Acta Agrobotanica

DOI: 10.5586/aa.1665

Publication history
Received: 2016-03-01
Accepted: 2016-04-28
Published: 2016-06-30

Handling editor
Agnieszka Grinn-Gofroń, Faculty of Biology, University of Szczecin, Poland

Authors' contributions
Both authors contributed to the writing and to the formulation of the main objectives in the study; TP conducted the field-sampling, whilst CAS formulated the mathematical model and made Fig. 1 in ArcGIS; TP and CAS jointly made the sensitivity tests and Fig. 2

Funding
The phytopathology clinic sample data reported here were funded by consultancies with each individual nursery described. Dr. C.A. Skjøth is supported by European Commission through a Marie Curie Career Integration Grant (project ID CIG631745 and acronym SUPREME).

Competing interests
No competing interests have been declared.

Copyright notice
© The Author(s) 2016. This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits redistribution, commercial and non-commercial, provided that the article is properly cited.

Citation
Pettitt TR, Skjøth CA. A simple model describes development of early peaks in oomycete zoospore inoculum detected in southern UK outdoors horticultural reservoirs. Acta Agrobot. 2016;69(2):1665. http://dx.doi.org/10.5586/aa.1665

Digital signature
This PDF has been certified using digital signature with a trusted timestamp to assure its origin and integrity. A verification trust dialog appears on the PDF document when it is opened in a compatible PDF reader. Certificate properties provide further details such as certification time and a signing reason in case any alterations made to the final content. If the certificate is missing or invalid it is recommended to verify the article on the journal website.

INVITED ORIGINAL RESEARCH PAPER

A simple model describes development of early peaks in oomycete zoospore inoculum detected in southern UK outdoors horticultural reservoirs

Tim R. Pettitt*, Carsten Ambelas Skjøth

Institute of Science and the Environment, University of Worcester, Henwick Grove, WR2 6AJ, Worcester, United Kingdom

* Corresponding author. Email: t.pettitt@worc.ac.uk

Abstract

The numbers of water-borne oomycete propagules in outdoor reservoirs used in horticultural nurseries within the UK are investigated in this study. Water samples were recovered from 11 different horticultural nurseries in the southern UK during Jan–May in 2 "cool" years (2010 and 2013; winter temperatures 2.0 and 0.4°C below UK Met Office 30 year winter average, respectively) and 2 "warm" years (2008 and 2012; winter temperatures 1.2 and 0.9°C above UK Met Office 30 year winter average, respectively). Samples were analyzed for total number of oomycete colony forming units (CFU), predominantly members of the families Saprolegniaceae and Pythiaceae, and these were combined to give monthly mean counts. The numbers of CFU were investigated with respect to prevailing climate in the region: mean monthly air temperatures calculated by using daily observations from the nearest climatological station. The investigations show that the number of CFU during spring can be explained by a linear first-order equation and a statistically significant r^2 value of 0.66 with the simple relationship: $[CFU] = a(T - T_b) - b$, where a is the rate of inoculum development with temperature T, and b is the baseload population at temperatures below T_b. Despite the majority of oomycete CFU detected being non-phytopathogenic members of the Saprolegniaceae, total oomycete CFU counts are still of considerable value as indicators of irrigation water treatment efficacy and cleanliness of storage tanks. The presence/absence of *Pythium* spp. was also determined for all samples tested, and *Pythium* CFU were found to be present in the majority, the exceptions all being particularly cold months (January and February 2010, and January 2008). A simple scenario study (+2 deg C) suggests that abundance of water-borne oomycetes during spring could be affected by increased temperatures due to climate change.

