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FULL RESEARCH PAPER

Quantification of airborne spores of *Monilinia fructicola* in stone fruit orchards of California using real-time PCR

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Abstract The fungal pathogen *Monilinia fructicola* causes blossom blight and fruit brown rot of stone fruits in California. In this study, spore densities in the air were monitored in six orchard/year combinations with Burkard spore traps. A real-time PCR assay was developed to efficiently quantify the dynamics of spore density in these orchards during the growing season. Different patterns of dynamics of spore density were observed in these orchards. A linear relationship between numbers of spores counted with a compound microscope and those determined with the real-time PCR assay was obtained, using the same samples of spore traps. Spore density in five of six orchard/year combinations ranged from 0.0 to 0.05 spores l^{-1}, except for that in orchard 4, which showed much higher values of spore density in the air, as well as higher values and wider range of incidences of blossom infection and fruit rot than those in the other orchards. The results demonstrated a potential method to quantitatively determine spore inoculum potential in orchards by using a real-time PCR assay.

Keywords Epidemiology · Inoculum · *Prunus* spp. · Spore quantification

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Introduction

The fungal pathogen *Monilinia fructicola* causes brown rot of stone fruit in California. Under favourable climatic conditions, ascospores and/or conidia of the pathogen can infect blossoms, causing blossom blight (Tate and Wood 2000), and conidia in summer can infect fruit, causing fruit rot (Byrde and Willetts 1977). Under conditions unfavourable for blossom blight development, blossoms bearing latent infections will develop into young fruit with latent infections (Northover and Cerkauskas 1994). The spore inoculum in mid and late season in stone fruit orchards in California originates from thinned fruit on the orchard floor (Hong et al. 1997; Luo et al. 2005). These spores can become airborne and infect immature fruit on trees under favourable weather conditions (Luo et al. 2005). When infection levels are high, fruit rot can appear early during the growing season and cause significant yield losses (Luo et al. 2001a, b).

Fungicides are still the primary management tools for brown rot of stone fruit in California (Michailides et al. 1997). Accurate disease prediction becomes important to estimate risk of disease development (Luo and Michailides 2001c) and to help growers make decisions on timing of fungicide sprays. Since blossom and fruit susceptibility and climatic conditions change over the season (Luo and Michailides, 2001b, c), the importance of spore inoculum to cause disease can vary (Luo and Michailides 2003) at the different growth stages of stone fruit as defined by Polito (1981). Prediction and risk assessment of disease development using a decision support system (Luo and Michailides 2001a) requires an accurate estimation of inoculum potential in orchards (Luo et al. 2001a, b), and the spore density in the air was determined to estimate dynamics of inoculum in stone fruit orchards (Luo et al. 2001a; 2005). The information on possible changes of inoculum availability during the season is also important for determining the critical time periods for efficient and timely fungicide sprays. Thus, an efficient and quick method to quantify spore densities of *M. fructicola* in stone fruit orchards is certainly needed.

Spore traps have been used for many years to monitor and quantify airborne fungal species (Kennedy et al. 2000; Maxwell et al. 2005; Rodríguez-Rajo et al. 2005). The conventional method to identify and quantify the pathogen's spores is to view and count spores with a compound microscope. However, microscopic methods are very labour-intensive and require individuals trained in fungal taxonomy. Certainly, these requirements hinder the processing of large numbers of samples needed for epidemiological studies at orchard and regional scales.

As an alternative to microscopic examination, PCR approaches have been developed using species-specific primers (McCartney et al. 2003) to amplify the target segment of DNA of the fungal species of interest. Normally, conventional PCR approaches have been used to determine if the spores of the species of interest exist in spore trap samples, but do not provide any quantitative information of the target DNA relating to the number of spores in the samples. When the quantity of spores is very small in a sample, sensitivity of conventional PCR is limited. We previously developed a more sensitive nested PCR method (Ma et al. 2003) to detect small amounts of target DNA of *M. fructicola* spores collected with spore traps. Using this method, densities of spores equal to or greater than a defined threshold (Luo et al. 2001b), which was set to help make decisions on fungicide sprays, can be detected. Although very sensitive, the use of the nested PCR method is still not sufficiently quantitative for studying inoculum dynamics during the season. A more efficient and quantitative method is therefore needed. In addition to high sensitivity in detection of target DNA, the real-time PCR method has successfully quantified airborne, seedborne and soilborne inoculum of other fungi (Bates et al. 2001; Cullen et al. 2001 Cullen et al. 2005; Van de Graaf et al. 2003).

