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ORIGINAL ARTICLE

LAMP for in-field quantitative assessments of airborne grapevine downy mildew inoculum

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Abstract

Aims: Cheap, rapid tools for measuring emissions of *Plasmopara viticola* sporangia directly in the field are required to protect grapevines efficiently and sustainably against downy mildew. To this end, we adapted an existing loop-mediated isothermal amplification (LAMP) protocol based on ITS2 sequences, coupled with a rotating-arm sampler and simple cell lysis, for the in-field measurement of airborne sporangia of *P. viticola*.

Methods and Results: We estimated the sensitivity and specificity of the molecular reaction with an unpurified DNA template in controlled conditions, using the droplet digital PCR (ddPCR) as a reference. We show that the LAMP lower limit of quantification is 3.3 sporangia.m^{-3} air sampled. Cell lysis in KOH solution was less efficient than CTAB for DNA extraction, but the repeatability of the method was good. We tested this protocol directly in a plot at Chateau Dillon (Blanquefort, France) in which we monitored *P. viticola* sporangia concentrations from March to October 2020 (88 samples which revealed concentrations ranging from 0 to 243 sporangia.m^{-3}). There was a significant quantitative correlation ($R^2 = 0.52$) between ddPCR and LAMP results.

Conclusion: LAMP analysis of an unpurified DNA matrix is a simple and reliable method for in-field estimations of the concentration of airborne *P. viticola* sporangia.

Significance and Impact of the Study: This study constitutes a first step towards the development of a regional grapevine downy mildew monitoring network in the vineyards of Bordeaux.

KEYWORDS

epidemiology, plant diseases, plant pathology, plant protection products, spores

INTRODUCTION

Grapevine downy mildew, caused by the oomycete *Plasmopara viticola*, is one of the most devastating diseases of grapevine worldwide. This disease is currently controlled by intensive fungicide applications, and grapevines are one of the most heavily treated types of agricultural production (Urruty et al., 2016). The environmental and health concerns associated with plant protection products have resulted in efforts to decrease their use substantially. One way of reaching this goal is to develop an accurate means of measuring pathogen pressure, to make it possible to adapt the doses and spatiotemporal scheduling of these treatments. Chen et al. (2020) showed that, statistically, waiting for the appearance of the first symptoms before applying the first plant protection product can reduce treatments by 50%, with no yield loss. Epidemic risk prediction models for grapevine downy mildew are currently

driven by observed and forecast weather data (Brischetto et al., 2021; Dalla Marta et al., 2005; Rossi et al., 2009). These models have proved useful for assessing epidemic risk at the regional scale. Observed or forecast meteorological data can be used only to qualify the climatological impact on the state of the system, without taking other parameters (pathogen, plant and soil) into account. The local presence of the pathogen, assessed regularly by monitoring, is certainly one of the most important parameters not currently taken into account that could help to optimize disease management strategies (Delière et al., 2015). It is, therefore, essential to develop efficient, reliable and easy-to-use tools for monitoring pathogens directly in the air. A number of methods are available for pathogen monitoring, including airborne spore trapping, which is an effective method for the early detection of outbreaks before symptoms appear on the plot (Munir et al., 2020; Rahman et al., 2017; Thiessen et al., 2016). Such monitoring can improve plant disease forecasting models (Van der Heyden et al., 2021). Spore identification and counting can be performed by multiple methods and with diverse technologies. Light microscopy is the oldest and most straightforward method for investigating airborne propagules. However, the taxonomic identification of spores based on morphological characters requires a high level of training and is very time-consuming. Another approach to the analysis of aerobiological samples involves targeting nucleic acid sequences (DNA or RNA) specific to the organism of interest by PCR. The molecular approach based on quantitative PCR has the advantage of being very sensitive, specific and quantitative. Loop-mediated isothermal amplification (LAMP; Notomi, 2000) is another molecular method based on a more complex set of primers than conventional PCR-based methods. It has the advantages of a very low detection threshold, making it possible to detect very small amounts of DNA, and of being accessible, cost-effective and easy to perform. LAMP assays have been successfully used on environmental air samples for the detection of *Erysiphe necator*, the causal agent of grapevine powdery mildew (Thiessen et al., 2018), *Uromyces betae*, the causal agent of sugar beet rust (Kaczmarek et al., 2019), *Oculimacula acuformis* and *O. yallundae,* which cause eyespot on cereals (King et al., 2021) and *Phytophthora infestans,* which causes late blight on potato and tomato (Arocha Rosete et al., 2021). Despite its demonstrated efficacy for detecting the presence of target organisms, its suitability for use in quantitative approaches remains a matter of debate because of its moderate ability to quantify small amounts of target DNA (Hardinge & Murray, 2020). Finally, digital droplet PCR (ddPCR) can be used for absolute quantification of the number of target sequences present in a sample (Hindson et al., 2011). This method is very precise but time-consuming. It also requires a high level of technical skill, and is, therefore, less suitable for cheap routine analyses of samples. Nevertheless, it remains the best reference method for nucleic acid quantification (Ristaino et al., 2020). Several real-time PCR tools based on ITS sequences have been developed for the quantification of *P. viticola* in plants (Valsesia et al., 2005) and in air samples (Carisse et al., 2020). A LAMP protocol for detecting *P. viticola* sporangia based on the ITS-2 sequence has been developed by Kong et al. (2016). Marimuthu et al. (2020) also used a LAMP assay, this time based on the CesA4 gene sequence, to detect the presence of *P. viticola* in leaf samples. Their primer set was recently successfully used to detect airborne *P. viticola* inoculum in air samples obtained from a suction trap placed in a vineyard plot (Basha et al., 2021).

