# Rapid sampling technique to monitor *Erysiphe necator* more effective than visual scouting

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## 1 Introduction

Knowledge of *Erysiphe necator* presence and severity in a vineyard can be used to guide management decisions (Carisse *et al*., 2009; Caffi *et al.*, 2012; Thiessen *et al*., 2018). However, visually scouting for *E. necator* can be very time-consuming and have a low probability of detecting disease (Mahaffee, *et al*., 2022). This is especially true during early season when *E. necator* can have small, diffuse colonies with few distinct signs or symptoms (Gadoury *et al*., 2012) which makes correct identification of disease difficult. Additionally, visual scouting cannot necessarily be considered as an accurate reflection of the presence of *E. necator* in a vineyard block due to the extensive time required and spatial distribution of diseased plants (Parnell *et al*., 2015; Mahaffee, *et al.,* 2022).

Finding monitoring techniques that are alternatives to visual assessment have long been a significant topic of research in plant pathology. Technological advances have led to options such as airborne spore sampling with PCR detection to determine pathogen presence. But even when these alternative methods are used for monitoring *E. necator* specifically (Falacy *et al*., 2007; Carisse *et al*., 2009; Thiessen *et al.,* 2016) they can be expensive and inflexible, and therefore, inaccessible or impractical to many commercial vineyards.

Integration into existing vineyard practices can make *E. necator* monitoring more cost effective and feasible. Throughout the growing season, workers are frequently in the vineyard conducting tasks that require extensive interaction with the canopy (e.g., shoot thinning and tucking, catch-wire movement, leaf removal, crop thinning, tissue nutrient sampling, crop estimates, pest and disease scouting). As these workers touch the canopy, fungal spores are potentially deposited onto their gloves.

This research examined whether worker gloves could be used as a sampler to monitor for *E. necator* in commercial vineyards. Specifically, the objectives were to assess whether swabbing worker gloves ('glove swabs') could be used as a more efficient tool for detecting the presence of *E. necator* in the vineyard in comparison to visual scouting.

## 2 Methods

Forty-three commercial vineyards were sampled during the growing season (May-September) in 2018-2020 (12 in OR 2018; 16 in OR, 5 in WA, 3 in CA 2019; 7 in OR 2020). Targeted areas included grower identified blocks with a history of higher levels of powdery mildew to increase the possibility of detecting *E. necator.*

Individual blocks were visually assessed for foliar disease incidence using a stratified sampling pattern (Thiessen *et al.,* 2016). Briefly, rows were selected by dividing the vineyard block into approximately three equal sections of rows (strata) and randomly selecting a row from each stratum.

Along each row, while simulating canopy maintenance, the researcher would estimate leaf incidence of *E. necator* by stopping at ten evenly spaced locations along a row and visually examining thirty leaves at each location. For each leaf where signs or symptoms were observed, a sterile cotton swab with a polystyrene handle (25-806 2PC; Puritan Medical) was rubbed over the putative *E. necator* colony and transported to the lab for quantitative PCR (qPCR) as described below. These 'leaf swabs' were used to assess the accuracy of visual assessment by a positive qPCR (Fig. 1). Hereafter, leaf swabs indicate when both visual and qPCR results were positive for *E. necator*.

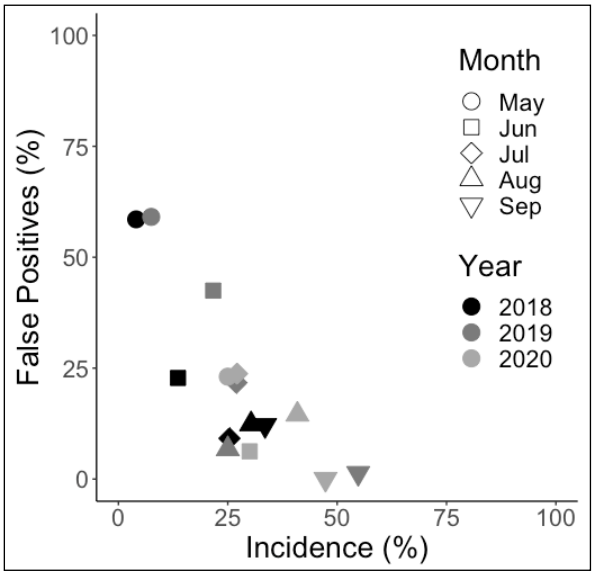


Figure 1: Visual misidentification of *Erysiphe necator.* False positive percentage calculated from the number of samples visually determined to have signs of *E. necator* compared to the number of samples having positive qPCR detection of *E. necator* presence (Theissen *et al*., 2016). Means from each month and year shown.

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At the end of each row sampled for leaf incidence, another sterile cotton swab was rubbed on the surface of the gloves worn while scouting and simulating canopy maintenance (Fig. 2). This 'glove swab' was processed like the leaf swabs and the results for the presence of *E. necator* was compared to the results of the leaf swabs from the row in which it was collected.


Figure 2: Worker gloves can act as a spore sampler for *E. necator* while performing standard canopy maintenance tasks (A). Collection and location information was recorded (B) and a sterile cotton swab was rubbed across glove surface to collect fungal spores that may have been deposited there after canopy manipulation (C-E). Video tutorial: https://youtu.be/VuEBF-1Md08

DNA from the leaf and glove swabs were extracted by placing the cotton tip in a 2 ml microfuge tube with 400 µL of autoclaved 5% Chelex 100 (Sigma) in 18MΩ water, then shaken for 5 min on a horizontal vortex for 5-min prior to boiling twice for 10 min with a 5 sec vortex in between.