Keywords

Pythiaceae; Saprolegniaceae; populations; seasonal-maxima; temperature

Introduction

Irrigation water, especially from surface sources such as reservoirs, ponds, and rivers has long been recognized as a significant source of infective plant pathogen inoculum, especially of oomycete genera such as *Pythium*, *Phytophthora*, and *Aphanomyces* [1]. Many horticultural nurseries treat their irrigation water to eliminate plant pathogens [2] and routinely need to test this water to confirm the efficacy of such treatments. Despite the presence of pathogenic species, the majority of the oomycete populations detectable in surface waters are not phytopathogens. Nevertheless, regardless of their

pathogenicity, generic counts of viable oomycete propagules before and after treatments such as slow sand filtration [3], are of considerable practical value in determining their efficacy [4]. For such testing to be cost-effective, planning and knowledge about the development of oomycete populations in time is vital. The majority of quantitative studies of oomycete populations in water use either baiting methods [5] or, more recently, quantitative PCR, with only a very few directly plating and quantifying viable colony forming units (CFU) [6–8]. Simple methods that can estimate CFU development as a function of environmental variables will enhance testing capabilities. In the UK, populations of oomycetes in lake water have been observed to show some periodicity with maxima falling in early spring, early summer, and autumn [9]. For effective testing of treated and untreated irrigation water to determine the efficacy of water treatments, a relatively large CFU count (>50 CFU L^{-1}) in the untreated reservoir water is desirable. This is an arbitrarily-set threshold based on experience, and equates to CFU counts of approximately 3–8 per plate; counts of less than this are insufficient to provide a confident control for the expected zero CFU counts from tests of water following effective treatment, or taken from clean storage tanks. Long-term planning that includes timing of sampling and forecasting of potential CFU in the water is therefore important. Here, using historic plant clinic data measuring total and potentially pathogenic oomycete CFU in water samples, we explore the potential impact of temperature on oomycete CFU numbers in horticultural reservoirs to determine the possibility of using air temperatures for population predictions. We develop a mathematical model to guide optimal sampling times and show how the model can also be used to assess the possible impact of climate change on population periodicity, thus enhancing long term structural planning.

Material and methods

Samples of untreated, unused ("raw") reservoir water were collected from 11 UK horticultural nurseries located in the southern UK (Fig. 1) and representing a wide range of production including woody and herbaceous perennial ornamentals, pot and bedding, and protected fruit and vegetables, as part of routine plant clinic tests for water treatment efficacy. These tests have been carried out on request for commercial nurseries throughout the UK since 1995 and normally consist of assessments of oomycete colonization of samples of water collected from water storage tanks pre- and post-treatment for plant pathogen control, from points of delivery to the plants and from raw water storage reservoirs. The reservoirs assessed were all located outdoors and consisted of either clay- or butyl-lined ponds ranging in volume from 300 to 7000 m^3 and varied in depth between 2 and 4 m. Water samples of 1 liter volume were assessed for viable oomycete propagule numbers using the membrane filtration-colony plating procedure [4,7], colonies were sub-cultured for identification to genus by morphology and culture characteristics, and were counted; count-data for members of the Saprolegniaceae and Pythiaceae were pooled and expressed as viable oomycete CFU L^{-1}. For this small study, data from 4 years were selected for analysis; 2 comparatively "cool" years, 2010 and 2013, with mean winter air temperatures 2.0 and 0.4°C below the UK Met Office 30 year average (http://www.metoffice.gov.uk/climate/

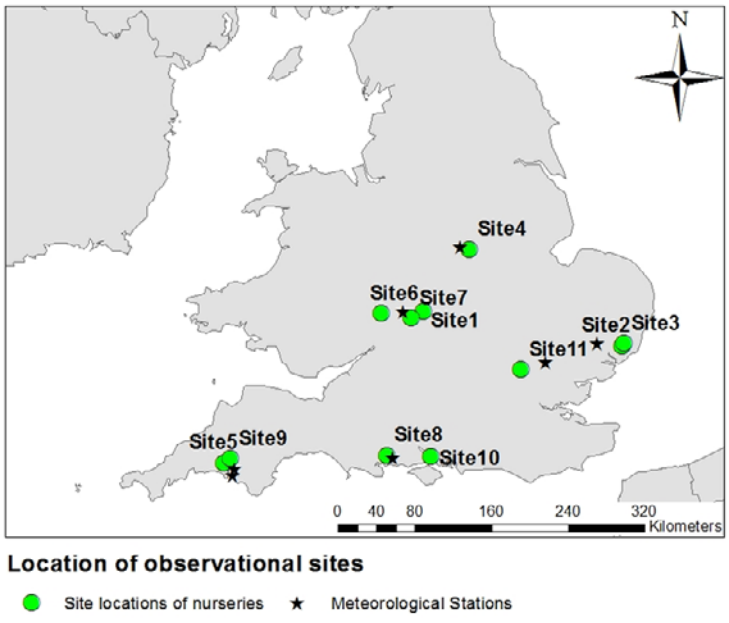

Fig. 1 Location of meteorological stations and nurseries from which the observations were taken in this study. Numbers of the nurseries correspond to the locations given in Tab. 1 and each site has an associated meteorological site.

