Quantification of Pythium populations in ginseng soils by culture dependent and real-time PCR methods

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1. Introduction

Panax quinquefolius L. (American ginseng) has become a popular herbal remedy for a variety of ailments, including the common cold (Predy et al., 2005). Canadian production, concentrated in Southern Ontario, is valued at over $57 million annually (1999–2003) and represents over 60% of world production (Agriculture and Agri-Food Canada, 2003). Crop loss due to disease can be significant (Howard et al., 1994; Reeleder et al., 2006a) with young ginseng seedlings being particularly susceptible to “damping-off”, caused by Rhizoctonia solani Kühn, Pythium irregulare Buisman and Pythium ultimum Trow (Reeleder and Brammall, 1994). R. solani is generally managed through the use of fungicides but this option is currently not available for control of Pythium spp. These pathogens, as well as plant parasitic nematodes, are instead managed largely through fumigation of the planting site prior to seeding (Ontario Ministry of Agriculture, Food and Rural Affairs, 2005; Reeleder et al., 2007). There are few reports documenting Pythium populations in North American ginseng soils, but a preliminary study found populations ranging from 87 to 850 colony forming units (CFU) per g soil (Reeleder et al., 2002). A recent study showed that populations in some ginseng fields are dominated by P. ultimum and the P. irregulare species complex, whereas P. heterothallicum is dominant in other fields (Reeleder et al., 2007). This latter species has not been isolated from diseased ginseng roots and may not be pathogenic (Reeleder and Brammall, 1994).
Pythium populations in soils and plant roots have been assessed using a variety of techniques. The simplest method is based on the number of CFU formed after dilution plating on selective media (Jeffers and Martin, 1986). However, this method is labour intensive, does not allow for discrimination among morphologically similar species, and can be hindered by competition from other soil organisms. More sophisticated immunological methods, such as ELISA, have also been employed for Pythium quantification (Yuen et al., 1998), but these methods are still culture dependent.

More recently, real-time PCR (qPCR) has been used for the fast, accurate and culture independent quantification of a variety of pathogens (including Pythium spp.) from plant tissues (Schaad and Frederick, 2002; Schena et al., 2004) and soils (Okubara et al., 2005; Schroeder et al., 2006; Lievens et al., 2006; Kernaghan et al., 2007). Although a variety of chemistries have been developed for quantitative real-time PCR (Ginzinger, 2002), SYBR green intercalating dyes represent the most flexible approach to the analysis of species assemblages.

Taxon specific PCR primers have been designed for a variety of Pythium species, for use in both conventional (Kageyama et al., 1997; Wang et al., 2003) and real-time PCR reactions (Schroeder et al., 2006). In the present study, we designed PCR primers specific to P. ultimum Trow and P. irregulare Buisman sensu stricto, the two most destructive Pythium species in southwestern Ontario ginseng plantations (Reeleder and Brammall, 1994). P. ultimum is fairly well circumscribed both morphologically and genetically (Van der Plaats-Niterink, 1981; Kageyama et al., 2007). However, the species concept of P. irregulare is currently under revision (Matsumoto et al., 2000; Garzón et al., 2005, 2007) and the occurrence of morphologically similar, but genetically divergent isolates (cryptic species) makes quantification difficult.

Although real-time PCR quantification methods are more sensitive and specific than culture based methods (Atkins et al., 2003; Ippolito et al., 2004; Kernaghan et al., 2007), plating on selective media still gives valuable and complementary data on soil pathogen populations. For example, relationships among DNA concentration, microbial biomass and infectivity may not necessarily be a direct indication of inoculum potential. The relationship between DNA concentration and inoculum potential should therefore be determined empirically for each pathogen species.

As the ability to accurately quantify populations of Pythium species directly from soils prior to ginseng planting would allow for more accurate assessments of disease risk and a reduction in the need for fumigation, we developed qPCR assays for P. irregulare and P. ultimum and used them in both artificially infested soil and naturally infested ginseng-cultivated soils. We also conducted concurrent estimates of inoculum potential using counts of colony forming units on semi-selective agar media.