Despite the great potential of LAMP for the quantitative monitoring of pathogens in field conditions, the LAMP protocols of both Kong et al. (2016) and Marimuthu et al. (2020) were limited to the qualitative detection of *P. viticola*. The aim of this study was to develop a reliable, rapid, affordable and transferable strategy for detecting and quantifying *P. viticola* spores from environmental samples for the robust assessment of plant health risks. We adopted the LAMP protocol of Kong et al. (2016) for use directly on lysates of cells extracted from environmental air samples captured by impaction samplers. Using ddPCR as a reference method, we demonstrate the suitability of LAMP for the quantitative monitoring of *P. viticola* sporangia, with 88 samples collected from Chateau Dillon in the 2020 growing season.

MATERIALS AND METHODS

Plasmopara viticola strains and culture conditions

DNA from eight pure strains of *P. viticola* from the UMR SAVE (Unité Mixte de Recherche Santé et Agroécologie du VignoblE) collection, collected from different locations, were used to test probes and primer specificity and to develop the LAMP and ddPCR assays. The strains used were Pv3968, Pv3375, Pv1343, Pv3719, Pv2649, Pv2282, Pv221 and Pv412 (Table S1). These biotrophic strains were grown on leaf cuttings (Cabernet Sauvignon), as previously described (Delmas et al., 2014; Delmotte et al., 2014).

Environmental samples

In addition to pure cultures, the LAMP method was assessed on *P. viticola* aerial sporangia captured in the

field with a Sporestick® rotating-arm sampler (*OptiSense* Limited). The Sporestick® was positioned 130 cm above ground level (at the limit of canopy) in a plot of Merlot grapevines in the Chateau Dillon vineyard (Blanquefort, France) from 13 March 2020 to 14 October 2020 (88 samples). This plot was chosen to maximize the chances of sampling *P. viticola*, as it had a strong history of downy mildew and contained several rows of grapevines not treated with fungicides. Two wood matches (40 mm × 2 mm) coated with white petroleum jelly (Cooper, CE N°232–373-2) were positioned vertically on the Sporestick® rotation arm for sporangia collection, and the rotor speed was set at 2400 rpm. With these parameters, 6.22 m^3 of air was sampled per hour (McCartney et al., 1997). The matches were replaced three times per week, every Monday (72 h of sampling), Wednesday (48 h of sampling) and Friday (48 h of sampling). The used matches were collected, placed in a 5 ml collection tube (Dutscher) and stored at −20°C until DNA extraction.

DNA extraction

We tested a simple rapid DNA extraction procedure based on cell lysis in potassium hydroxide (KOH), which we compared with the most reliable and robust CTAB DNA extraction protocol. For each method tested, matches coated with petroleum jelly were included in the extraction tubes to evaluate the potential inhibitory effects of petroleum jelly or matches on PCR performance.

