Received: 24 June 2020 | Revised: 5 January 2021 | Accepted: 7 January 2021

DOI: 10.1111/efp.12673

SHORT COMMUNICATION

Forest Pathology WILEY-BLACKWELL WILEY

Oomycetes in rhizosphere soil of ornamental plants from retail nurseries in Southern Sweden

Michelle Cleary[1] | Mimmi Blomquist[1] | Marylise Marchand[2] | Johanna Witzell[1]

[1]Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences (SLU), Alnarp, Sweden

[2]French National Institute for Agriculture, Food, and Environment (INRAE), Cestas, France

Correspondence
Michelle Cleary, Swedish University of Agricultural Sciences (SLU), Southern Swedish Forest Research Centre, Sundsvägen 3, 230 53 Alnarp, Sweden.
Email: Michelle.Cleary@slu.se

Funding information
Svenska Forskningsrådet Formas, Grant/Award Number: 2015-01192

Editor: H. Tuğba Doğmuş-Lehtijärvi

Abstract

Trade of herbaceous and woody ornamental plants is recognized as a principal pathway for the introduction of alien plant pathogens, such as soil-borne *Phytophthora* and *Pythium* species (oomycetes). The rhizosphere soil of container-grown plants obtained from 13 nurseries and garden centres in southern Sweden were sampled and the presence of *Phytophthora* and *Pythium* species determined using traditional baiting and isolation. DNA sequencing of isolates revealed five *Phytophthora* taxa including *P. cinnamomi*, *P. citricola* s.l., *P. plurivora*, *P. undulata* and a non-identified *Phytophthora* sp., as well as *Phytopythium* cf. *citrinum* and several species of *Pythium*. *Phytophthora cinnamomi* was detected on two different ornamental plants at the same nursery and is a first record of *P. cinnamomi* in Sweden. The survey revealed that a diversity of potentially pathogenic oomycetes is associated with ornamental plants available for sale in Sweden and emphasizes the need to recognize potential risks if these organisms get introduced in nature.

1 | INTRODUCTION

Oomycetes (commonly known as 'water moulds') include several plant pathogens belonging to the genera *Phytophthora*, *Pythium* and *Phytopythium*. *Phytophthora* pathogens in particular are an increasingly important threat to forests and other natural and semi-natural ecosystems in Europe causing devastating declines and dieback of important tree species (Jung, Colquhoun & Hardy, 2013). Human activities, including the international plant trade, have facilitated spread of *Phytophthora* species. Nursery stock of woody ornamentals may inadvertently contain infectious propagules in soil, organic debris and on roots, which may serve as vectors to gardens, urban landscapes and natural habitats where they are subsequently planted (Jung et al., 2016). Once established, air- and soilborne *Phytophthora* species can attack other, potentially new, hosts causing foliar dieback or root and collar rot, which affects the growth, vitality and survival of woody plants and trees. Recent analyses revealed several alien *Phytophthora* species in southern Swedish landscapes (Cleary et al., 2017). Here, we wanted to evaluate the risk of potentially important pathogenic *Phytophthora* species entering natural environments via planting of ornamental nursery stock.

2 | MATERIALS AND METHODS

Between June and August 2017, herbaceous and woody ornamental plants showing chlorosis, withering and/or necrotic lesions on foliage were anonymously purchased from 13 horticultural garden centres or nurseries in southern Sweden (Figure 1). Plants (totally 44) represented the following taxa: *Acer palmatum*, *Buxus sempervirens*, *Euonymus fortunei*, *Fragaria* spp., *Hebe ochracea*, *Ilex* spp., *Pinus mugo*, *Rhododendron* spp., *Stewartia pseudocamellia*, *Vaccinium* spp. and *Viburnum furcatum*.