### Table 1 – Species used in this study and the results of conventional PCR (using primers ITS1/ITS4) and testing of taxon specific primers

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession #</th>
<th>Source</th>
<th>Conventional PCR and presence of restriction sites</th>
<th>qPCR</th>
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<tr>
<td></td>
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<td></td>
<td>Pf/PiR, Pu/PuR</td>
<td></td>
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<td>Isolates used for Pythium primer design and/or testing</td>
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<td>491</td>
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<td>598</td>
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<tr>
<td>P. irregulare (Group II)</td>
<td>PID008</td>
<td>SCFPRC</td>
<td></td>
<td>–</td>
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<td>Pythium paroecandrum</td>
<td>568</td>
<td>CCFC</td>
<td></td>
<td>–</td>
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<tr>
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<td>SCFPRC</td>
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<td>Pythium intermedium</td>
<td>PID091*</td>
<td>SCFPRC</td>
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<td>–</td>
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<td>SCFPRC</td>
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<td>SCFPRC</td>
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<td>P. intermedium</td>
<td>PID306*</td>
<td>SCFPRC</td>
<td></td>
<td>+</td>
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+ : Amplification of product with predicted size and melting temperature; – : no amplification of target; *: isolates sequenced; F: presence of FspI restriction site; B: presence of BccI restriction site.
Eppendorf Mastercycler (Brinkmann Instruments, Mississauga, ON) thermocycler in 50 μL reactions containing 1 U Platinum® Taq DNA Polymerase, 1× PCR buffer, 200 μM dNTP mix (all from Invitrogen, Burlington, ON), 2.5 mM MgCl₂, 0.8% BSA, 0.4 μM each of primers ITS 1 and ITS 4 (White et al., 1990) and 5 μL template DNA. Cycling parameters were 94 °C for 2 min followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, followed by 72 °C for 10 min.

PCR products generated from Pythium isolates from south-western Ontario ginseng soils (PID003, PID018, PID091, PID095, PID096, PID306) (Table 1) were purified with the MinElute PCR Purification Kit (Qiagen, Mississauga, ON) and sequenced on an ABI 377 Sequencer (Applied Biosystems, Foster City, CA) using BigDye® Terminator chemistry (Applied Biosystems); Genbank accession numbers DQ083527–DQ083532.

Reference DNA was obtained from fresh mycelium of P. irregulare (PID 008) and P. ultimum (PID 095) (Table 1) by extracting with the DNeasy Plant Mini Kit (Qiagen) and quantifying with an Eppendorf BioPhotometer calibrated (yeast) control extract. All reactions received a 15 min pre-denaturation step. Primers ITS 1 and ITS 4 (White et al., 1990) were used to amplify DNA from pure cultures and soil. PCR reactions were performed using P. irregulare (PID008) and P. ultimum (PID095) for 2 weeks in clarified V8 broth. Twenty mycelial mats were then washed with sterile water and vacuum filtration, macerated using autoclaved mini-blenders and suspended in approximately 40 mL/kg soil in a large plastic bag and mixed by vigorously shaking, then allowing the mixed soil to air dry. The resulting inoculum was then further mixed with pasteurized field soil in different proportions. The resulting soil dilutions were 100, 50, 10 and 1%. Each dilution was then divided into six sub-samples, air dried in the greenhouse and stored at 4 °C until plating and DNA extraction.

Samples of naturally infested soils (ranging from sands to sandy loams) were collected at seven sites in south-western Ontario. At each site, one 441 m² plot was constructed and divided into 49.9 m² quadrats. Approx. 2 L of topsoil was removed from each quadrat. Sampling locations were recorded by GPS. Soil samples from within each site were pooled, passively air dried at room temperature, mixed with a commercial cement mixer, sieved, and mixed again. All equipment, including mixer and sieve were sterilized with household bleach (diluted with water to 0.5% sodium hypo-chlorite) between soils. Soils were placed in plastic bags and stored at 4 °C until used. DNA was extracted and purified from 5 g subsamples of each artificially and naturally infested soil as described in Kernaghan et al. (2007).

