de Bary) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, which was isolated from 4% of the samples from four hosts (apple, apricot, myrobalan and pear), *Globisporangium intermedium* (de Bary) Uzuhashi, Tojo & Kakish. (from apple, apricot, myrobalan, pear and plum), *G. heterothallicum* (W.A. Campb. & F.F. Hendrix) Uzuhashi, Tojo & Kakish. (from pear), *G.* cf. *mamillatum* (Meurs) Uzuhashi, Tojo & Kakish. (from pear and apricot), *G. spiculum* (B. Paul) Uzuhashi, Tojo & Kakish. (from apricot), *Phy. citrinum* (B. Paul) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque (from apple and pear), *Phy. helicoides* (Drechsler) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque (from apple), *Pythium* cf. *coloratum* Vaartaja (from pear), *Py.* cf. *diclinum* Tokun. (from apple) and *Py. folliculosum* B. Paul (from cherry). As well, *Phytopythium litorale* (Nechw.) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, *Phy.* cf. *ostracodes* (Drechsler) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, and *Pythium* spp. were also isolated.

Out of 16 samples of irrigation water taken from four fruit orchards, 15 (94%) contained oomycetes, and *Phytophthora* pathogens were found in nine samples (56%). The most frequently isolated were *Phytophthora lacustris* and *P. citrophthora*, followed by *P.* cf. *hydropathica* C.X. Hong & Gallegly (new for the Czech Republic), *P. plurivora* and *P. bilorbang* Aghighi, G.E. Hardy, J.K. Scott & T.I. Burgess. Three species (*P. bilorbang*, *P.* cf. *hydropathica* and *P. citrophthora*) were only isolated from irrigation water. As well, *Phytopythium helicoides*, *Phy. litorale* and *G. heterothallicum* were also isolated from irrigation water.