Tab. 1 Study sites, their geographical location (degrees and decimal degrees), altitude, and the matching climate station from the GSOD data set.

Site	Examined years	Latitude	Longitude	Altitude (m a.s.l.)	Met station and USAF-ID	Altitude (m)
1	2008, 2010, 2012, 2013	52.183904	−1.740765	62	Pershore (35290)	31
2	2008, 2010, 2012, 2013	52.120020	1.345290	28	Wattisham (35900)	89
3	2008, 2010, 2012, 2013	52.145220	1.363379	31	Wattisham (35900)	89
4	2008, 2010, 2012, 2013	52.825492	−1.186343	64	Nottingham/East Midlands (34185)	93
5	2008, 2012, 2013	50.450539	−4.288463	96	Plymouth Mount Batt (38270)	50
6	2008, 2010, 2012	52.094832	−2.359566	137	Pershore (35290)	31
7	2008, 2010, 2012, 2013	52.101351	−1.897717	38	Pershore (35290)	31
8	2008, 2010, 2012, 2013	50.793996	−1.932456	15	Bornemouth/Hirrn (38620)	10
9	2008, 2010, 2012	50.500445	−4.195292	67	Plymouth Mount Batt (38270)	50
10	2008, 2010	50.845397	−1.276739	15	Bornemouth/Hirn (38620)	10
11	2008, 2013	51.779483	−0.130747	74	Stansted Airport (36830)	106

uk/summaries); and 2 "warm" years, 2008 and 2012, with mean winter air temperatures 1.2 and 0.9°C above the UK Met Office 30 year average.

Meteorological observations were obtained from the data set Global Summary of the Day (GSOD), which is exchanged as part of the World Weather Watch Program within the World Meteorological Organization (WMO). Each monitoring site was then matched with the nearest meteorological site (Tab. 1, Fig. 1) and monthly mean values of 2-meter temperature (air temperatures measured at WMO standard height, 1.25–2 m, above the ground; http://www.wmo.int/pages/themes/climate/climate_data_and_products.php) were calculated for each site.

Oomycete CFU counts from each sampling site were paired with their relevant monthly mean air temperatures (Tab. 1) using a simple mathematical relationship based on the following three assumptions: (***i***) the monthly increase in CFU in late-winter and spring is linearly related to monthly mean temperatures; (***ii***) CFU development only happens above a certain temperature threshold (***iii***) there will be a small baseload of CFU in the water, despite low temperatures. The suggested mathematical model has the following form:

$$[CFU] = a(T - T_b) - b \qquad \textbf{Eq. 1}$$

where a is the rate of inoculum development with temperature T, and b is the baseload population at temperatures below T_b. We found the optimal settings of the model parameters and tested the sensitivity of these parameters using typical climatological temperatures found in the southern UK during spring.

Results

Monthly mean numbers of oomycete CFU L^{-1} in spring increased with monthly mean air temperature after an initial lag phase over the temperature range 0 to approximately 5°C. This relationship was well described using a linear first-order equation (Eq. 1). Using monthly mean air temperature data and varying the values for a, b, and T_b, this equation was used to predict oomycete CFU counts, and the best fit was found with a slope of 60 CFU / $T - T_b$, a baseload population of 20 CFU, and a base temperature of 4.5°C (Tab. 2). These parameters gave a statistically significant r^2 value of 0.66 (Fig. 2) and a good description of the overall pattern in CFU development during spring.

Tab. 2 Sensitivity study of the mechanistic model.