For pure cultures, sporangia were collected from fresh sporulating leaves with a cotton swab and suspended in 3 ml of distilled water. The samples were placed in an ice bath to prevent zoospore germination until there were at least 2 $\times 10^6$ sporangia.ml^{-1}. Sporangium concentration was checked with a haemocytometer under a light microscope. Serial dilutions (eight series for each strain i.e. Pv412 and Pv221) were performed to obtain sporangium suspensions with concentrations of 10^6; 5 $\times 10^5$; 5 $\times 10^4$; 5 $\times 10^3$ and 5 $\times 10^2$ sporangia.ml^{-1}. Cells were lysed in KOH in 5 ml sampling tubes: 200 µl of each sporangium solution was diluted in 1800 µl 0.3 mol.l^{-1} KOH (dilution factor 1:10). Three stainless steel balls and two matches coated with petroleum jelly were added. The tubes were then shaken for 1 min on an Orbital shaker at 430 rpm. Suspensions of 1 and 10 sporangia were obtained by removing sporangia one-by-one from dehydrated pure cultures under a binocular loupe and placing them directly in 5 ml sampling tubes containing 2 ml of 0.3 mol/L KOH. Three stainless steel balls and two matches coated with petroleum jelly were added and the tubes were shaken for 1 min on an Orbital shaker at 430 rpm. We then removed 50 µl of the lysis solution and mixed it with 450 µl of ultrapure water. This dilute suspension served as a template for the amplification reactions. For field samples, lysis was performed directly in the 5 ml sampling tube. We added 2 ml of 0.3 mol/l KOH and three stainless steel balls and the tubes were shaken for 1 min on a plate shaker. We then removed 50 µl of the lysis solution and mixed it with 450 µl of ultrapure water. This dilute solution served as a template for the amplification reactions.

CTAB extraction was performed in 2 ml Eppendorf tubes, with pure cultures only. Briefly, we diluted 100 µl of each 10^6 sporangia per ml suspension (see the previous paragraph) in 900 µl CTAB buffer (CTAB 2% PVPP), and we diluted 20 µl of each of three sporangium suspensions to concentrations of 5 $\times 10^5$, 5 $\times 10^4$ and 5 $\times 10^3$ sporangia per ml (see the previous paragraph), in 980 µl CTAB buffer. Suspensions of 10 sporangia were obtained by removing sporangia one-by-one from dehydrated pure cultures under a binocular loupe and transferring them directly to 2 ml Eppendorf tubes containing 1 ml CTAB buffer (CTAB 2% PVPP). Two matches coated with petroleum jelly were added to each tube, and the tubes were then incubated at 65°C for 2 h. We added 500 µl of chloroform-isoamyl alcohol (24:1 mixture) and the tubes were shaken gently for 10 min. Samples were centrifuged for 10 min at 17,949 *xg* and 4°C, and the aqueous phase was then transferred to a fresh 2 ml Eppendorf tube (almost 650 µl of supernatant). A two-thirds volume of cold isopropanol (−20°C) was added (1085,5 µl) and the tubes were shaken gently for 10 min before being placed at −20°C overnight to precipitate the DNA. The next day, the tubes were centrifuged for 10 min at 17,949 x *g* and 4°C. The supernatant was removed and the DNA pellets were washed with 800 µl of 70% ethanol and centrifuged for 10 min at 17,949 x *g*. The DNA pellets were dried under aseptic conditions and solubilized in 2000 µl distilled water. For a comparison of this method with extraction in KOH, we diluted 50 µl of the resulting DNA suspension in 450 µl of distilled water (1:10 dilution).

Real-time LAMP reaction

Primers targeting the ITS2 sequence of *P. viticola* have been proposed for LAMP (Kong et al., 2016, Table S2) and were used in this analysis. We used the LAMP protocol described by Kong et al. (2016), except that the master mix was purchased from Optigene (GspSSD2.0 Isothermal Mastermix ISO-004). The reaction mixtures contained 1.4 µM FIP, BIP and B-LOOP, 0.2 µM F3 and B3, 15 µl MasterMix ISO-004, 2.5 µl PCR grade water and 5 µl lysate or extracted DNA for a final volume of 25 µl. As a negative control, the DNA template was replaced with 5 µl PCR grade water. The highest concentration on the standard curve (10^6 sporangia per ml) was

used as a positive control. The reaction was allowed to proceed at 65°C for 45 min in a Genie® HT thermocycler (OptiGene Limited). The reaction time (Rt, in minutes) was defined as the point on the amplification curve at which the linear phase of matrix amplification was effective (point of inflection of the sigmoid fluorescence curve with respect to time).