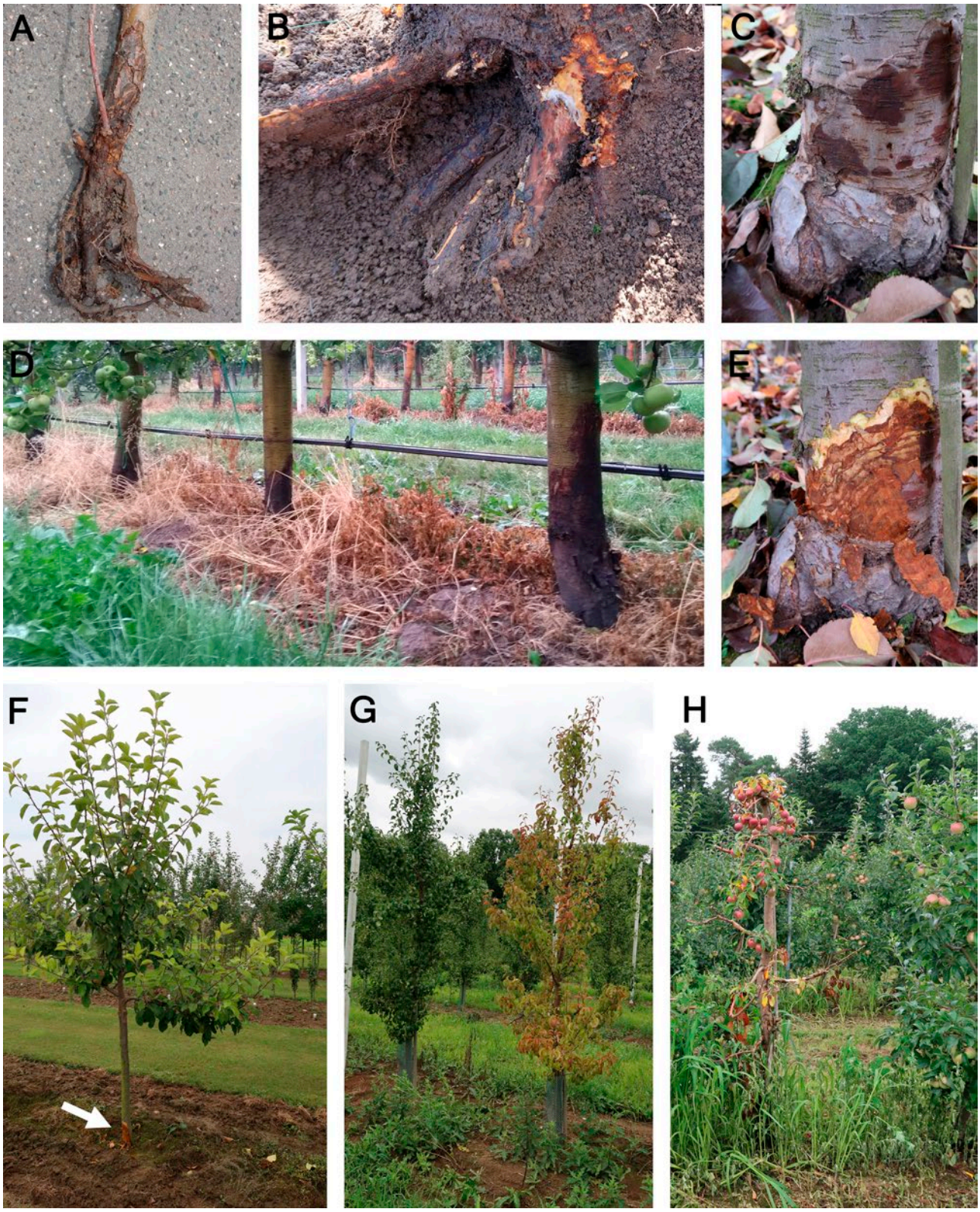

Figure 1. Characteristic symptoms of *Phytophthora* root and collar rot of *Malus domestica* (A–F, H) and *Pyrus communis* (G): A, root and collar rot of a young tree; B, root rot of a mature tree; C, characteristic tar spots on a tree collar; D, some apple orchards severely damaged by the disease; E, collar rot after the removal of bark (tree from Picture C); F, young tree with yellowed foliage resulting from collar rot (arrow); G, characteristic foliage yellowing of a pear tree; H, heavily diseased trees are characterised by production of small fruits

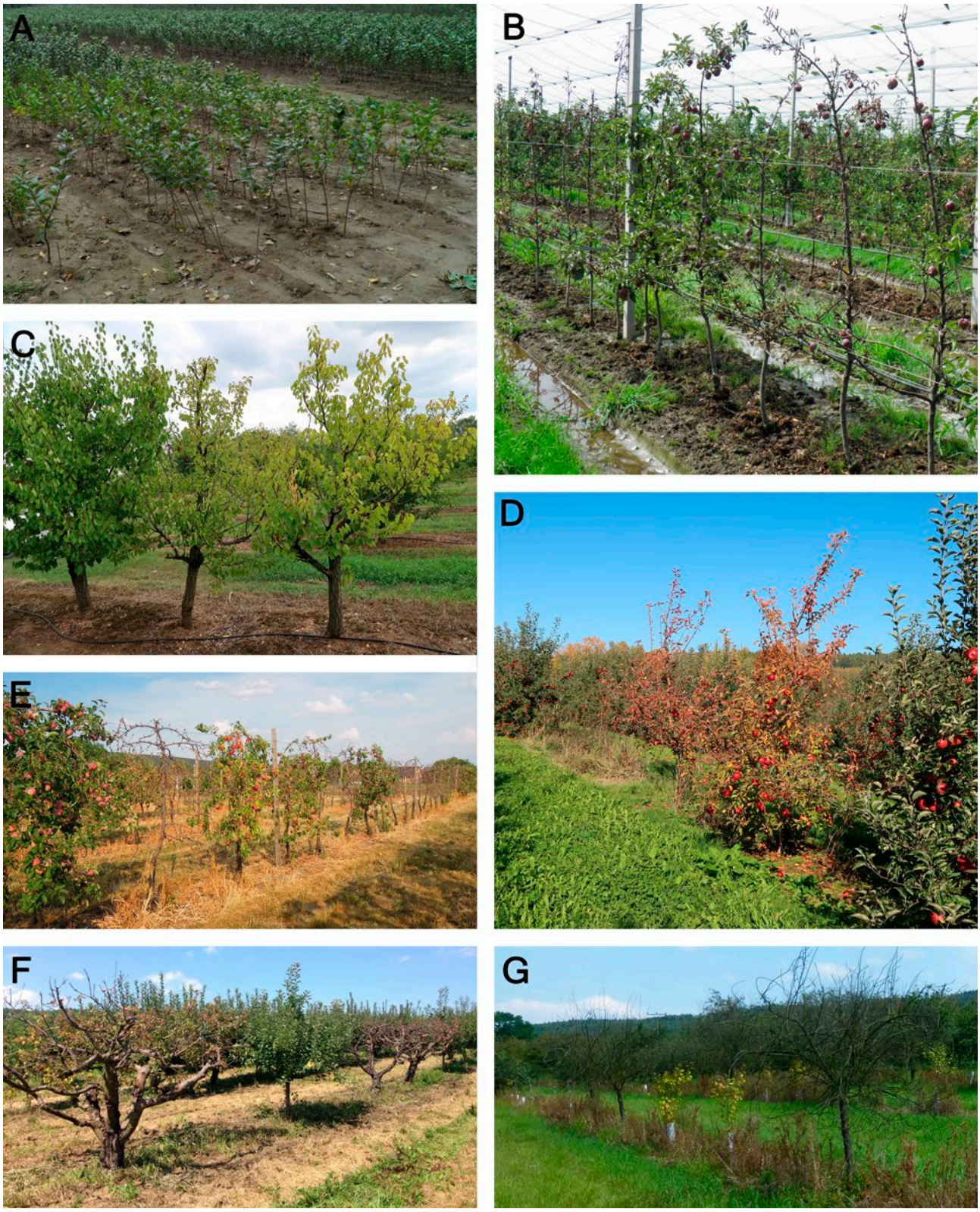

Figure 2. Characteristic damage of *Malus domestica* fruit nurseries and orchards (A, B, D–F) and *Prunus domestica* (C, G): A, yellow foliage of infected seedlings, B, an excess of water promoted the disease caused by *P. chlaymdospora* × *amnicola* in a young orchard; C, new disease outbreaks are apparent due foliage yellowing; D, premature leaf senescence and shedding of infected trees; E, characteristic disease focus in an orchard row; F, killed mature trees; G: attacked replanting in a damaged orchard.