The objectives of this study were to develop a real-time PCR assay to quantify spore density of *M. fructicola* from spore trap samples collected from stone fruit orchards in California, and to study the inoculum dynamics during the growing season in stone fruit orchards by implementing this real-time PCR assay.

Materials and methods

Orchards

Four stone fruit (*Prunus* spp.) orchards located at the Kearney Agricultural Centre, University of California in Parlier, USA and the surrounding areas were selected. orchard 1 had two peach and two nectarine varieties; orchard 2 had a peach and a nectarine variety; orchard 3 had one plum variety; and orchard 4 had 16 peach and nectarine varieties, but only six varieties were used in this study. Table 1 lists all the varieties in these orchards and the corresponding bloom and harvest dates from 2003 to 2005. No fungicide sprays were applied in any of the orchards. In each orchard and year, a Burkard spore trap (seven-day recording volumetric spore trap) (Burkard Manufacturing Co. Ltd, Rickmansworth, Hertfordshire, UK) was placed between the trees in a row at 1.5 m height from the orchard ground, corresponding to the middle height of the tree

Table 1 Summary of experiments conducted in six stone fruit orchards/years

Year	Orchard	Crop	Variety	Date of full bloom	Mean incidence of blossom infection (%)[a]	SD	Date of harvest	Mean incidence of fruit rot (%)[b]	SD
2003	1	Nectarine	May Glo	12-Feb	0.00	0.00	5-Jun	0.00	0.00
		Peach	Flavorcrest	8-Mar	9.39	4.39	27-Jun	2.00	0.30
		Nectarine	August Glo	10-Mar	11.24	6.33	20-Aug	3.50	1.10
		Peach	O'Henry	11-Mar	0.00	0.00	20-Aug	0.00	0.00
	2	Peach	Elegant Lady	6-Mar	0.57	0.85	22-Jul	5.00	2.20
		Nectarine	Fantasia	7-Mar	1.86	1.56	28-Jul	7.40	3.50
	3	Plum	Fravorrich	27-Feb	0.00	0.00	NA	0.00	0
2004	2	Peach	Elegant Lady	10-Mar	7.93	5.39	20-Jul	3.10	3.00
		Nectarine	Fantasia	10-Mar	8.56	5.22	26-Jul	15.60	7.70
	4	Peach	Mid Pride	26-Feb	0.64	1.30	9-Jul	0.00	0.00
		Peach	Spring Lady	5-Mar	16.31	10.15	10-Jun	35.00	18.00
		Peach	Belle of Georgia	12-Mar	20.49	6.50	9-Aug	0.33	0.57
		Peach	White Lady	12-Mar	6.03	4.89	14-Jul	0.00	0.00
		Peach	Late Dwarf	12-Mar	27.82	19.73	16-Sep	2.30	1.90
		Peach	Queencrest	8-Mar	11.92	8.61	28-May	9.80	9.60
2005	1	Nectarine	May Glo	10-Feb	0.00	0.00	4-Jun	0.00	0.00
		Peach	Flevorcrest	27-Feb	0.00	0.00	28-Jun	5.70	1.80
		Nectarine	August Glo	28-Feb	1.27	2.69	15-Aug	8.20	6.60
		Peach	O'Henry	2-Mar	0.00	0.00	18-Aug	0.50	0.30

A Burkard spore trap was placed in each orchard to collect spores of *Monilinia fructicola* in the air. Average incidences of blossom infection and fruit rot were recorded for each variety

[a] About 500 blossoms were arbitrarily collected from seven to ten trees for each variety. The average blossom infection (%) was calculated from five to seven containers

[b] Data were recorded from ten trees for each variety at 2 weeks before harvest. About 50–70 fruits per tree were counted

canopy. The spore trap tape used to capture spores was coated with Vaseline just prior to placement in the trap and replaced weekly. After removal of the tape from each trap, it was cut into seven equal daily segments, each segment placed on a microscope slide and, stored in a Petri dish at 4°C until processed. Three spore traps were used in 2003: one each was placed in orchard 1, orchard 2, and orchard 3; two each in orchard 2 and orchard 4 in 2004; and one in orchard 1 in 2005.