Probe and primer design for ddPCR

The region from ITS1 to ITS2 (DQ665668.1) was previously used to design LAMP primers. For the creation of comparable locus conditions between LAMP and ddPCR, we targeted a similar amplicon with the ddPCR primers and probe (the probe and primer sequences are summarized in Table S2 and Figure S1), with a slight shift in the ITS2 sequence to optimize the amplification reaction and *Taq*Man probe detection (Bustin et al., 2009; Raymaekers et al., 2009; Rodríguez et al., 2015). ITS2 sequences from *P. viticola* and three other fungal pathogens of grapevine—*Erysiphe necator*, *Guignardia bidwellii* and *Botrytis cinerea*—were downloaded from the NCBI GenBank database and Mega-X software was used to align these sequences, to ensure primer specificity. A 230 bp amplicon was generated by this reaction and detected with a *Taq*Man probe.

Droplet digital PCR

The 22 µl reaction mixtures contained 0.7 µM forward and reverse primers, 0.5 µM *Taq*Man probe, 11 µl probe buffer mix, 4.36 µl PCR grade water and 2 µl lysate or solutions of extracted DNA at various concentrations. For the negative control, the DNA template was replaced with 2 µl PCR grade water. The reaction mixture was emulsified in a 70 µl oil droplet for the *Taq*Man probe (Bio-Rad), with a QX200 droplet generator. We transferred 40 µl of this emulsion to a Bio-Rad ddPCR plate. PCR amplification began with 5 min at 95°C, followed by 40 cycles of 30 s at 95°C, 1 min at 60°C and 30 s at 72°C, and a final 10-min period at 98°C. The plate was read immediately with a QX200 Droplet Reader. The results were analysed with Quantasoft software, using a fluorescence threshold of 2500 to differentiate between positive and negative droplets. The positive droplets were counted to obtain an absolute value for DNA concentration.

Specificity

We assessed the specificity of the primers and probes for *P. viticola*, by performing amplifications on DNA from other oomycetes genetically related to *P. viticola* from the genera *Phytophthora* (*Ph.*), *Pythium* (*Py.*), *Peronospora* (*Pe.*), *Bremia* (*Br.*) and *Plasmopara* (*Pl.*): *Ph. humicola, Ph. cambivora, Ph. inundata, Ph. cinoramomi, Ph. quercinia, Ph. megasperma, Ph. cactorum, Py. intermedium, Py. ultimum, Pe. pisi, Br. lactucae* and *Pl. halstedii*. We also assessed the specificity of the probe and primers with ascomycetes responsible for other cryptogamic diseases of grapevines likely to be present in the environmental samples: *Erysiphe necator*, *Botrytis cinerea* and *Guignardia bidwellii*. These amplifications were performed with the LAMP primer set and the ddPCR primers and probe.

Sensitivity and standard curve for real-time LAMP

We assessed the LAMP PCR performances of two DNA matrices (KOH lysates vs DNA extracted in CTAB), using sporangium suspensions at various concentrations, with the construction of standard curves. In total, eight serial dilutions were lysed with KOH for each of two strains (Pv221 and Pv412). Three serial dilutions of Pv221 were obtained with the CTAB procedure. The DNA solutions obtained were then stored at −20°C until analysis by LAMP and ddPCR. The sensitivity of LAMP was estimated with a simplified approach based on a previous study (Forootan et al., 2017). The limit of detection (LoD) was defined as the lowest concentration at which 95% of the samples were detected. The limit of quantification (LoQ) was defined as the lowest concentration at which 100% of the samples were detected and the coefficient of variation (CV) for reaction time was below 35%, with

$$CV = 100 \times SD / \mu$$

where μ is the mean Rt for a concentration and *SD* is its standard deviation.