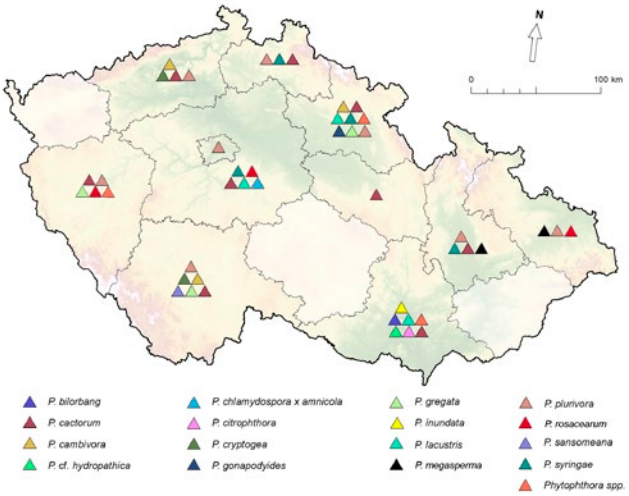

Figure 3. Regional map of the occurrence of *Phytophthora* spp. in fruit orchards (including irrigation water) in the Czech Republic. The regions without data are in dull colour.

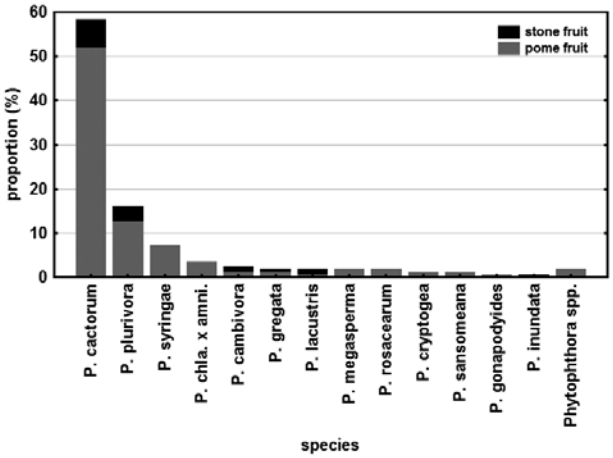

Figure 4. Frequency of occurrence of *Phytophthora* in fruit trees in the Czech Republic.

The investigation also showed that 71% of sampled lots of *ex vitro*-produced nursery stock contained plants infected by oomycetes, whereas *in vitro* plants were free of these organisms. *Phytophthora cactorum*, the only species of the genus isolated from nursery stock, was the most frequently isolated oomycete, identified in 46% of sampled groups of *ex vitro*-produced plants, and 25% of other positive samples were infested only by *Pythium* spp. (Table 2). *Phytophthora cactorum* was identified in planting material from all source countries in frequencies from 36 (the Netherlands) to 67% (Poland) of tested lots (Table 2). Some pathogenic species from *Pythium* s. l. were also obtained from nursery stock. The most frequent were *Phytopythium vexans* (29% of samples) and *Globisporangium intermedium* (14%). The other less-frequently isolated species were *Phy. citrinum*, *Py. emineosum* and *Py. litorale* (Table 2).

Some fungal pathogens were isolated from 37 (9.5 %) of samples of damaged roots and collars of fruit trees during this study. Identified pathogens causing collar and stem diseases included *Cadophora luteo-olivacea* (J.F.H. Beyma) T.C. Harr. & McNew (from collar rot of pear), *Calosphaeria pulchella* (Pers.) J. Schröt. (from canker of apricot), *Cylindrocladiella parva* (P.J. Anderson) Boesew. (from collar rot of plum), *Diaporthe eres* Nitschke, *D. rudis* (Fr.) Nitschke and *Diaporthe* sp. (from canker of apple and pear), *Valsaria insitiva* (Tode) Ces. & De Not. (from canker of apple and peach), *Leucostoma persoonii* (Nitschke) Höhn. (from canker of apple**,** apricot and peach), *Neonectria* sp. (from canker of plum), *Peyronellaea obtusa* (Fuckel) Aveskamp, Gruyter & Verkley (from canker of pear), *Trametes versicolor* (L.) Lloyd (silver leaf of plum). Identified root rot pathogens included *Didymella pomorum* (Thüm.) Qian Chen & L. Cai (from apricot), *Fusarium oxysporum* Schltdl. and *F. sporotrichioides* Sherb. (from apricot), *Nectria haematococca* Berk. & Broome 1873 (from apple and pear), *Roesleria subterranea* (Weinm.) Redhead (from pear), and *Valsaria insitiva* (rot of main roots of apricot). Some of these pathogens (*Cylindrocladiella parva*, *Diaporthe rudis*, *Peyronellaea obtusa* and *Roesleria subterranea*) or the associated diseases (collar rot of pear caused by *Cadophora luteo-olivacea*, silver leaf of plum caused by *Trametes versicolor,* root rot of apricot caused by *Didymella pomorum*) are the first records for the Czech Republic.

Factors potentially affecting the numbers of diseased trees were not systematically evaluated. However, it was apparent that younger orchards (up to 10 or 15 y old) were more damaged than older orchards. Diseased trees were more frequent in places with locally unsuitable soil conditions (poor drainage, high water table), and these trees apparently served as long-term reservoirs of pathogens infection in affected orchards. Unsuitable applications of water to tree collars or their direct surroundings by drip irrigation systems were also associated with collar rot development.