	Bias; R^2						
Slope/BaseT	20	40	60	80	100	120	140
2.0	68; 0.63	−26; 0.62	−120; 0.62	−214; 0.62	−308; 0.61	−402; 0.62	−497; 0.62
2.5	77; 0.64	−7; 0.63	−92; 0.62	−177; 0.62	−261; 0.62	−346; 0.62	−431; 0.62
3.0	85; 0.65	10; 0.64	−66; 0.63	−141; 0.63	−217; 0.63	−292; 0.63	−368; 0.63
3.5	92; 0.66	26; 0.65	−41; 0.65	−108; 0.64	−175; 0.64	−242; 0.64	−309; 0.64
4.0	99; 0.66	41; 0.66	−17; 0.66	−76; 0.66	−135; 0.66	−194; 0.65	−253; 0.65
4.5	106; 0.66	55;0.66	**4; 0.66**	−48; 0.66	−99; 0.66	−150; 0.66	−202; 0.66
5.0	112; 0.65	69; 0.66	25; 0.66	−20; 0.66	−64; 0.66	−108; 0.66	−153; 0.66
5.5	117; 0.63	81; 0.65	44; 0.66	8; 0.65	−30; 0.65	−67; 0.65	−104; 0.66
6.0	122; 0.61	90; 0.64	59; 0.64	28; 0.64	−3; 0.65	−34; 0.65	−66; 0.65
6.5	125; 0.55	99; 0.61	73; 0.62	46; 0.62	19; 0.63	−7; 0.63	−34; 0.63
7.0	129; 0.50	107; 0.55	85; 0.57	63; 0.58	41; 0.59	18; 0.59	−4; 0.59

Best performing values are marked with bold.

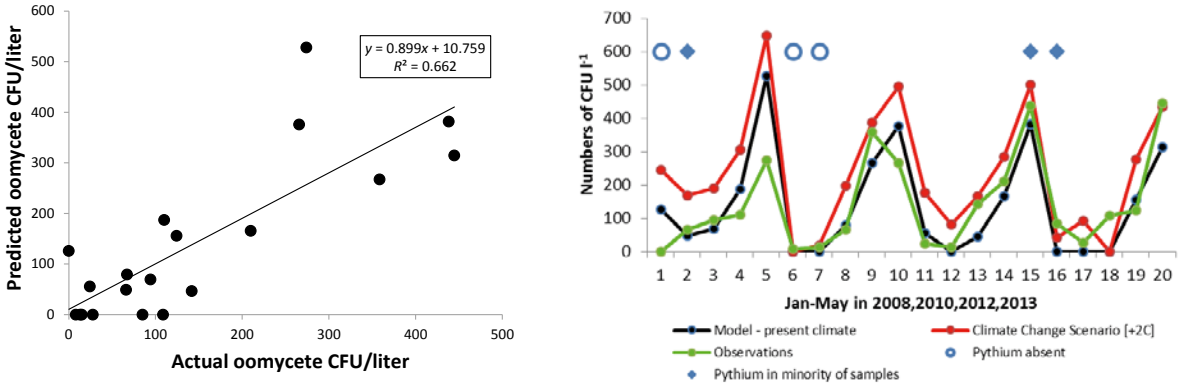

Fig. 2 Left – calibrated values of the mechanistic model for describing number of colony forming units. Right – results from the mechanistic model using a climate change scenario (+2 deg C) compared with actual and predicted observations. Figure also indicates months where *Pythium* spp. were not detected.

Whilst the majority of CFU appeared to be from the Saprolegniaceae (majority *Saprolegnia*, with some *Achlya* and *Leptolegnia*), *Pythium* (Pythiaceae) CFU were also detected, especially in samples collected in March, April, and May (Fig. 2). Although appearance of *Pythium* CFU in water samples could not be conclusively linked to temperature, the months when this genus was not detected, or was not present in the majority of samples, were the earlier months of January and February, especially in the coolest year, 2010.

The model also was used to predict CFU counts under a modest climate change scenario of +2 deg C (Fig. 2). These predictions indicated that whilst the 50 CFU L^{-1} were unlikely to be exceeded until March in 2008 and 2010, and April in 2012 and 2013, this count might be exceeded in January in the warmer years and in March and February in the cooler years 2010 and 2013, respectively.