Natural infection of blossoms and fruit

Infection of blossoms was monitored in each orchard yearly when a spore trap was installed in the orchard. At full bloom, a total of about 500 blossoms were arbitrarily collected from seven to ten trees of each variety in each orchard in each year, except for orchard 4 where the three available trees of each variety were used. Blossoms were soaked in 500 ml of 1% solution of commercial bleach (0.525% sodium hypochlorite) for 3 min and washed with sterilized water five times each for 2 min. Plastic containers ($40 \times 24 \times 12$ cm) were sterilized by washing them with 10% bleach twice. Two layers of wet paper towels were placed on the bottom of each container. About 60–70 blossoms were placed individually on top of the wet paper towels in each container that was covered to maintain >95% RH. The blossoms in the containers were incubated at 23–25°C for 5–7 days. For each container, the incidence of blossom infection (%) was calculated by the number of blossoms showing typical sporulation of *M. fructicola* on the peduncle and hypantheum divided by the total number of incubated blossoms multiplied by 100. Mean incidence of blossom infection for each variety was calculated from replicated containers.

Two weeks before harvest for each variety, 10 trees of each variety in each orchard (only three

trees of each variety in orchard 4) were arbitrarily selected. The incidence of visible fruit rot in each tree was assessed on 50–70 fruit, and the mean incidence of fruit rot was calculated for each variety.

DNA extraction from spore trap samples

To reduce the number of testing samples, the tape segments of 3 days were combined into one sample. Each segment was cut into 1×2 cm pieces and placed in a 2 ml FastDNA tube containing 1.8 ml 0.1% Nonidet (Sigma- Aldrich, St. Louis, MO, USA) and garnet matrix. The tubes were incubated at 55°C for at least 20 min, and then shaken for 5 min in an Eppendorf mixer. The tubes were centrifuged at 14,000 rpm for 10 min and the supernatants were decanted. Extraction of DNA from the spores was performed using the FastDNA Kit (Q-BIO gene Corp. Irvine, CA) by adding 300 µl Cell Lysis/DNA solubilizing solution for yeast, algae, and fungi to each FastDNA containing centrifuged spores and tape segments described above. The mixture was shaken in the Prep Cell Disruptor (QbioGene, Irvine, CA, USA) 10 times for 40 s at 4.5 m s^{-1} with 2 min cooling in ice after the 5th shaking cycle. Extraction of DNA was performed using the FastDNA Kit according to the product manual. The extracted DNA was dissolved in 10 µl of H_2O for PCR amplification with the real-time PCR assay described below.

Real-time PCR assay

A *M. fructicola*–specific primer pair RTMfF (5′-ATGAGCGCTTCCAGAATGAT-3′)/RTMfR (5′-TTCAGAAGCTGTTTGGGGA-3′) was designed based on a *M. fructicola*-specific DNA fragment amplified by a microsatellite M13 (Ma et al. 2003). This primer pair was expected to generate a 390 bp PCR product from *M. fructicola* DNA but not from other microorganisms. Ten isolates each of *M. fructicola*, the closely related species *M. laxa*, and isolates of *Botrytis cinerea*, *Botryosphaeria dothidea*, and *Alternaria alternata* were used to determine the specificity of this primer pair. Real-time PCR amplifications were performed in the DNA Engine Opticon 2 System (Bio-Rad Laboratories, Hercules, CA, former MJ Research, Waltham, MA) using the SYBR Green I fluorescent dye. Amplifications were conducted in 50 µl volumes containing 25 µl SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA), 4 µl template DNA extracted from spores, and 4 µl of each forward and reverse primers (4 µM each). The following parameters were used for real-time PCR amplifications: an initial pre-heat for 3 min at 95°C, followed by 50 cycles at 94°C for 15 s, 64°C for 25 s, 72°C for 30 s, and 73°C for 1 s in order to detect and quantify the fluorescence at a temperature above the denaturation of primer–dimers. After the amplifications were completed, melting curves were obtained based on a standard protocol according to manufacturer's instructions and used to confirm the signal from the target product without containing primer–dimers.