Influence of rootstocks and isolates on the extent of damage

The difference in the extent of damage caused by different *Phytophthora* pathogens in ´M26´ and ´St. Julien´ rootstocks was highly significant ($a = 8.7 \times 10^{-8}$). The mean extent of necrosis in ´M26´ rootstock was 15.0 mm, whereas the mean for ´St. Julien´ was 21.6 mm. The origin of the isolates (pome or stone fruits) did not have

Table 1. Overview of *Phytophthora* spp. isolated from fruit trees (host species, cultivars and rootstocks) and diseases they caused (C: collar rot, R: root rot) in the Czech Republic.

Phytophthora species	Identified host and disease			
	Species, cultivar	Disease	Rootstock	Disease
P. cactorum	*Cydonia oblonga*	R	*Cydonia oblonga*	C,R
	Malus domestica	C,R	Geneva G11	R
	Malus domestica ´Bohemia´	R	JTE	C
	Malus domestica ´Braeburn Lochbuie´	R	M9	C,R
	Malus domestica ´Champion´	R	M26	R
	Malus domestica ´Golden Delicious´	R	MA	C,R
	Malus domestica ´Heliodor´	R	MM106	C,R
	Malus domestica ´Idared´	R	*Malus domestica*	C,R
	Malus domestica ´Jonagold´	R	P14	C,R
	Malus domestica ´Luna´	C,R	Pajam	R
	Malus domestica ´Melba´	R	*Prunus avium*	R
	Malus domestica ´Minerva´	C,R	*Prunus cerasifera*	R
	Malus domestica ´Red Jonaprince´	C,R	St. Julien	R
	Malus domestica ´Red Topaz´	C		
	Malus domestica ´Remo´	R		
	Malus domestica ´Rozela´	C,R		
	Malus domestica ´Selena´	R		
	Malus domestica ´Topaz´	C,R		
	Prunus armeniaca	C,R		
	Prunus avium	R		
	Prunus avium ´Burlat´	R		
	Prunus cerasus ´Fanal´	R		
	Prunus cerasus ´Újfehértoi Fürtös´	R		
	Prunus domestica	C,R		
	Prunus persica	R		
	Prunus persica ´Favorita Morettini´	R		
	Pyrus communis	C,R		
	Pyrus communis ´Alexander Lucas´	C,R		
	Pyrus communis ´Bohemica´	C,R		
	Pyrus communis ´Conference´	C,R		
P. cambivora	*Malus domestica*	C,R	M9	R
	Prunus cerasus	C,R		
P. cryptogea	*Malus domestica* ´Topaz´	C,R	M9	R
	Malus domestica ´Topaz Red´	R	MM106	R
	Prunus domestica ´Reine Claude d'Althan´	R	*Prunus cerasifera*	R
P. gonapodyides	*Malus domestica* ´Orion´	C,R	-	
P. gregata	*Malus domestica* ´Champion´	R	M26	R
	Malus domestica ´Orion´	C,R	*Prunus mahaleb*	R
	Prunus cerasus	R		
P. chlamydospora × *amnicola*	*Malus domestica*	R	-	
	Malus domestica ´Braeburn´	R		
	Malus domestica ´Golden Delicious´	R		
P. inundata	*Prunus domestica*	R	-	
P. lacustris	*Malus domestica* ´Topaz´	R	MM106	R
	Prunus armeniaca	C,R	St. Julien	C,R
P. megasperma	*Malus domestica*	R	-	

(Continued)

statistically significant effects on the average length of necroses in either rootstock (data not presented).

The virulence of particular isolates in different rootstocks is shown in Table 3. Most of the isolates caused greater damage to ´St. Julien´ than to ´M26´. These differences were usually (with the exceptions of the strains *P. cambivora* 819 and *P. lacustris* 791) statistically supported. The greatest differences in virulence towards the

Table 1. (Continued).

Phytophthora species	Identified host and disease			
	Species, cultivar	Disease	Rootstock	Disease
P. plurivora	*Malus domestica*	R	JTE	C,R
	Malus domestica ′Bohemia′	C,R	M9	R
	Malus domestica ′Idared′	R	M26	R
	Malus domestica ′James Grieve′	C,R	*Prunus cerasifera*	R
	Malus domestica ′Red Topaz′	C,R		
	Malus domestica ′Topaz′	C,R		
	Prunus cerasifera nigra	R		
	Prunus cerasus ′Újfehértoi Fürtös′	C,R		
	Prunus domestica	R		
	Prunus domestica ′Haganta′	R		
	Prunus domestica ′Reine Claude d'Althan′	R		
	Pyrus communis	R		
	Pyrus communis ′Alexander Lucas′	C,R		
P. rosacearum	*Malus domestica*	R	M26	R
	Malus domestica ′Selena′×′Rozela′	R		
P. samsomeana	*Malus domestica* ′Topaz Red′	R	M9	R
	Pyrus communis ′Alexander Lucas′	R		
P. syringae	*Malus domestica*	R	M9	
	Malus domestica ′Melodie′	R		
	Malus domestica ′Topaz′	R		
Phytophthora spp.	*Malus domestica* ′Rozela′	R	M26	C,R
	Malus domestica ′Topaz′	R,C		
	Malus domestica ′Topaz Red′	R		

rootstocks were detected in *P. gregata* (necrosis in ′St. Julien′ was by 57% longer), *P. gonapodyides* (48%) and *P. plurivora* (46%). However, for apple ′M26′ rootstock, the three most virulent isolates were *P. cactorum* 795, *P. cactorum* 809 and *P. syringae* 945. The differences in virulence to rootstock of *P. cactorum* 795, and *P. syringae* 945 were statistically significant (Table 3).