Discussion

The approach of matching nearby meteorological stations has worked well in a number of studies on airborne spores [10] and pollen [11] when the focus is on overall climate in specific regions of Europe including the UK (e.g., [12]). Here the assumption that the mean monthly air temperature reflects the temperature in the aquatic environment in the nurseries assessed seems also to have worked well.

The good fit between mean monthly air temperature and mean oomycete CFU counts can be explained directly in terms of changes in water temperature as there is a reasonably close tie-up between monthly mean air temperatures and recorded lake epilimnion temperatures [13]. The samples assessed in this study were all collected from the surface waters of reservoirs that were of depths broadly equivalent to the epilimnion or superficial layer of a lake, and so might be expected to be more responsive to changes in air temperature and less bound by the complexities of stratification seen in larger bodies of water [14].

For carrying out tests to determine the efficacy of irrigation water treatments, an ideal number of oomycete CFU in untreated reservoir water would be ≥50 CFU L^{-1}. Water tests are costly and time-consuming, whilst accurate results, as early in the growing season as possible, are essential. Using the model described above it was possible to predict that the earliest potential timing of such tests for accuracy, based on the numbers of CFU in untreated water, would be once the mean monthly air temperature has reached 5.67°C.

The climate change scenario predicted earlier increases in CFU. This suggests that a consequence of climate change will be increased CFU development early in the spring. Similar phenological changes have been observed for a number of different species [15]. The results from this particular study suggest that climate change would affect suggested sampling periods and in some cases mitigation strategies as a consequence of the possible increased pathogen load implied by the larger concentrations of total oomycete CFU in the water, although such potential links between total oomycetes and pathogen genera require further investigation.

Conclusions

- It was possible to use monthly mean air temperatures to predict monthly mean oomycete CFU counts in horticultural reservoir waters.
- Predicted CFU counts can be used to identify optimum timings for early-season water tests for oomycetes.
- A modest climate change scenario (+2 deg C) indicated that the optimum timing for water testing would be earlier by as much as 1 month.

Acknowledgments

We acknowledge the valuable comments on the manuscript from Professor John Newbury. We acknowledge NOAA for providing access to the GSOD meteorological archive.

References

1. Hong CX, Moorman GW. Plant pathogens in irrigation water: challenges and opportunities. Crit Rev Plant Sci. 2005;24:189–208. http://dx.doi.org/10.1080/07352680591005838
2. Stewart-Wade SM. Plant pathogens in recycled irrigation water in commercial plant nurseries and greenhouses: their detection and management. Irrigation Science. 2011;29:267–297. http://dx.doi.org/10.1007/s00271-011-0285-1
3. Hunter PJ, Calvo-Bado LA, Parsons NR, Pettitt TR, Petch GM, Shaw E, et al. Variation in microbial communities colonizing horticultural slow sand filter beds: implications

for filter function. Irrigation Science. 2012;31:631–642. http://dx.doi.org/10.1007/s00271-012-0339-z