Statistical analysis

These analyses were performed on vineyard air samples. Positive and negative LAMP (L+, L−) and ddPCR (D+, D−) analyses are presented in a contingency table (Table 1). These data were subjected to Bayesian analysis (Yuen & Hughes, 2002) with sensitivity defined as the true positive proportion (TPP) = (L+P+)/(L+P+)+(L+P−), specificity defined as the true negative proportion (TNP) = (L−P−)/(L−P−)+(L−P+), and precision defined as the number of correct diagnoses

TABLE 1 Results for the analysis of air samples by LAMP and ddPCR presented in Figure 3

	L+	L−	Total
D+	48	12	60
D−	7	21	28
Total	55	33	88

Abbreviations: ddPCR, droplet digital PCR; LAMP, loop-mediated isothermal amplification.

(TPP + TNP) divided by the total number of tests, thus, precision = (L + P+) + (L−P−)/(L + P+) + (L + P−) + (L−P−) + (L−P+). The degree of concordance was assessed by calculating the positive likelihood ratio (LR[+] = TPP/FPP) and the negative likelihood ratio (LR[−] = FNP/TNP), with the false positive proportion (FPP) = (L + P−)/(L + P+) + (L + P−) and the false negative proportion (FNP) = (L−P+)/(L−P−) + (L−P+). A good likelihood was considered to be an LR+ greater than 1 (the higher the value the better), and an LR− as close as possible to 0. The following probabilities were determined: (i) when *P. viticola* was detected by both LAMP and ddPCR: Pr(L+/D+) = (L+D+)/L+, (ii) when *P. viticola* was detected by neither ddPCR nor LAMP: Pr(L−/D−) = (L−D−)/L−, (iii) when *P. viticola* was detected by LAMP but not by ddPCR: Pr(L+/D−) = (L + D-)/L+ and (iv) when *P. viticola* was detected by ddPCR but not by LAMP: Pr(L−/D+) = (L−D+)/L−. The observed (C obs. = precision) and random (C al.) concordances were used to calculate the Kappa (*K*) coefficient of concordance (Desquilbet, 2012) with C obs. = precision = (L+D+) + (L−D−)/(L+P+) + (L+P−) + (L−P−) + (L−P+); C al. = (L+) x (D+)/[(L + P+) + (L + P−) + (L − P−) + (L − P+) + (L−) × (D−)]/[(L + P+) + (L + P−) + (L−P−) + (L − P+)] /[(L + P+) + (L + P−) + (L−P−) + (L−P+)] and finally, *K* = (C obs.−C al.)/(1−C al.). *K* can be used to evaluate the degree of true agreement between the results for the two methods, LAMP and ddPCR, with $K < 0$ indicating very poor concordance and $K = 1$ indicating perfect concordance.

RESULTS

Specificity and sensitivity of LAMP on *P. viticola*

All French and European *P. viticola* strains were detected by LAMP. All the other oomycetes and ascomycetes tested gave negative LAMP results. The LoD of our LAMP method on *P. viticola*, defined as the lowest sporangium concentration for which 95% of the samples were detected, was between 50 and 500 sporangia per ml of lysis solution, for 87.5% and 100% amplification, respectively (Figure 1). In other words, the LoD of this method is between 100 and 1000 sporangia stuck on the collectors. The LoQ of our method, defined as the lowest concentration for which 100% of samples were detected, with a CV ≤ 35%, was 500 sporangia per ml of lysis solution (CV = 15.6%), corresponding to 1000 sporangia stuck on the collectors.

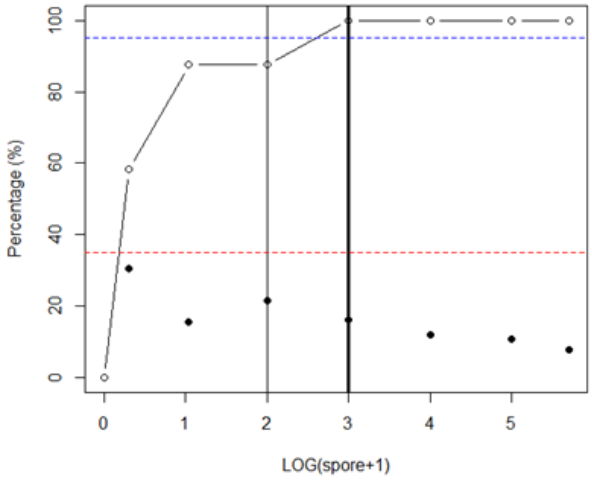

FIGURE 1 Sensitivity of the loop-mediated isothermal amplification assay for *Plasmopara viticola*. Each white dot (○) represents the percentage of samples positively amplified for 16 different sporangium suspensions (eight for the Pv221 strain and eight for the Pv412 strain). Each black dot (●) represents the coefficient of variation (CV) calculated on the same set of suspensions ($n = 16$). The horizontal dashed lines represent the threshold values for a CV of 35% (red line) and for 95% amplification (blue line). The limit of detection is between the two vertical black lines. The thicker line represents the limit of quantification.