Virulence of Phytophthora *pathogens to rootstocks*

In both infection experiments (′St. Julien′ and ′M26′), the mean necrosis lengths differed significantly among the isolates, and hypotheses of the equality of mean values of necrosis length were rejected ($P < 0.001$). The differences among the isolates were greater in ′St. Julien′ rootstock than in ′M26′.

In ′M26′ rootstock, the most pathogenic strains were *P. lacustris* (isolate no 791; mean necrosis length = 21.2 mm), *P. chlamydospora* × *amnicola* (801; mean = 20.0 mm), and *P. cryptogea* (812; mean = 17.0 mm). The isolate *P. syringae* (945) was the least pathogenic (Table 3). Differences in virulence among particular isolates were less important, and only three homogeneous groups (at $a = 0.05$), with overlaps, were established (Figure 5).

In ′St. Julien′, the most pathogenic strains were *P. gregata* (isolate 865; mean necrosis length = 35.6 mm) *P. chlamydospora* × *amnicola* (801; mean = 33.8 mm), and *P. sansomeana* (868; mean = 26.9 mm) (Table 3). The isolate *P. syringae* (945) was again the least pathogenic. Differences in virulence among particular isolates were important and five homogeneous groups ($a = 0.05$) were established (Figure 5).

DISCUSSION

The presented outcomes clearly demonstrated the importance of Oomycete pathogens in fruit orchards in the Czech Republic. It is very likely that the outcomes of this study (51% of positively tested trees, 17 *Phytophthora* and 13 *Pythium* s. l. identified species) are underestimated because of the limited number and locations of sampling, absence of sampling of asymptomatic trees, and potential displacing of *Phytophthora* pathogens by secondary fungal pathogens in lesions. Moreover, many species of *Pythium* s. l. are susceptible to hymexazol (Kato *et al*., 1990), which was used in the isolation medium utilized in this study. Documented tree losses reached 15 % or more diseased or dead trees in the most affected, usually younger, orchards (Figure 2). This was

Table 2. Overview of *Phytophthora* and *Pythium* s. l. isolations from commercial nursery material.

Host species	Rootstock	Country of origin	Year	Pathogen
Cydonia oblonga	Kwee Adams	NL	2018	*P. cactorum, Phy. vexans*
Cydonia oblonga	MA	NL	2017	*P. cactorum*
Malus domestica	A2	NL	2018	-
Malus domestica	Geneva G11	FR	2017	*P. cactorum, Phy. vexans*
Malus domestica	MM106	NL	2018	-
Malus domestica	MM106	NL	2017	*Py. litorale*
Malus domestica	M26	NL	2018	*P. cactorum, Phy. citrinum,*
Malus domestica	M9	CZ	2018	*P. cactorum*
Malus domestica	M9	CZ	2018	*G.* cf. *intermedium*
Malus domestica	M9	FR	2018	*P cactorum, Phy. vexans*
Malus domestica	M9	FR	2018	*Phy. vexans*, *Py.* cf. *litorale*
Malus domestica	M9	NL	2017	*Phy. vexans*
Malus domestica	M9	NL	2018	*P. cactorum, Phy. vexans*
Malus domestica	M9	NL	2018	-
Malus domestica	M9 Emla	FR	2018	*Py.* cf. *litorale*
Malus domestica	P14	PL	2018	*P. cactorum*, *Py. emineosum*
Prunus avium		NL	2017	-
Prunus avium		NL	2018	*Phy.* cf. *vexans*
Prunus cerasifera		CZ	2017	*P. cactorum*
Prunus cerasifera		CZ	2018	-
Prunus cerasifera		PL	2018	*P. cactorum*, *G. intermedium*
Prunus cerasifera	M29C/in vitro	IT	2017	-
Prunus domestica	St. Julien	NL	2017	*P. cactorum, Phy. vexans*
Prunus domestica	St. Julien	NL	2018	-
Prunus domestica	Wavit®/in vitro	IT	2017	-
Prunus domestica	Wavit®/in vitro	IT	2018	-
Prunus domestica × *canescens*	Gisela® 5/in vitro	IT	2017	-
Prunus domestica × *canescens*	Gisela® 6/in vitro	IT	2017	-
Prunus mahaleb		PL	2017	-
Prunus mahaleb		PL	2018	*P. cactorum*, *G. intermedium*
Prunus spinosa	WUR S766	NL	2018	-
Pyrus caucasica		PL	2017	*G.* cf. *intermedium*
Pyrus caucasica		PL	2018	*P. cactorum*

surprising, as no important problems potentially connected with oomycetous pathogens have been previously reported in the area. Moreover, *Phytophthora* pathogens have not been recently highlighted as important phytopathogenic problems in fruit production in other Central European countries, including Poland or Germany which are important fruit producers in the region. The last important *Phytophthora* outbreak in fruit trees was reported in temperate Europe more than 60 years ago (e.g., Braun, 1952; Buddenhagen, 1955; Smith, 1953). Effective control measures were developed, and more resistant rootstock began to be used, so *Phytophthora* problems became less important in modern intensive orchards (Smith *et al*., 1988). Although the most frequent *Phytophthora* pathogen (*P. cactorum*) is the same, the Czech outbreak is likely to be recent as the main problems occur in orchards planted after 2000, and is probably independent of previously described outbreaks (e.g., Braun, 1952; Buddenhagen, 1955; Smith, 1953).