2.3. Collection and preparation of soil

Artificially infested soil was prepared using Fox loamy sand (Brunosolic Gray Brown Luvisol; Typic Hapludalf; 0.9% OM) from the Agriculture and Agri-food Canada research farm at Delhi, Ontario (42°47′N, 80°38′W). Soil was steam pasteurized at 74 °C for 30 min using a Lindig soil treatment system (Lindig Manufacturing, St. Paul, MN), air-dried, sieved through a 4 mm mesh, mixed and stored at room temperature until used. Soil dilution (1 part soil in 4 parts 0.25% water agar) procedures were carried out using P. ultimum (PID095) and P. ultimum (PID095) were grown for 2 weeks in clarified V8 broth. Twenty mycelial mats were then washed with sterile water and vacuum filtration, macerated using autoclaved mini-blenders and suspended in approximately 40 mL/kg soil in a large plastic bag and mixed by vigorously shaking, then allowing the mixed soil to air dry. The resulting inoculum was then further mixed with pasteurized field soil in different proportions. The resulting soil dilutions were 100, 50, 10 and 1%. Each dilution was then divided into six sub-samples, air dried in the greenhouse and stored at 4 °C until plating and DNA extraction.

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2.4. qPCR

qPCR was performed on extracts of samples of the artificially infested soil using SYBR green chemistry on a Roche Diagnostics LightCycler™ I. Twenty microlitres reaction mixtures were prepared in glass capillaries (Roche Diagnostics) and included 2 μL soil DNA extract, 9.8 μL Quantit-Test SYBR Green PCR Master Mix (Qiagen), 0.1 μL HotStarTaq DNA Polymerase (Qiagen) and 0.6 mM each of primers PiF and PiR or PuF and PuR. Reactions using PuF/PuR received 1 mM MgCl₂ in addition to the 2.5 mM present in the QuantiTect Master Mix.

For each primer pair, PCR reactions were carried out on DNA extractions from each of the six subsamples taken from each soil dilution, as well as on a dilution series of reference DNA (Quantified DNA from pure culture) and the negative (yeast) control extract. All reactions received a 15 min pre-treatment at 95 °C, then 60 cycles of 94 °C for 15 s, 64 °C for 15 s, 72 °C for 25 s for the PiF/PiR primer set and 94 °C for 15 s, 54 °C for 30 s, 72 °C for 35 s for the PuF/PuR primer set. For all
reactions, temperature transition rates were 20 °C/s and the fluorescent signal was acquired at 72 °C. After cycling, melting curves were generated by increasing the temperature from 65 to 95 °C at a rate of 0.1 °C/s with continuous acquisition of the fluorescent signal.

For the naturally infested soils, qPCR was carried out as for artificially infested soils, except that each crude DNA extract (one from each of four subsamples of each of the seven soils) was split into three parallel extraction replicates after the first centrifugation and prior to purification and amplification. This allowed for increased precision in situations with very low levels of target DNA.

2.4.1. Post amplification characterization of qPCR products
qPCR products amplified from soil extracts were run on agarose gels and stained with ethidium bromide to confirm the product size. For each soil sample which successfully amplified using either primer set, one of the amplicons from the three parallel extractions for that sample was restricted with either FspI or Bcc I (as above), to further confirm the identity of the amplified DNA. One real-time amplicon produced with each primer set, was also randomly selected and sequenced.

2.4.2. Analysis of qPCR data
qPCR data generated by the light cycler was analyzed using LinRegPCR software (Ramakers et al., 2003). For each set of PCR reactions (each Light Cycler carousel) the logarithms of the initial fluorescence (N0 calculated by LinRegPCR) for the serial dilutions of quantified reference DNA were plotted against the logarithms of their actual DNA concentrations. The equation of the resulting line was then used to estimate the initial concentration of target DNA templates from the soil samples. This approach does not require (or assume) equal PCR amplification efficiencies and accounts for any variation in amplification efficiency among sets of PCR reactions (Ramakers et al., 2003).

2.5. Dilution plating
The number of colony forming units per gram dry soil was determined for both artificially and naturally infested soils using P5ARP soil dilution plates as described above, but using soil:water agar dilutions of 1:10 and 1:100. Three 10 g aliquots of each soil were oven-dried at 104 °C for 3 days. Oven-dry weights were used to determine soil moisture; plate count data were then adjusted to a CFU per g dry soil basis. Isolates of Pythium spp. from each field were identified morphologically on the basis of Middleton (1952), Waterhouse (1967) and Van der Plaats-Niterink (1981). Pearson correlations between the resulting CFU data and the qPCR data were calculated in SPSS release 13.0 (SPSS Inc. Chicago, IL) using non-transformed data.