To create a standard curve for quantitative determination of the number of *M. fructicola* spores from spore trap samples with the primer pair RTMfF/RTMfR, an isolate of *M. fructicola* MS22 was cultured in Petri dishes (10×150 mm) containing acidified (2.6 ml of a 25% vol/vol lactic acid solution per litre) potato dextrose agar (APDA) and incubated at 23 ± 2°C for 5 days in the dark. The pathogen was then sub-cultured on new Petri dishes under the same conditions for 5 days. Conidia of *M. fructicola* were harvested by pouring 3 ml sterile distilled water in each Petri dish and the spore density of the suspension was adjusted to 5×10^7 spores ml^{-1}. This spore suspension was used to make 10-fold serial dilutions of 2.5×10^7, 2.5×10^6, 2.5×10^5, 2.5×10^4, and 2.5×10^3 spores ml^{-1}, respectively. To make similar spore trap samples as those collected from fields, 20 µl of each of these spore suspensions was pipetted onto the surface of a segment (same size as a real sample of spore trap tape) of a new spore trap tape affixed to a glass slide coated with Vaseline. These slides were incubated at 23–25°C for 1 h. The DNA of spores from these tape segments on slides was extracted using the procedure described above. The final numbers of spores in the reaction tubes were 2×10^1, 2×10^2, 2×10^3, 2×10^4, and 2×10^5 conidia, respectively. These series dilutions of DNA samples were used with real-time PCR to

generate a standard curve to quantify spores from spore trap samples.

Comparison between the conventional and the real-time PCR methods

To compare the results of spore density determined with the real-time PCR and those with the conventional method, tapes from the spore trap were prepared as described above. A conidial suspension of *M. fructicola* of 5×10^5 spores l^{-1} was prepared. Various numbers of drops (5–20) of the spore suspension (about 5–20 μl) were arbitrarily placed on each of these spore trap tapes to prepare tapes with different number of spores. These tapes were kept at 23 ± 2°C for 4 h to dry. The entire surface of each tape was viewed under a compound microscope (250 ×) to count the total number of spores. Counting spores from a slide with a microscope began from the left-upper corner of the slide. The slide was continuously moved from the left end to the right end while counting the spores under the microscope. When counting spores for one line was finished, the field of the microscope was moved to the next line of the slide and spore counting was continued from the right end to the left end of the slide of this line. The whole slide was scanned in this way to obtain the total number of spores on the slide. The same tapes were then processed using the real-time PCR assay to determine the corresponding number of spores for each of them.

Data analysis

For each spore trap sample collected from orchards, the detected quantity of DNA was used to calculate the corresponding number of spores by using the standard curve of the real-time PCR assay. The spore density (spores l^{-1}) of *M. fructicola* in the air per sample (P) which represented the average of 3 days was calculated using the formula provided by the manufacturer for the specific trap model (10 l air min^{-1}) used in this study: $P = N/(10\ l\ min^{-1} \times 24\ h \times 60\ min \times 3\ days)$, where N is total number of spores from a 3-day sample determined with the real-time PCR assay.

A plot of spore density vs. sampling dates was obtained for each orchard/year combination. In comparing between the conventional and the real-time PCR methods, a linear regression between number of spores counted under the microscope and number of spores determined with the real-time PCR assay was conducted with the REG procedure of SAS (version 9.1, SAS Institute, Cary, NC). The average blossom infection and fruit rot incidence, as well as the corresponding standard deviation were also calculated for each variety of each orchard/year combination.

Results

Real-time PCR for quantification of *M. fructicola* spores

When the DNA template of ten isolates each of *M. fructicola*, *M. laxa*, *Botryosphaeria dothidea*, and *Alternaria alternata* was used to test the specificity of species-specific primer pair RTMfF/RTMfR, only *M. fructicola* isolates showed an expected 390-bp PCR product. No products were generated by this primer pair from other species, which demonstrated that this primer pair is specific to *M. fructicola*. In each sample, a strong linear relationship was obtained between standard curves of C(T) values and spore quantity in a range from 20 to 200,000 spores per sample (Fig. 1).

Comparison between conventional method and the real-time PCR assay

A linear relationship between number of spores counted with the compound microscope and the corresponding number of spores determined with the real-time PCR assay was obtained (Fig. 2). The range of number of spores counted with the microscope was from less than 1,000 to over 6,000 spores on these tape samples. The r^2 value was 0.60 with $P = 0.002$ for this regression.