FIGURE 1 Rhododendron spp. plants purchased from garden centres and ornamental nurseries showing symptoms of dieback, chlorosis and necrotic leaf spots

The plants were likely both imported to and produced in Sweden. At the laboratory, soil was shaken loose from the roots of each plant and approximately 400 g of soil was sampled, transferred to plastic boxes (15 cm × 15 cm 8 cm) and then flooded with 500 ml of distilled water. Fresh young leaflets of *Fagus sylvatica*, *Quercus robur* or *Rhododendron* 'Cunningham's White' were, after a few hours, placed on the surface of the water as baits and incubated at room temperature (approximately 21°C). Baits were checked daily and if characteristic necrotic spots or lesions appeared, leaves were blotted dry on tissue paper, cut into small pieces and placed on *Phytophthora* selective PAR(PH)-V8 agar amended with 10 μg/L rifampicin, 1 ml/L dimethyl sulfoxide [DMSO], 250 μg/L sodium ampicillin, 5 μg/L pimaricin, 100 μg/L pentachloronitrobenzene [PCNB], 50 μg/L hymexazol and 10 μg/L benomyl. Mycelia emerging from leaf pieces were subcultured to fresh potato dextrose agar (PDA) to obtain pure cultures. Plugs of PDA containing hyphae were transferred to 50 ml Falcon tubes of Malt extract broth and incubated at room temperature. After 2 weeks, mycelia was harvested, filtrated in deionized water, transferred to 2 ml tubes and lyophilized for 48 hr and ground to fine powder in a Fast Prep-24 bead beater. Genomic DNA was extracted using the E.Z.N.A SP Plant Kit following the manufacturer's instruction, and the concentration of extracted DNA was measured using NanoDrop® ND1000 (Wilmington, USA). PCR amplification of the internal transcribed spacer (ITS) region of the ribosomal DNA gene was performed using the universal ITS4 and ITS6 that target conserved regions in the 18S and 28S rDNA genes and the final cleaned PCR products were sent for Sanger sequencing. DNA sequences were manually aligned and edited using BioEdit and then compared with other *Phytophthora* sequences in the GenBank (National Centre for Biotechnology Information, NCBI) using BLAST (Basic Local Alignment Search Tool) and putatively identified based on sequence identity 99% or above. The sequences obtained for all isolates putatively identified as *Phytophthora*, *Pythium* or *Phytopythium* were registered in the GenBank (Accession no.'s MT663314-MT6633325 and MW366725-MW366737, Table 1).

3 | RESULTS AND DISCUSSION

From the 13 nurseries, we recovered 38 isolates of which 10 were identified as *Phytophthora* species and 13 as *Pythium* or *Phytopythium* species (Table 1). The majority of the *Phytophthora* isolates came from three plant taxa: *S. pseudocamellia*, *V. corymbosum* 'Darrow' and several species and varieties of *Rhododendron*. *Phytophthora plurivora*, a common species associated with tree damage in southern Sweden, was detected at two nurseries and from more than one plant species. Up to two different species of *Phytophthora* were recovered from an individual *Rhododendron* plant from the same nursery. *Phytophthora cinnamomi* was recovered from the rhizosphere soil of two individual plants (*R. luteum* 'Whitethroat' and *S. pseudocamellia*) at the same nursery and is a first record for this pathogen in commercially available ornamental planting stock in Sweden.

The survey provides a first look at the diversity of oomycetes that may move with ornamental plants commonly available in plant trade in Sweden and emphasizes the need to recognize the risks to forest health due to these organisms. *Phytophthora* species are highly capable of surviving long periods in soil and in potting substrates, especially in the presence of live plant roots (Jung, Vettraino, et al., 2013; Puértolas et al., 2018). In the relatively densely populated area of southern Sweden, planting of ornamental plants in private and public gardens and green areas is common and frequent, and *Phytophthora* damage has been increasingly observed in deciduous trees growing in the vicinity of such plantings (Cleary et al., 2017). It thus seems likely that nursery plants have contributed to spread of *Phytophthora* pathogens to the wider landscape—a pathway that has been pointed out also in other studies (Simamora et al., 2018).