The recent increase in *Phytophthora* activity could be connected to several factors. The most important is likely to be the increase of imports of infected rootstocks, as plant trade is a main pathway for spreading these pathogens (Brasier, 2008). In our investigation, the *ex vitro*-plant material from all tested countries was found to be infected, and *P. cactorum* was identified in 46.4% of tested sample lots. Another factor affecting the importance of alien *Phytophthora* spp. is likely to be changing

Table 3. Mean necrosis lengths (median and standard deviations) caused by individual isolates (isolates from pome fruit are marked by the symbol *) on ´M26´ and ´St. Julien´ rootstocks and differences in pathogenicity toward both rootstocks.

Species	Isolate No.	Mean necrosis length (mm)		Probability (*P*)
		M26	St. Julien	
P. cactorum	795	13.76 (10.34)	8.73 (2.19)	0.045
*P. cactorum**	809	16.79 (13.03)	11.38 (5.29)	0.097
P. cambivora	815	13.04 (9.30)	20.60 (7.62)	0.008
*P. cambivora**	819	11.43 (6.54)	13.89 (9.36)	0.341
*P. cryptogea**	812	16.97 (3.52)	22.61 (5.66)	< 0.001
*P. gonapodyides**	864	12.91 (6.69)	24.93 (10.82)	< 0.001
*P. gregata**	865	15.24 (4.25)	35.56 (11.38)	< 0.001
P. chlamydospora × *amnicola**	801	19.98 (7.84)	33.81 (11.76)	< 0.001
P. inundata	924	14.05 (8.96)	24.46 (11.56)	0.003
P. lacustris	791	21.16 (12.79)	26.26 (13.32)	0.225
P. plurivora	789	13.05 (6.65)	24.36 (8.19)	< 0.001
*P. sansomeana**	868	15.54 (5.73)	26.93 (6.67)	< 0.001
*P. syringae**	945	11.03 (4.71)	6.73 (7.77)	0.042