Real-time PCR quantification of *M. fructicola* spores in air samples taken from stone fruit orchards

Using the real-time PCR method, we quantified *M. fructicola* spores on spore trap tapes. The

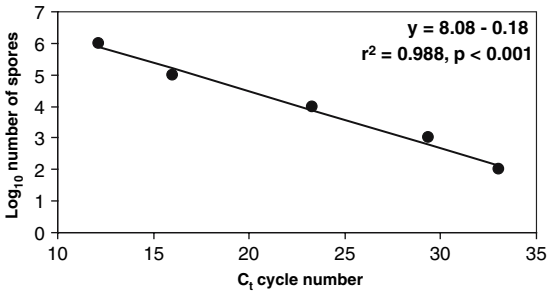

Fig. 1 An example of an output graph of a real-time PCR assay showing a standard curve by plotting the threshold cycle (C(T)) vs. the amount of log number of spores of *M. fructicola* amplified with the species-specific primer pair RTMfF/RTMfR in real-time PCR

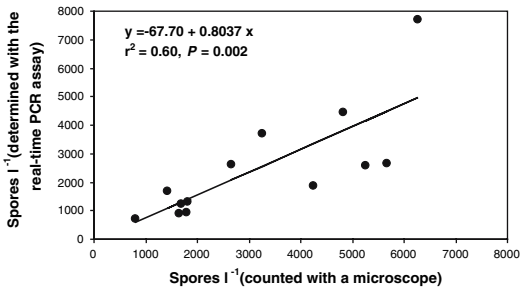

Fig. 2 Linear relationship between number of spores of *M. fructicola* counted with a compound microspore and those determined with the real-time PCR assay

results showed that the pattern of spore density in the air varied among orchards (Fig. 3). In general, spore densities ranged from 0 to 0.05 spores l^{-1} in orchards 1, 2, and 3, and for orchard 4 in 2004, which reached over 0.30 spores l^{-1} (Fig. 3). In 2003, spore densities in orchard 1 were relatively high from February to April, but no spores were detected later in the season except during the first week of August. In 2005, comparatively low spore densities were observed in spring and high spore densities were observed from mid to late July. In orchard 2, spores were detected in air samples from early February to late spring (April) in 2003, as well as on several days during the middle of the season. Spores were also observed in late season (August). However, lower spore densities were observed in the spring of 2005, on many days in mid season (May) but not in late season. In orchard 3 in 2003, no spores were observed in the spring, but they were observed in mid (April to May) and late season (July to August). In orchard 4, spores were detected throughout the entire 2004 growing season. High spore densities in the air were observed in March and again in July, and relatively low densities of spores were observed on some days of April, May, and June.

Blossom infection and fruit rot

In 2003, blossom infection was detected in two of four varieties in orchard 1. No blossom infection was found in orchard 3, but a low level of blossom infection was observed in orchard 2 (Table 1). All these varieties also showed fruit rot at harvest, while no fruit rot was observed on the varieties, which showed no blossom infection in spring. In 2004, all varieties in the two orchards showed blossom infection, but no fruit rot was observed on two varieties in orchard 4 (Table 1). In 2005, consistent results of blossom infection on Mayglo and O'Henry were obtained as in 2003, even though a very low level of fruit rot was recorded on O'Henry. Generally, these two last varieties demonstrated resistance to brown rot. However, the mean incidence of blossom infection on Flavorcrest was 9.39% in 2003, but was 0.0 in 2005 (Table 1). In orchard 2, the two varieties showed moderate levels of blossom and fruit infections in two years (Table 1).

Discussion

This study reports the development of a real-time PCR assay to quantify efficiently spore densities of *M. fructicola* in the air and provide results of the implementation of the assay in six stone fruit orchard/year combinations. The method may serve also as an alternative to the conventional method, which relies on counting spores with a microscope as was done in a previous study (Luo et al. 2005). The number of spores on tape samples determined with the real-time PCR assay were linearly correlated with those counted with a compound microscope. Thus, the real-time PCR assay, if adapted, can serve as a potential method for quantifying fungal spore densities not only for *M. fructicola*, but for other fungal species as well.