2.6. Sequencing of dilution plate colonies
Due to the presence of cryptic species in the P. irregulare complex, not all of the isolates morphologically identified as P. irregulare possessed the PiF/PiR primer sites and would therefore not be detected by our assay. In order to correlate our qPCR and CFU data sets from naturally infested soils, it was necessary to determine the proportion of colony forming units that possessed PiF/PiR primer sites and adjust the qPCR data for P. irregulare from naturally infested soils accordingly. Representative dilution plate colonies morphologically identified as P. irregulare were sequenced using the primer sets ITS1/ITS4 or ITS5/ITS4 (White et al., 1990) as described above. The 83 resulting ITS sequences were then aligned with reference sequences from Genbank using Clustal X (Thompson et al., 1997) and then manually adjusted using Bioedit (Ver. 5.0.6) (Hall, 1999). Maximum parsimony analysis was then performed using PAUP* version 4beta 10 (Swafford, 1998) with Phytophthora cinnamomi Rands as an outgroup, TBR branch swapping and 100 bootstrap replications.

3. Results
3.1. Specificity of qPCR primers
qPCR using the taxon specific primer pairs PiF/PiR and PuF/PuR on P. irregulare (PID008) and P. ultimum (PID095) DNA from pure cultures (at between 100 and 1 pg/μL extract) produced amplicons with melting points at 87.5 and 82.3 °C, respectively. The melting profiles of P. irregulare amplicons also included a secondary peak at 82 °C, likely due to a highly AT rich region in the center of the amplicon (see Li et al., 2003). This melting profile is also predicted by the analysis of P. irregulare ITS sequences using the Poland algorithm (Steger, 1994). Post amplification gel electrophoresis also revealed that all amplicons were of the predicted sizes. Average PCR efficiencies, calculated using LinRegPCR (Ramakers et al., 2003), were 1.51 and 1.68 (of a maximum possible value of 2.0) respectively.

Testing of the two primer pairs against a range of related species indicated that they were highly specific under the amplification parameters used. Only DNA from P. irregulare (Groups I and II) and P. ultimum resulted in qPCR amplicons using PiF/PiR and PuF/PuR, respectively (Table 1). All DNA extracts tested produced conventional PCR products using the non-specific primers ITS1/ITS4 (White et al., 1990), indicating the presence of amplifiable templates.

3.2. Specificity of restriction digests
FspI and BccI digests of conventional PCR products generated from 17 Pythium isolates using primers ITS1–ITS4 (Table 1) resulted in characteristic restriction fragment patterns for P. irregulare (Groups I and II) and P. ultimum, respectively. The restriction patterns clearly distinguished the two target amplicons from each other and from those of all other Pythium species tested (Fig. 1).

Although the taxon specific primers used for qPCR amplification of the target Pythium species (PiF/PiR and PuF/PuR) are internal to the ITS primers used for the conventional PCR, one characteristic restriction site was still present in each of the qPCR amplicons, allowing us to confirm target amplification by digesting the qPCR products with FspI and BccI.
3.3. qPCR of DNA extracted from soils

qPCR amplification from the soils artificially inoculated with the two Pythium species resulted in amplicons of the same size and melting temperatures as those from pure culture. PCR efficiencies of reactions using templates derived from artificially inoculated soil were also very similar to those from pure culture. Average target DNA concentrations for *P. irregulare* DNA ranged from 13.7 pg/μL ± 2.42 in the soil inoculated at 100% to .04 pg/μL ± .01 in the soil inoculated at 1% and *P. ultimum* ranged from 19.51 pg/μL ± .52 to .43 pg/μL ± .23.

PCR efficiencies of amplifications from naturally infested soil extracts were also very similar to those from pure culture, and again resulted in amplicons of the predicted size. However, amplification of extracts from the fourth set of subsamples using the PiF/PiR primer set exhibited very poor PCR efficiency, perhaps due to prolonged storage of the soil samples. Data from this replication were not included in the analyses.

Average concentrations of *P. irregulare* DNA ranged from $3.7 \times 10^{-5}$ pg/μL soil extract ± $4.5 \times 10^{-5}$ (SE) in soil G, to .04 pg/μL ± .03 in soil F. *P. ultimum* DNA ranged from .21 pg/μL ± .05 in soil C to 1.2 pg/μL ± .57 in soil G.