The species *P. cinnamomi* that was isolated from two of our samples is one of the 100 worst invasive alien species due to its wide host range, and its global distribution which has been a direct result of the anthropogenic movement of trees, woody ornamentals and herbaceous plants (Burgess et al., 2017; Jung, Colquhoun & Hardy, 2013). Though it has not been detected in urban or natural settings in Sweden, its detection in the limited material screened in this study

TABLE 1 List of plant taxa that were collected from 13 horticultural nurseries and garden centres and analysed for oomycetes in the rhizosphere soil using classical techniques. *N* = no oomycetes identified

Plant Retailer	Plant species[1]	No. of plants	No. of isolates	Putative *Phytophthora* spp. detected	Putative *Pythium* or *Phytopythium* spp. detected	GenBank Accession no.
1	*Rhododendron* sp.	1	-	-	-	
2	*Rhododendron* spp.	3	-	-	-	
3	*Rhododendron* spp.	2	2	N	N	
4	*Rhododendron* spp.	3	5	N	N	
5	*Rhododendron* spp.	2	6	*P. citricola* s.l.	*Pythium* sp.	MT663314
				Phytophthora sp.		MT663315, MW366725
				P. undulata		MT663316, MT663317
				P. plurivora		MT663318
6	*Fragaria* x *ananassa* 'Korona'	2	2	-	*Pythium sp.*, *Phytopythium cf. citrinum*	MW366726, MW366729
	Rhododendron x 'Nova zembla'	1	1	-	*P. dissotocum*	MW366728
	Vaccinium angustifolium 'North blue'	1	1	-	*P. diclinum*	MW366727
7	*Rhododendron* x 'Nova zembla'	1	1	*P. undulata*	-	MT663319
	Rhododendron hanceanum x R. keiskei	1	1	*P. undulata*	-	MT663320
	Rhododendron 'Gunter Dinger'	1	1	-	*P. undulatum*	MW366730
	Acer palmatum 'Osakazuki'	1	-	-	-	
8	*Buxus sempervirens*	1	-	-	-	
	Euonymus fortunei 'Blondy'	1	-	-	-	
	Hebe ochracea 'James Stirling'	1	-	-	-	
	Ilex sp.	1	1	-	*Pythium* sp.	MW366734
	Pinus mugo	1	-	-	-	
	Rhododendron spp.	2	2	-	*Pythium spp.*,	MW366731, MW366732
	Rhododendron 'Bad Eilsen'	1	1	-	*P. coloratum*	MW366733
	Rhododendron degronianum ssp. *yakushimanum* 'Morning Red'	1	-	-	-	
	Rhododendron luteum 'Whitethroat'	1	1	*P. cinnamomi*	-	MT663321
	Stewartia pseudocamellia	1	1	*P. cinnamomi*	-	MT663322
	Viburnum furcatum	1	-	-	-	
9	*Azalea japonica* 'Petticoat'	2	1	*N*	*N*	
10	*Rhododendron* x 'Nova zembla'	1	1	*N*	*N*	
	Rhododendron x 'Dreamland'	1	1	*N*	*N*	
11	*Fragaria* x *ananassa*	1	1	-	*P. dissotocum*	MW366736
	Rhododendron 'Shamrock'	1	1	-	*P. coloratum*	MW366737
	Rhododendron sp.	1	1	*N*	*N*	
	Vaccinium corymbosum 'Patriot'	1	1	-	*Pythium sp.*	MW366735
12	*Fragaria vesca*	1	1	*N*	*N*	
	Rhododendron 'Mogambo'	1	1	*N*	*N*	

(Continues)

TABLE 1 (Continued)

Plant Retailer	Plant species[1]	No. of plants	No. of isolates	Putative *Phytophthora* spp. detected	Putative *Pythium* or *Phytopythium* spp. detected	GenBank Accession no.
13	*Rhododendron* 'Roseum Elegans'	2	2	*P. plurivora*	-	MT663323, MT663324
	Vaccinium corymbosum 'Darrow'	1	1	*P. plurivora*	-	MT663325

[1]Nursery material represents plants that are likely both produced in Sweden and imported.

suggests that it may already be prevalent in plant retailers in Sweden and thus its establishment and expression may only be a matter of time. The climate in the southern-most region of Sweden (Scania) is mild, with relatively warm, wet winters and longer spring and autumns. Recent CLIMEX model predictions (Burgess et al., 2017) suggest that if introduced to these conditions, *P. cinnamomi* could persist.