One of the potential applications of this method is the estimation of inoculum potential

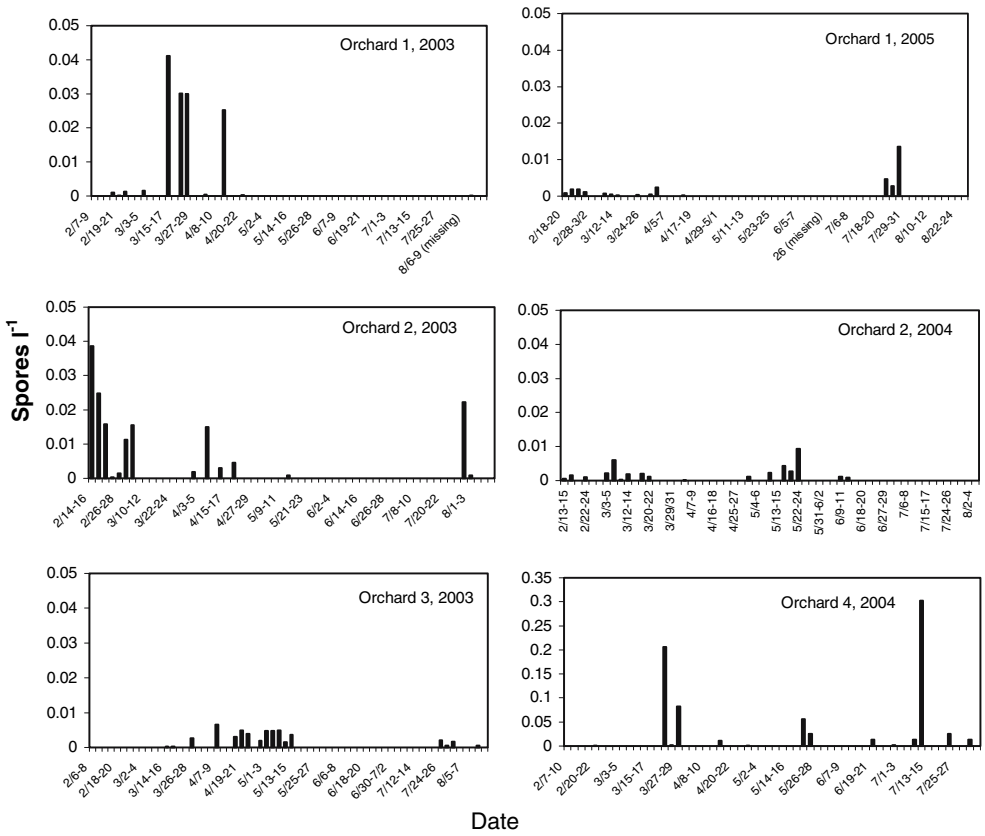

Fig. 3 Spore density of *M. fructicola* in the air of six stone fruit orchard/year combinations estimated by a real-time PCR assay. Samples of three consecutive days were combined into one sample which was processed with the real-time PCR assay. Mean daily spore density for each 3-day period is plotted for each orchard

in orchards to predict risks of blossom infection and fruit rot (Luo and Michailides 2001b, c; 2003) and to help growers in making decisions on fungicide sprays. Spore densities in the air of orchards can be quantified at critical periods in the season (Luo and Michailides 2001b; Luo et al. 2001a) in a timely and efficient manner with the real-time PCR assay, while such results cannot be obtained with the conventional method. Using this real-time PCR method, many more samples (representing more orchard locations) could be processed quickly, providing an opportunity to study the dynamics of inoculum potential on a regional scale. Since we had determined in a previous study (Luo et al. 2005) the critical time periods for quantifying latent infection in blossoms and immature fruit, using the overnight freezing incubation technique (Michailides et al. 2000; Michailides et al. 2005), the real-time PCR method developed in this study may provide a more accurate estimate of risks of blossom blight and fruit rot in an orchard. For example, a previous study (Ma et al. 2003) using a nested PCR assay to estimate spore density level in the air demonstrated that about 170 spores per slide (about 0.01 spores l^{-1}) could be used as a spore density threshold for making a decision on fungicide application to reduce risk of blossom infection. The spore density determined with the real-time PCR assay will be more precise in implementing this threshold.