A *cytB* qPCR assay was used to detect the presence of *E. necator*. This assay targets a 428 bp mitochondrial amplicon with multiplexed TaqMan QSY probes for wild-type and the G143A mutation (Miles, *et al.,* 2021). This *cytB* qPCR assay is also comparable to the Unc qPCR (Thiessen *et al.,* 2016) and sensitive to a single spore (data not shown). *Erysiphe necator* was considered to be present if either the G-143 or A-143 probe was detected.

A two-class latent class analysis (LCA) (Turechek *et al.,* 2013; Thiessen *et al.,* 2016) was performed to construct a reference standard from the data when neither test can be considered the 'gold-standard' for the detection of *E. necator* in the field. Samples collected during each year (3) were considered as independent populations to avoid over-parameterization of the data. Statistical analyses were conducted using R 4.0.3 (R Core Team 2020) and the R package poLCA (Linzer and Lewis 2011).

## 3 Results

A total of 1,501 leaf swabs and 926 glove swabs were collected resulting in 920 paired row transects, split across years and states (Table 1). Overall combined agreement (when a paired glove and leaf swab both detected the presence or absence of *E. necator*) was 58.5%, with a high degree of association between the two sample types (Odds Ratio: 11.0). Only 1.96% of glove swab samples did not detect *E. necator* in the field when there was leaf swab detection of the pathogen. In 39.6% of the transects, there was no visual detection while the glove swabs detected *E. necator*.

The latent class analysis, which does not assume that either testing condition necessarily reflects the accurate condition response and statistically constructs a reference standard, indicated that glove swabs were more likely to correctly identify the presence of *E. necator.* The test sensitivity, or the conditional probability that a positive, *E. necator* DNA present, glove swab or leaf swab result reflected a truly positive field disease condition was 0.994 and 0.455, respectively. The test specificity, or the conditional probability that a negative, *E. necator* DNA absent, glove swab or leaf swab result reflected a truly negative field condition was 0.999 and 0.936, respectively.

## 4 Conclusions

Glove swabs appear to be more effective as a spore sampler for *Erysiphe necator* than visual scouting. Glove swabs can be collected in a section of the vineyard far more quickly than it takes to visually assess that area for *E. necator*. Covering more area, more efficiently, allows for an increased chance of disease detection (Parnell, *et al.,* 2015).

Additionally, especially when visually scouting during the early season when disease incidence is low, *E. necator* is often falsely identified based on symptoms (Fig. 1), even when collected by trained scouts. On top of the false identification, there is a low probability of finding disease in the field, particularly when disease is below 5% (Mahaffee, *et al*., 2022). Since glove swabs can detect *E. necator* at low disease levels and be collected in conjunction with normal vineyard management practices, they can be an effective tool in decision support systems to monitor disease before it can be visually observed.

Glove swabs as spore samplers are also a more cost-effective and flexible tool than other samplers, such as airborne inoculum spore traps (Thiessen, *et al.,* 2016). Like other spore samplers, the glove swabs can also be used to monitor for *E. necator* throughout the season to evaluate the efficacy of a spray program.

With this assay, the glove swab samples can be used to monitor for QoI fungicide resistance caused by the G143A allelic change. Other SNPs of interest could also be investigated through these samples; though the glove swabs sample from an unknown number of individuals, so are only suitable for tests for pooled samples.

This technique also opens the possibility that other implements that contact the canopy could be adapted to become a spore sampler and used to monitor for other organisms. This approach would also like translate to numerous other cropping systems.

| Year | State | Test[ab] | | Glove swab[a] Positive | Glove swab[a] Negative | Fisher's exact test[c] | Odds ratio[d] | Agreement[e] |
|---|---|---|---|---|---|---|---|---|
| 2018 | OR | Leaf swab | Positive | 92 (28%) | 9 (3%) | <0.001 | 12.24 | 66% |
| | | | Negative | 102 (31%) | 123 (38%) | | | |
| 2019 | OR | Leaf swab | Positive | 90 (29%) | 3 (1%) | <0.001 | 11.48 | 49% |
| | | | Negative | 156 (51%) | 60 (19%) | | | |
| | WA | Leaf swab | Positive | 18 (16%) | 2 (2%) | 0.005 | 6.58 | 51% |
| | | | Negative | 54 (47%) | 40 (35%) | | | |
| | CA | Leaf swab | Positive | 12 (34%) | 2 (6%) | 0.07 | 5.20 | 63% |
| | | | Negative | 11 (31%) | 10 (29%) | | | |
| 2020 | OR | Leaf swab | Positive | 72 (53%) | 2 (2%) | <0.001 | 11.42 | 68% |
| | | | Negative | 41 (30%) | 21 (15%) | | | |

Table 1: *Erysiphe necator* detection results for 'glove swab' samples compared to visual disease collection of *E. necator* directly from the leaves on a 'leaf swab' sample. A qPCR assay with the multiplexed TaqMan *cyt*B G143A assay (Miles *et al.,* 2021) was used for *E. necator* detection for both techniques.
[a] A positive detection was indicated if either the G-143 wild-type or A-143 mutant-type allele was detected in the sample by the *cyt*B qPCR assay as described in the text. A negative sample did not have detectable amolification of either the G-143 or A-143 allele. Numbers describe the count (and percentage) of samples for which *E. necator* DNA was detected or not detected for each sampling method.
[b] Leaf swab results describe aggregate of all leaf swab results from the paired vineyard row associated with the glove swab.
[c] Fisher's exact test was used to assess the null hypothesis that a glove swab detection of *E. necator* is not associated with a leaf swab detection of *E. necator*.
[d] Odd's ratio was used to describe the degree of association between the two techniques' detection of *E. necator,* representing the odds that a glove swab will have the same outcome as the leaf swab.
[e] Agreement was defined as the percentage of both sample test types determined to be either positive or negative.

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