### 3.3.1. Post-amplification characterization of qPCR products from soil

qPCR amplification of the two Pythium species pathogen species from the naturally infested soils again resulted in the same melting profiles as those from pure culture. Gel electrophoresis of the qPCR amplicons produced with the two primer sets revealed that they were all of the predicted size. Restriction digestions using BccI and FspI also indicated that the target DNA had been amplified. Restriction of the 508 bp *P. irregulare* (Groups I and II sensu Matsumoto et al., 2000) amplicons with FspI resulted in fragments of ~450 and 50 bp, whereas restriction of the 407 bp *P. ultimum* amplicons with BccI resulted in fragments of ~290 and 120 bp. Sequences of randomly selected real-time amplicons produced using the two primer sets also aligned well with sequences derived from pure culture.

### 3.4. Colony forming units

Plating of soils artificially inoculated with either *P. irregulare* or *P. ultimum* onto P5ARP media gave average CFU counts ranging from 42.5 ± 3.6 propagules/100 mg dry soil in the 100% infested (non-diluted) soil to 8.7 ± 2.1 in the most diluted soil (1% inoculum) and from 62.1 ± 7.4 to 7.9 ± .55 propagules/100 mg dry soil, respectively.

Plating of the naturally infested soils onto P5ARP media resulted in average *P. irregulare* CFU counts (based on morphology) ranging from 23.4 ± 3.7 (SE) propagules/100 mg dry soil in soil E to 3.6 ± 1.1 propagules/100 mg dry soil in soil A. *P. ultimum* CFU counts ranged from 14.9 ± 3.1 in soil E to .62 ± .36 in soil D.

### 3.5. Analysis of sequences derived from dilution plates

The maximum parsimony analysis of the *P. irregulare* ITS sequences obtained from the colonies growing on P5ARP dilution plates clearly divided the colonies into two groups; those which possessed the PiF/PiR primer sites and those which did not (Fig. 2). Overall 64% of the *P. irregulare* isolates sequenced contained the qPCR primer sites and would have been detectable by our qPCR assay. Proportions of isolates which possessed the primer sites ranged from 10% in soil G to 100% in soil A (Fig. 3).

### 3.6. Comparison of qPCR and CFU measures of Pythium populations

In the soils artificially infested with either *P. irregulare* or *P. ultimum*, both the DNA concentrations and CFU counts decreased with increasing inoculum dilution. The changes in the two measures were positively correlated (Pearson correlation) with $r = .998$, $p = .011$, $n = 4$ for *P. irregulare* and $r = .974$, $p = .026$, $n = 4$ for *P. ultimum*.

However, the relationship between measured pathogen concentration and soil dilution was much stronger for DNA measures than for CFU (Fig. 4). In the case of *P. ultimum* a linear regression of DNA concentration on inoculum dilution (not shown) gives $r^2 = .980$; $p = .001$, while CFU on inoculum dilution gives $r^2 = .921$; $p = .040$.

Concentrations of *Pythium* DNA were also positively correlated with CFU counts in the seven naturally infested soils; $r = .761$, $p = .046$, $n = 7$ for *P. irregulare* (after correction for presence/absence of qPCR primer sites) and $r = .931$, $p = .002$, $n = 7$ for *P. ultimum*. However, the CFU data indicates that the
Fig. 2 – One of the most parsimonious trees based on the ITS sequences from 83 colonies from seven ginseng soils (A–G), isolated on selective media and morphologically identified as *Pythium irregulare* (labeled with a PID number). Consistency index (CI) = 0.876, rescaled consistency index (RCI) = 0.852, retention index (RI) = 0.972. Isolates in the upper clade possess the PiF/PiR priming sites and are therefore detectable by the real-time PCR assay. Reference sequences from Genbank and bootstrap values above 70% are included.
naturally occurring *P. irregulare* populations are somewhat higher than those of *P. ultimum*, while the average concentration of *P. irregulare* DNA was much lower than that of *P. ultimum* DNA (Fig. 5).

### 4. Discussion

We have used both real-time PCR and dilution plating on selective media in order to estimate populations of *P. irregulare* and *P. ultimum* in ginseng soils in south-western Ontario. In general, there was good agreement between the data sets produced using the two techniques. However, the direct comparison of qPCR and dilution plate data proved difficult for *P. irregulare*, due to the presence of cryptic species within the "*P. irregulare* complex" (Garzón et al., 2007).