A total of 13 isolates from soils were identified as *Pythium* (12) or *Phytopythium* (1) species. Some *Pythium* species are known as causal agents of damping-off disease of tree seedlings in nurseries, but they may also be pathogens on herbaceous or woody plants in forest settings (Chavarriaga et al., 2007), sometimes causing symptoms that are indistinguishable from those caused by *Phytophthora*. The possible risks associated with movement of alien *Pythium* species from ornamental plants to forest landscape thus warrant further studies.

In conclusion, our results stress the need to consider ornamental plantings as a potential pathway for alien, tree-pathogenic oomycetes to spread into Swedish nature. To minimize this risk, nurseries and plant retailers need affordable detection tools that allow rapid, on-site screening of nursery stock for the presence of plant pathogenic oomycetes.

ACKNOWLEDGEMENTS

The authors would like to thank A. Riebe, M. Ghasemkhani and M. Elsafy for technical assistance. The work was funded by FORMAS—The Swedish Research Council, Grant # 2015-01192.

ORCID

Michelle Cleary https://orcid.org/0000-0002-0318-5974

REFERENCES

Burgess, T. I., Scott, J. K., Mcdougall, K. L., Stukely, M. J. C., Crane, C., Dunstan, W. A., Brigg, F., Andjic, V., White, D., Rudman, T., Arentz, F., Ota, N., & Hardy, G. E. S. J. (2017). Current and projected global distribution of *Phytophthora cinnamomi*, one of the world's worst plant pathogens. *Global Change Biology*, *23*, 1661–1674. https://doi.org/10.1111/gcb.13492

Cleary, M., Blomquist, M., Vetukuri, R. R., Böhlenius, H., & Witzell, J. (2017). Susceptibility of common tree species in Sweden to *Phytophthora cambivora*, *P. plurivora* and *P. cactorum*. *Pathology*, *47*, e12329. https://doi.org/10.1111/efp.12329

Chavarriaga, D., Bodles, W. J. A., Leifert, C., Belbahri, L., & Woodward, S. (2007). *Phytophthora cinnamomi* and other fine root pathogens in north temperate pine forests. *FEMS Microbiology Letters*, *276*, 67–74. https://doi.org/10.1111/j.1574-6968.2007.00914.x

Jung, T., Colquhoun, I. J., & Hardy, G. E. S. J. (2013). New insights into the survival strategy of the invasive soilborne pathogen *Phytophthora cinnamomi* in different natural ecosystems in Western Australia. *Pathology*, *43*, 266–288. https://doi.org/10.1111/efp.12025

Jung, T., Orlikowski, L., Henricot, B., Abad-Campos, P., Aday, A. G., Aguín Casal, O., Bakonyi, J., Cacciola, S. O., Cech, T., Chavarriaga, D., Corcobado, T., Cravador, A., Decourcelle, T., Denton, G., Diamandis, S., Doğmuş-Lehtijärvi, H. T., Franceschini, A., Ginetti, B., Green, S., … Peréz-Sierra, A. (2016). Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of *Phytophthora* diseases. *Pathology*, *46*, 134–163. https://doi.org/10.1111/efp.12239

Jung, T., Vettraino, A. M., Cech, T. L., & Vannini, A. (2013). The impact of invasive *Phytophthora* species on European forests. In K. Lamour (Ed.), *Phytophthora: A global perspective* (pp. 146–158). CABI.

Puértolas, A., Boa, E., Bonants, P. J. M., & Woodward, S. (2018). Survival of *Phytophthora cinnamomi* and *Fusarium verticillioides* in commercial potting substrates for ornamental plants. *Journal of Phytopathology*, *166*, 484–493. https://doi.org/10.1111/jph.12708

Simamora, A., Paap, T., Howard, K., Stukely, M., Hardy, G., & Burgess, T. (2018). *Phytophthora* Contamination in a Nursery and Its Potential Dispersal into the Natural Environment. *Plant Disease*, *102*, 132–139. https://doi.org/10.1094/pdis-05-17-0689-re

How to cite this article: Cleary M, Blomquist M, Marchand M, Witzell J. Oomycetes in rhizosphere soil of ornamental plants from retail nurseries in Southern Sweden. *Forest Pathology*. 2021;51:e12673. https://doi.org/10.1111/efp.12673