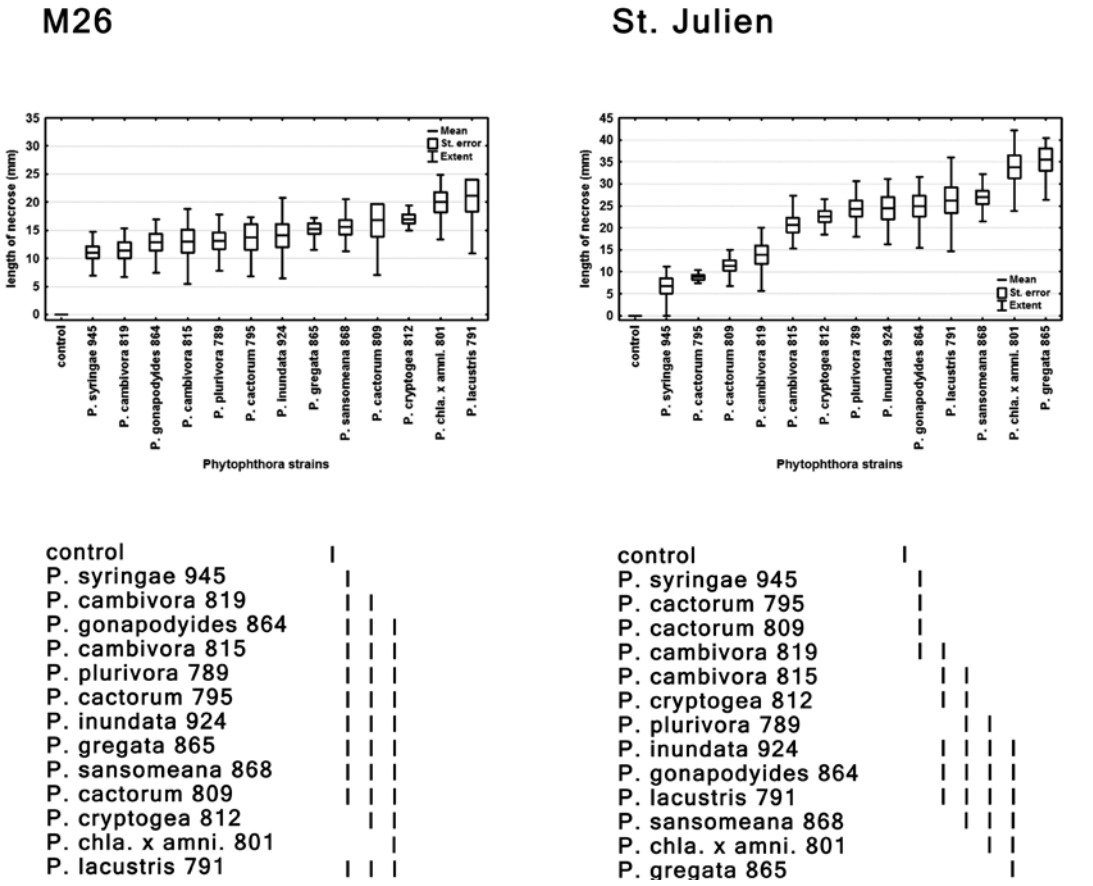

Figure 5. Virulence of *Phytophthora* isolates in ´M26´ and ´St. Julien´ rootstocks. The means, quartiles, extents and outliers are presented in the graphs and homogeneous groups in the tables.

environmental conditions (Desprez-Loustau *et al*., 2016) in Central Europe. Especially in the last 5 years, severe dry summers have occurred in the Czech Republic (Šercl *et al*., 2018), which has led to water stress on hosts and increased susceptibility to pathogens. The increased use of irrigation in orchards has provided conditions suitable to *Phytophthora* disease development. The recent Czech findings are similar to the situation in the Mediterranean region and the Balkan Peninsula, where severe root and collar rots and locally important losses caused by *Phytophthora* spp. were identified (Elena and Paplomatas, 1999; Pane *et al*., 2009; Nakova, 2010a; Kurbetli *et al*., 2017). These led to investigation of their control, and evaluation of the resistance of rootstocks, in the region (Thomidis, 2001; Boughalleb *et al*., 2006; Thomidis *et al*., 2008; Belisario *et al*., 2009).

There is poor awareness of *Phytophthora* pathogens in fruit production in the Czech Republic. These pathogens have not been systematically monitored and the causes of individual declines have apparently been misidentified, so no effective control measures have been developed. This has resulted in a limited number of fungicides effective against soil oomycetes to be registered for use in fruit orchards in this country (Anonymous, 2018). As well, the frequently used rootstocks have not been assessed for susceptibility to the most frequently occurring naturalized oomycetes. The poor knowledge and pressure for gain have led to the problems being overlooked. Implementation of unsuitable procedures in orchards and the growing of fruit trees without regard to locally unsuitable sites (high water tables, heavy or impermeable clay soils) which serve as long-term infection reservoirs in infected orchards, and repeated replanting with susceptible rootstocks, have also exacerbated disease problems.

Among the isolated pathogens, *P. cactorum* was the most frequently occurring species. This usually damaged apple trees (affecting about a half of the fruit orchards in the area; Buchtová, 2015), but it was also the most frequently occurring pathogen in stone fruit trees. This species is the most important pathogen of apple trees (Smith *et al*., 1988; Harris, 1991; Sutton *et al*., 2014), but also damages other hosts (Smith *et al*., 1988; Ogawa *et al*., 1995; Erwin and Ribeiro, 1996). Moreover, *P. cactorum* is still frequently introduced with nursery stock. The next most important and well-known *Phytophthora* pathogens of apple trees are *P. syringae* (causing losses in cool European areas; Smith *et al*., 1988; Harris, 1991), *P. plurivora*, *P. megasperma* Drechsler, *P. roseacearum* E.M. Hansen & W.F. Wilcox, *P. cryptogea*, *P. cambivora* and *P. gonapodyides* (Smith *et al*., 1988; Harris, 1991; Erwin and Ribeiro, 1996; Hansen *et al*. 2009; Sutton *et al*., 2014) were also isolated. *Phytophthora citrophthora*, another known pathogen of pome and stone fruit in Southern Europe (Elena and Paplomatas, 1999; Thomidis, 2001; Pane *et al*. 2009; Nakova, 2010b), was identified in only in irrigation, but could also damage trees in adjacent irrigated orchards.

Other isolated less recognized pathogens (*P. gregata*, *P. chlamydospora* × *amnicola*, *P. inundata*, *P. lacustris* and *P. sansomeana*) were isolated from pome or stone fruit on only a few occasions. *Phytophthora gregata* was described in 2011 from dying swampy vegetation in Australia (Jung *et al*., 2011), and is known to cause root rot in forest and ornamental plants in different stands in the Czech Republic (CCPO, 2018). This pathogen was found to cause root and collar rot in ′Orion′ apple and root rot in ′Champion′ apple and cherry. The hybrid *P. chlamydospora* × *amnicola*, first isolated in 2009 and 2010 from water in Australia and South Africa (Nagel *et al*., 2013), was isolated from only a few orchards of one company, but the affected plantations were heavily damaged, with very frequent death of trees (Figure 2B). *Phytophthora inundata*, first known in Europe as *P.* sp. O-group, was originally described in 2003 (Brasier *et al*. 2003), and is known as a pathogen of some fruit trees including almond and olive (Safaiefarahani *et al*., 2013). The pathogen was found to cause root rot of plum in the Czech Republic. *Phytophthora lacustris*, formerly named P. taxon Salixsoil, is known as a moderately pathogenic species frequently distributed in riparian stands, usually causing root rot in some European native trees. The pathogen is also a severe fine root pathogen of peach seedlings, but it was found to be non-pathogenic to cherry and myrobalan (Nechwatal *et al*., 2013). We identified *P. lacustris* as a cause of collar rot and death of mature apricot trees and root rot of apple Topaz′. *Phytophthora sansomeana* was originally described from soybean in Midwestern states of the United States of America, and was also identified from Douglas-fir, alfalfa fields, weeds, gerbera and soil (Hansen *et al*., 2009; Rahman *et al*. 2014). This pathogen was found to cause extensive root rot in apple ′Topaz red′ on M9 rootstock and pear ′Alexander Lucas′.