Comparison of the quantification performance of LAMP relative to ddPCR as a reference

Two standard curves were obtained with eight serial dilutions of two strains of *P. viticola*: Pv412 and Pv221. Given the quantitative nature of this evaluation, concentrations below the LoQ were removed from the linear regression analysis. In total, 32 samples each of Pv221 and Pv412 were positively detected by LAMP and ddPCR. The quantitative relationship between the Rt obtained by LAMP and the logarithm base 10 of the number of copies of ITS2 obtained by ddPCR for the samples is presented in Figure 2, with an R^2 of 0.791. The Pv412 curve has an R^2 of 0.829 between the Rt of LAMP and the logarithm base

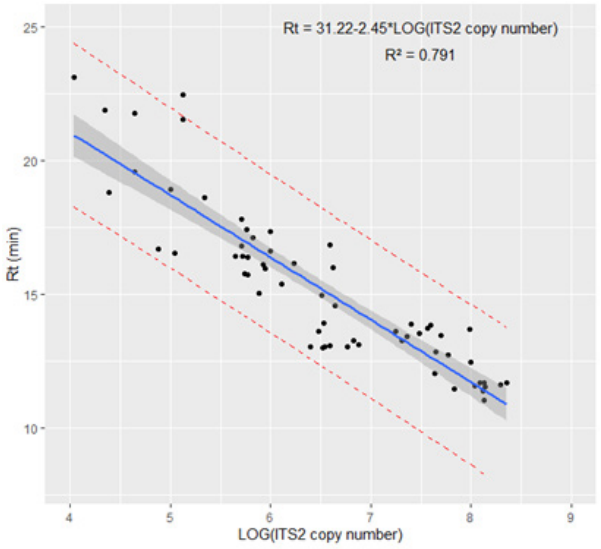

FIGURE 2 Linear regression between the Rt value obtained by loop-mediated isothermal amplification and the log(ITS2 copy number) obtained by droplet digital PCR ('0' and concentrations under the limit of quantification excluded).

10 of ITS2 copy number detected by ddPCR. Similarly, the Pv221 curve has an R^2 of 0.775 (Figure S2).

Comparison of LAMP PCR results for different templates: Crude KOH cell lysate and DNA extracts obtained with the CTAB method

For CTAB extracts, the R^2 of the curve between the Rt of LAMP and the logarithm base 10 of ITS2 copy number detected by ddPCR was 0.878, with a $p < 0.001$. For KOH lysates, the R^2 value was 0.796, with a p value of <0.001 (Figure S3).

Performance of LAMP on environmental DNA

From 13 March 2020 to 14 October 2020, 88 environmental samples were collected from a vineyard managed without fungicide treatment. They contained 0 to 243 sporangia per m^3. These samples were analysed by both ddPCR and LAMP: 68% of samples were positive with ddPCR (60/88) and 62% were positive with LAMP (55/88), but only 55% (48/88) were positive with both techniques, and 24% were strictly negative with both techniques (21/88) (Figure 3a). During the early stages of the epidemic, before 15 May 2020 (stage 19 on the Eichhorn and Lorenz scale [E.L. 19/BBCH 60]), 37.5% of samples tested positive for ddPCR versus only 8.3% for LAMP (Figure 3a). The 46 samples positive with both methods and with an Rt below 30 min were used to create a standard curve for evaluating the relationship between LAMP Rt and the logarithm (base 10) of ITS2 copy number. The environmental sample standard curve had an R^2 of 0.523 (Figure 3b).