4. Büttner C, Bandte M, Pettitt TR. Filtration and centrifugation for detection of plant pathogens in irrigation water. In: Hong CX, Moorman GW, Wohanka W, Büttner C, editors. Biology, detection and management of plant pathogens in irrigation water. Saint Paul, MN: American Phytopathological Society; 2014. p. 139–148.
5. Werres S, Ghimire SR, Pettitt TR. Baiting assays for detection of *Phytophthora* species in irrigation water. In: Hong CX, Moorman GW, Wohanka W, Büttner C, editors. Biology, detection and management of plant pathogens in irrigation water. Saint Paul, MN: American Phytopathological Society; 2014. p. 125–138.
6. MacDonald JD, Ali-Shtayeh MS, Kabashima J, Stites J. Occurrence of *Phytophthora* species in recirculating nursery irrigation water. Plant Dis. 1994;78:607–611. http://dx.doi.org/10.1094/PD-78-0607
7. Pettitt TR, Wakeham AJ, Wainwright MF, White JG. Comparison of serological, culture, and bait methods for detection of *Pythium* and *Phytophthora* zoospores in water. Plant Pathol. 2002;51:720–727. http://dx.doi.org/10.1046/j.1365-3059.2002.00759.x
8. Reeser PW, Sutton W, Hansen EM, Remigi P, Adams GC. *Phytophthora* species in forest streams in Oregon and Alaska. Mycologia. 2011;103:22–35. http://dx.doi.org/10.3852/10-013
9. Hallett IC, Dick MW. Seasonal and diurnal fluctuations of oomycete propagule numbers in free water of a freshwater lake. J Ecol. 1981;69:671–692. http://dx.doi.org/10.2307/2259691
10. Skjøth CA, Sommer J, Frederiksen L, Gosewinkel Karlson U. Crop harvest in Denmark and Central Europe contributes to the local load of airborne *Alternaria* spore concentrations in Copenhagen. Atmos Chem Phys. 2012;12:11107–11123. http://dx.doi.org/10.5194/acp-12-11107-2012
11. Grewling Ł, Jackowiak B, Nowak M, Uruska A, Smith M. Variations and trends of birch pollen seasons during 15 years (1996–2010) in relation to weather conditions in Poznań (western Poland). Grana. 2012;51:280–292. http://dx.doi.org/10.1080/00173134.2012.700727
12. Skjøth CA, Bilińska D, Werner M, Malkiewicz M, Adams-Groom B, Kryza M, et al. Footprint areas of pollen from alder (*Alnus*) and birch (*Betula*) in the UK (Worcester) and Poland (Wrocław) during 2005–2014. Acta Agrobot. 2015;68(4):315–324. http://dx.doi.org/10.5586/aa.2015.044
13. Livingstone DM, Lotter AF. The relationship between air and water temperatures in lakes of the Swiss Plateau: a case study with palæolimnological implications. J Paleolimnol. 1998;19:181–198. http://dx.doi.org/10.1023/A:1007904817619
14. Piccolroaz S, Toffolon M, Majone B. A simple lumped model to convert air temperature into surface water temperature in lakes. Hydrology and Earth System Sciences. 2013;17:3323–3338. http://dx.doi.org/10.5194/hess-17-3323-2013
15. Thackeray, SJ, Sparks, TH, Frederiksen, M. Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. Glob Chang Biol. 2010;16:3304–3313. http://dx.doi.org/10.1111/j.1365-2486.2010.02165.x

Model opisujący wczesne piki zoospor *Oomycetes* w zbiornikach wodnych szkółek ogrodniczych na południu Wielkiej Brytanii

Streszczenie

Celem badań było określenie liczby występujących w wodzie propagul lęgniowców w zbiornikach szkółek ogrodniczych w Wielkiej Brytanii. Próbki wody pozyskano w okresie od stycznia do maja, w 11 szkółkach ogrodniczych zlokalizowanych w południowej Anglii. Analizy wykonano w lat "zimnych" (2010, 2013; z temperaturą w zimie odpowiednio 2.0 i 0.4°C poniżej 30-letnich średnich wieloletnich dla miesięcy zimowych) oraz dwóch "ciepłych" (2008 i 2012; z temperaturą w zimie odpowiednio 1.2 i 0.9°C powyżej 30-letnich średnich wieloletnich dla miesięcy zimowych; wg UK Met Office). Próbki analizowano pod względem całkowitej liczby propagul tworzących kolonię (CFU), z dominacją rodziny Saprolegniaceae i Pythiaceae, które w wynikach przedstawiono jako wspólną średnią miesięczną. Liczby CFU badano w odniesieniu do panujących warunków klimatycznych w regionie m.in. średniej miesięcznej temperatury. Mimo że większość badanych rodzin lęgniowców nie jest fitopatogenna, rodziny Saprolegniaceae i Oomycete wykorzystywane są jako wskaźniki skuteczności uzdatniania wody oraz określania stopnia czystości zbiorników wykorzystywanych do nawadniania

gleby w szkółkach ogrodniczych. Scenariusz wzrostu temperatury o 2°C sugeruje, że wzrost *Oomycetes* w wodach wiosną może wynikać ze wzrostu temperatur spowodowanych zmianami klimatycznymi.