An investigation of oomycete contamination of commercial nursery stock revealed a high proportion of *Phytophthora* infections (up to 61%). Infected plants were identified in all tested domestic and foreign sources of planting material, with the exception of *in vitro*-produced rootstocks.

The most frequent pathogens of nursery stocks were *P. cactorum*, *Phytopythium vexans* and *Globisporangium intermedium*. Coincidentally, *P. cactorum* was the most frequent *Phytophthora* species distributed in fruit

orchards in the Czech Republic. Similarly, *Phy. vexans* and *G. intermedium* were the most frequently isolated species among *Pythium* s. l. spp. from orchards. This supports the suggestion that plant trade is the main pathway for introducing plant pathogens (e.g., Brasier, 2008; Bienapfl and Balci, 2014), and the possibility that the trade and planting of infected material is the main factor shaping oomycetous communities of orchards after their establishment. The role of later infections (from irrigation water and mechanization) could be secondary. Development and use of appropriate disease management measures should not be focused only on orchards, where, the long-term effects of permissible controls can be questionable, but should focus on fruit tree nurseries as the main sources of infection. On the other hand, although a brief investigation of the diversity of oomycetes from irrigation water did not confirm irrigation water to be the main source of pathogens, the recording of potentially important species (*P. plurivora*, *P. citrophthora*) highlights this source of inoculum of pathogenic oomycetes (e.g., Ivors and Moorman, 2014).

Both of the tested rootstocks (´M26´ and ´St. Julien´) are frequently used in the Czech Republic. The apple rootstock ´M26´ has been evaluated as less susceptible (Utkhede, 1986; Bielenin, 1995) to intermediately and highly susceptible (Browne and Mircetich, 1993; Biggs *et al*., 2012) to different *Phytophthora* species, whereas ´St. Julien´ rootstock has been evaluated as having medium susceptibility to *P. citrophthora* and as more resistant to *P. cactorum* (Elena and Tsipouridis, 2000). However, in the present study ´St. Julien´ was evaluated as being generally more susceptible than ´M26´ to *Phytophthora* spp. Nine out of eleven tested species caused more extensive damage to ´St. Julien´ than ´M26´ rootstocks. Only two species, *P. cactorum* and *P. syringae,* caused larger necroses in ´M26´ than in ´St. Julien´. These two pathogens are probably specialised to apple trees, and this result is in accordance with evaluations of these pathogens as the most important in apple trees, *P. cactorum* worldwide and *P. syringae* in northwestern Europe (Harris, 1991). This is also in accordance with fact that ´St. Julien´ is relatively resistant to *P. cactorum* (Elena and Tsipouridis, 2000). On the other hand, generalisation at the host genus level (*Malus* vs. *Prunus*) is not possible due to variation in susceptibility of different rootstocks and cultivars (see Utkhede, 1986; Browne and Mircetich, 1993; Boughalleb *et al*., 2006).

The outcomes of pathogenicity tests (Table 3, Figure 5) indicated that the lesser-known and rarely isolated species, described from other continents (*P. gregata*, *P. chlamydospora* × *amnicola*, *P. inundata*, *P. lacustris* and *P. sansomeana*), were often the most virulent in the present study. They were the most virulent species in both tests. Information on the biology and hosts of these species is scarce (see above), and they are, with exception of *P. inundata* (Safaiefarahani *et al*., 2013), not known as important fruit tree pathogens. Although the differences in susceptibility among rootstocks and virulence between *Phytophthora* species were statistically significant, these results should be verified by field inoculation experiments, which would can take months or years to provide reliable results. However, the identified damage caused by these pathogens in the field, verified by the inoculation experiments, showed that these pathogens could pose potential threats to fruit orchards in the future.

This study documented a contemporary increase in the activity of many *Phytophthora* pathogens in fruit orchards in central Europe. The involvement of some well-known species (especially *P. cactorum*, *P. plurivora*) as well as new pathogens in Europe (*P. chlamydospora×amnicola*, P. *sansomeana*), causing important losses in different regions of the Czech Republic, has been confirmed. The increase of *Phytophthora* activity is probably related to the trade and planting of infected nursery stock, to climate change and water stress, to disregard of the problems caused by *Phytophthora* diseases in fruit orchards by stakeholders, and to inappropriate disease management. This situation needs further investigation focusing on the biology of these pathogens and the development of effective disease management, including the investigation and breeding of rootstocks for resistance.

ACKNOWLEDGEMENTS

We thank Mrs Lyliya Fedusiv and Šárka Gabrielová for their excellent technical assistance, Mr Vladimír Zýka for the preparation of Figure 3, and two anonymous reviewers for their comments and suggestions. This work was supported by the Technology Agency of the Czech Republic project No. TH02030521.

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