Statistical analysis

The results of detection by LAMP and ddPCR on the vineyard samples are presented in Table 1. The sensitivity (TPP) was 48/60 = 0.8, specificity (TNP) was 21/28 = 0.75, and precision was (48 + 21)/88 = 0.78. The FPP was 7/28 = 0.25 and the FNP was 12/60 = 0.2. The LR(+) was 3.2 and the LR(−) was 0.27. The calculated probabilities were Pr(L+/D+) = 0.87, Pr(L−/D−) = 0.64, Pr(L+/D−) = 0.13 and Pr(L−/D+) = 0.36. The Kappa coefficient (K) was 0.53.

DISCUSSION

We adapted the specific and sensitive LAMP method for direct use on cell lysates and assessed its suitability for use in quantitative analyses by comparison with ddPCR as a reference, for the detection of *P. viticola* in environmental samples. The correlation coefficients of the standard curves obtained here were similar to those reported in previous LAMP studies (Thiessen et al., 2018; Kaczmarek et al., 2019; Arocha Rosete et al., 2021; King et al. 2021) and were not dependent on the strain analysed. Hardinge and Murray (2020) reported that Rt variability in LAMP increases with decreasing target DNA concentration. We observed the same pattern, with an LoD at 95%, corresponding to the presence of more than 100 sporangia and an LoQ of 1000 sporangia.

It is important to have some idea of the performance of LAMP with a nonpurified DNA matrix when considering its application in the field, although this performance is rarely assessed. Grabicoski et al. (2020) showed that the type of nucleic acid extraction influenced the correlation coefficient of the linear regression between the amount of DNA and the Rt value, with better results obtained after a purification step. Indeed, DNA clean-up steps can remove cellular debris and DNA-binding proteins that interact with polymerase enzymes or disrupt their replication activities. Grabicoski et al. (2020) reported an R^2 of 0.753 between the logarithm of the amount of nonpurified DNA matrix for *Sclerotinia sclerotiorum* and LAMP results. We obtained similar results for KOH cell lysates (R^2 = 0.796).

The performance of a monitoring method depends on its detection limits (LoD and LoQ) being well known.

The LoD for sporangia ranged from 100 to 1000 sporangia stuck on the matches, corresponding to 0.33–3.34 sporangia per m^3 of air sampled. This upper range value corresponds to the LoQ of our method, according to Forootan et al. (2017). This value (3.34) is of the same order of magnitude as the concentration of 2.52 sporangia per m^3 is considered to be the threshold above which the probability of disease occurrence in the vineyard exceeds 0.5 (Brischetto et al., 2020). LAMP can, therefore, be considered a relevant method for detecting sporangia in the air at levels consistent with those present when disease symptoms first appear. Nevertheless, it is not possible, on the basis of current knowledge, to conclude with confidence that this threshold can be used in all situations, to optimize decisions about treatment. The formulation of a new RDD taking into account not only the concentration of sporangia in the air but also other local parameters, such as weather forecasts, the susceptibility of the grape variety and the physiological state of the plant, will probably be necessary to guarantee that epidemics are kept below an acceptable economic threshold.