Our qPCR primers for *P. irregulare* were designed to target the most common members of the *P. irregulare* complex; essentially those defined by Matsumoto et al. (2000) as “Group I” and “Group II” and more recently by Garzón et al. (2005) as *P. irregulare* sensu stricto. However, sequences from *P. irregulare*...
colonies formed on PaARP indicated the presence of another, morphologically similar but less common, strain of P. irregularare, which does not possess our primer sites (genetically similar to “Group IV” of Matsumoto et al., 2000). This resulted in overestimations of colony forming units with respect to the strains targeted by our qPCR primers. This problem was overcome by further sequencing of isolates from the dilution plates and performing maximum parsimony analysis (Fig. 3) to determine the intraspecific placement of each isolate. The proportion of isolates detectable by our qPCR assay was then calculated for each soil, and the dilution plate data corrected accordingly.

The maximum parsimony tree clearly divides the isolates recovered from the dilution plates into two groups. The upper clade, the members of which possess our qPCR primer sites, is equivalent to “Group I” and “Group II” of Matsumoto et al. (2000), while the lower clade is analogous to their “Group IV”. We did not detect any members of “Group III” in our soils. The upper clade of Fig. 3 is also analogous to the “P. irregularare sensu stricto” clade of Garzón et al. (2005), while the lower clade is analogous to their “P. irregularare sensu lato”. Garzón et al. (2007) have also proposed a new species within the P. irregularare complex on the basis of ITS and cox II sequences as well as AFLP data. These isolates form the uppermost subclade of our tree and represent approximately 6% of the dilution plate isolates sequenced. On the basis of the above phylogenetic analyses, it seems likely that other new species will soon be delineated within the P. irregularare complex.

The proportions of the two P. irregularare types differed greatly across the seven soils analyzed, with four being dominated by isolates with our primer sites and three dominated by isolates without our primer sites (Fig. 4). Although there is no obvious reason for this variation, the soils do vary somewhat in soil characteristics and cropping histories (see Kernaghan et al., 2007).

Although pathogen DNA concentrations and CFU counts were positively correlated in both the artificially and naturally infested soils, the range of DNA concentrations for P. ultimum in the naturally infested soils was much lower than that of P. irregularare, even though the range of CFU counts for the two species was similar (Fig. 5). Reasons for this discrepancy may include differences in extraction efficiency, or a higher germination rate of propagules produced by P. irregularare relative to those of P. ultimum. This is supported by the differences in the DNA:CFU ratios for the artificially infested soils (Fig. 2), in which both the DNA and CFU values for P. ultimum infested soils decrease rapidly with soil dilution, while CFU counts from P. irregularare infested soils remain fairly high, relative to DNA concentrations, over the dilution series. Also, P. irregularare grew more rapidly on the selective medium used and may have masked the presence of P. ultimum colonies in some cases, resulting in an underestimate of P. ultimum colonies.

Another possible explanation for differences in the DNA:CFU ratios between the two pathogens may lie in differences between the copy number of the ribosomal DNA gene clusters (which includes the targeted ITS region) per nucleus, which would result in differences among calculated DNA concentration to biomass ratios across species (Atkins et al., 2003). In fungi, rDNA gene cluster copy numbers have been estimated at approximately 100 per cell in Cochliobolus heterostrophus (Drechsler) Drechsler (Teuchiyama and Taga, 2001), and found to vary up to 400% among isolates of Glomus intraradices Schenck & Smith (Corradi et al., 2006). Although numbers of rDNA copies per nucleus have not been determined for Oomycetes, it is likely that variation in the number of rDNA repeats per nucleus will affect the ratio between calculated DNA concentrations and the actual concentration of the pathogen in the soil.

The difference between the ratio of calculated DNA concentration to the number of colony forming units in P. irregularare and P. ultimum exemplifies the need for caution when attempting to infer disease risk directly from pathogen DNA concentrations.

Taken alone, data on soil pathogen DNA concentrations are useful for relative comparisons across samples, but do not necessarily reflect fungal biomass, viability or inoculum potential. However, if a consistent relationship between DNA concentration and the number of colony forming units on selective media can be established, then data on DNA concentration can be used as a faster and more accurate proxy for the more time consuming culture based assessments of inoculum potential.

Although the incidence of disease will also depend upon plant susceptibility (e.g. seedling age), as well as on local environmental conditions, estimates of soil inoculum potentials by real-time PCR (after calibration by dilution plating) will improve the speed and accuracy of disease risk assessment and help to reduce unnecessary pesticide application.

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