There are several advantages of the real-time PCR method over the conventional method: (i) with the real-time PCR method possible errors in spore identification which are likely to occur when using the microscopic method are avoided, (ii) although a number of spores in a sample may be lost during the DNA extraction, this number was low according to our observations, (iii) counting spores on a trap tape segment using a

compound microscope can take up to 2 h. Specifically, for samples of spore trapping in a 180-day season, up to 360 h may be required to process all the samples from an orchard. However, since we combined the samples of three consecutive days into one, essentially we had only 60 samples, representing the entire sampling season, and one person would need only 3–4 days to obtain the results by using the real-time PCR assay. The estimated cost of the real-time PCR assay per sample is about US $3 for consumables, exclusive of labour cost. Although the conventional method of counting spores with the microscope does not require the use of any reagents such as those needed in the PCR assay, processing large samples with the former method could be impossible due to high labour costs and time.

Efficiency of the spore sampling and enumeration procedures could be further improved by reducing the time and cost associated with sampling and the real-time PCR assay. The results from the six orchards/years demonstrated that it might not be necessary to operate the spore traps during the entire season. Because no spores were observed in most orchards from late May to late June, it may not be necessary to sample spores during this period in California, except in orchards where the time interval from fruit thinning to irrigation is very short (Luo et al. 2005).

It was difficult to make conclusions in this study on correlations between spore density in the air and blossom infection and fruit rot levels, since only six orchard/year combinations were used and microclimatic conditions among these orchards might be different. Results from more orchards in multiple locations and years are still needed to obtain information on these possible relationships, even though, there was a general trend that such relationships may occur. For instance, since in orchard 4 there were many different peach and nectarine varieties that varied in fruit maturity dates (May to September), fruit rot occurred throughout the season as the different varieties matured, and resulted in a significant accumulation of spores in the orchard air over time (data not shown). Thus, in this orchard spore density increased during the season and was much higher than the densities in the other orchards studied (Fig. 3).

There might be several possible reasons why the r^2 value in the regression between the numbers of spores counted with the microscope and those determined with the real-time PCR assay (Fig. 2) was relatively low ($r^2 = 0.60$). First, counting spores with a microscope may be less accurate and repeatable (Luo et al. unpublished), and secondly spores may be lost during the DNA extraction process that may cause an inherent variation among all samples, even if this proportion is very low, as shown in previous observations (Luo et al., unpublished). However, the stability of the real-time PCR assay for samples of exactly the same number of spores by using separate DNA extraction has not yet been evaluated. Figure 2 implies that quantification of spore counts by using the real-time PCR assay was inherently lower than by counting with a compound microscope (slope of regression = 0.80), and might be due to the two reasons discussed above. Obviously, more samples and tests are needed to evaluate the real-time PCR method. Although it might not be sufficient to evaluate the accuracy of the real-time PCR assay by using only spore counts with a microscope as a standard, the linear correlation between the molecular and the conventional methods suggests that the real-time assay can substitute for the conventional method in quantifying airborne inoculum spore potential in stone fruit orchards.

Standard PCR methods using species-specific primers have been used previously to detect and identify airborne fungal spores (Calderon et al. 2002; Williams et al. 2001). However, these methods provide only qualitative data without any information on changes in the quantity of spores in the air during the season. The change of inoculum density during the season is an important determinant of disease epidemics. Since the real-time PCR assay can be used to process large numbers of samples, it could potentially be a very useful tool in many epidemiological studies. Real-time PCR has also been previously used to quantify fungal inoculum in seeds (Bates et al. 2001), soil (Cullen et al. 2001; 2005), water (Van de Graaf et al. 2003), and plant tissues (Cullen et al. 2001, 2005; Van de Graaf et al. 2003). The present study extended its use to quantitative epidemiological research in which a molecular

method was applied to efficiently determine the dispersal dynamics of pathogen inoculum during a season.

The critical steps in a real-time PCR assay for processing spore trap samples may vary depending on the pathosystem under study and the materials used for the assay. The most critical step in our study was the extraction of sufficient DNA from the spore trap tapes in order to obtain detectable amount of DNA. This assay can detect as few as 20 spores of *M. fructicola* per spore trap tape segment/slide from the field, and can detect a DNA amount corresponding to only two spores per sample from a pure culture of *M. fructicola* (Ma et al. 2003). Thus, the protocol we developed provides data of sufficiently high quality to meet the needs of most epidemiological studies on inoculum dispersal dynamics.

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