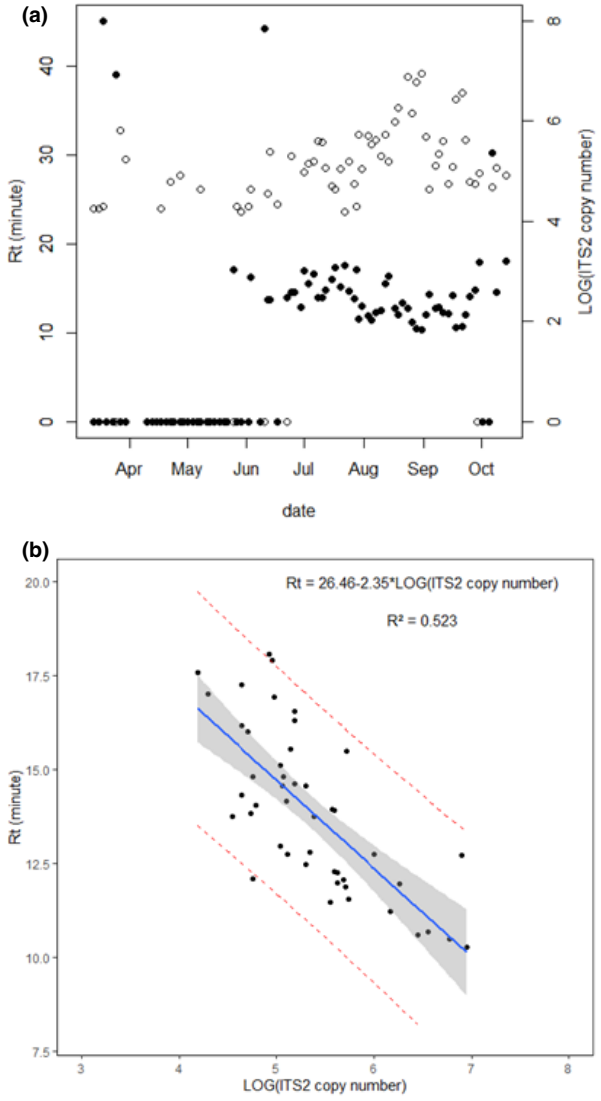

FIGURE 3 Results of loop-mediated isothermal amplification (LAMP) and droplet digital PCR (ddPCR) analyses of a series of samples from the untreated plot at Château Dillon, from 13 March 2020 to 14 October 2020. (a) Time series representation of 88 samples in LAMP (●, left axis) and ddPCR (○, right axis). (b) Linear regressions between the Rt value obtained by LAMP and the log(ITS2 copy number) obtained by ddPCR ('0' and rt values above 30 min excluded).

Our results for technical measurement performance were consistent with those of Ristaino et al., 2020, who found that ddPCR was the most accurate technique available. This difference in sensitivity supports the use of ddPCR, which remains the reference technique for quantifying nucleic acids, particularly at low concentrations. However, the time required to perform ddPCR, mostly due to the time required to generate the microdroplets needed for absolute nucleic acid quantification, remains a major constraint for detection and quantification in the field. Another digital PCR method (QuantStudio Absolute Q Digital PCR, AppliedBiosystems) also involves microdroplet generation and has a low throughput (16 samples). In this context, LAMP coupled with simplified DNA extraction by cell lysis in KOH remains the most suitable technology for in the field monitoring of *P. viticola*. The KOH extraction protocol provides a usable DNA template within 15 min that can be handled directly in the field, with gloves and protective glasses. The development of a, 2020 LAMP plate (96 samples) and the isothermal amplification cycle together take about 2 h.

The Bayesian analysis of the results was based on the 88 air samples collected in the vineyard, covering the entire campaign, from 13 March (stage 3 E.L. scale/BBCH 5) to 14 October 2020 (stage in 2020).

39 E.L. scale/BBCH 89. With a value of 3.2, the LR(+) is higher than 1, indicating a good capacity of LAMP to detect the probable presence of the pathogen, and thus a potential epidemic risk. The LR(−) was 0.27; it was, therefore, positive and close to 0, indicating that LAMP can also establish the probable absence of the pathogen and, therefore, a low epidemic risk of clonal secondary contamination. The Kappa coefficient, *K*, indicates moderate concordance, according to Desquilbet (2012). Over the whole-sampling campaign, the probability of obtaining a false negative was 0.36. These mid-quality scores indicate the relative performance of LAMP for detecting low concentrations of sporangia in the air early in the epidemic (before 15 May 2020). This risk of not detecting the onset of an epidemic process with an isolated trap is, in our view, too high. We propose an attenuation of this risk by increasing the number of traps, a strategy highlighting the value of organizing the analysis and interpretation

of airborne sporangia levels around a large network of measurements.

In conclusion, LAMP on KOH lysates is an analytical method highly suitable for reliable assessments of the risk of *P. viticola* infection in field conditions. The design of this tool represents a first step towards the implementation of a regional network of sporangia traps facilitating quantitative monitoring of the pathogen. The direct assessment of the pathogen over such a large scale, coupled with the weather-driven modelling approach currently in use, should improve our ability to predict the risk of epidemics. We thus plan to develop decision support system rules taking into account local data for the number of sporangia captured at the vineyard plot scale, and considering these numbers within the global epidemic context. Such improvements in risk assessment are the key to decreasing the use of plant protection products by adapting control strategies to the specific local situation of each wine-producing area. The technology presented here represents a first step towards achieving this goal.

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CONFLICT OF INTEREST

This manuscript has not been published elsewhere and is not under consideration for publication by any other peer-reviewed journal. All the authors have approved its content and declare that they have no conflict of interest.

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