Computer Vision for High-Throughput Quantitative Phenotyping: A Case Study of Grapevine Downy Mildew Sporulation and Leaf Trichomes

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ABSTRACT

Quantitative phenotyping of downy mildew sporulation is frequently used in plant breeding and genetic studies, as well as in studies focused on pathogen biology such as chemical efficacy trials. In these scenarios, phenotyping a large number of genotypes or treatments can be advantageous but is often limited by time and cost. We present a novel computational pipeline dedicated to estimating the percent area of downy mildew sporulation from images of inoculated grapevine leaf discs in a manner that is time and cost efficient. The pipeline was tested on images from leaf disc assay experiments involving two *F*₁ grapevine families, one that had glabrous leaves (*Vitis rupestris* B38 × ‘Horizon’ [RH]) and another that had leaf trichomes (Horizon × *V. cinerea* B9 [HC]). Correlations between computer

vision and manual visual ratings reached 0.89 in the RH family and 0.43 in the HC family. Additionally, we were able to use the computer vision system prior to sporulation to measure the percent leaf trichome area. We estimate that an experienced rater scoring sporulation would spend at least 90% less time using the computer vision system compared with the manual visual method. This will allow more treatments to be phenotyped in order to better understand the genetic architecture of downy mildew resistance and of leaf trichome density. We anticipate that this computer vision system will find applications in other pathosystems or traits where responses can be imaged with sufficient contrast from the background.

Downy mildew of grapevine (*Vitis* spp.), caused by the obligate biotrophic oomycete *Plasmopara viticola* (Berk. & M. A. Curtis) Berl. & de Toni, is a major disease in humid grape-growing regions around the world. The disease is particularly devastating to *Vitis vinifera* cultivars, which lack genetic resistance to the disease. On the other hand, most native North American species (e.g., *V. riparia*) and certain *V. vinifera* hybrids with North American species have genetic resistance to downy mildew (Cadle-Davidson 2008) and are commonly used as parents in resistance breeding efforts and in genetic studies. The pathogen can only infect the abaxial side of leaves and developing berries because it enters via stomata. With leaves, the stomata are located on the abaxial surface whereas, on developing berries, the stomata are only present before developing into lenticels, after which infection is no longer possible (Kennelly et al. 2005). After leaf infection, three possible symptoms or signs may appear. The first is the hypersensitive response, which presents as black necrotic specks on the underside of the leaf (Bellin et al. 2009). The second is sporulation of the pathogen, which appears as white fuzz on the abaxial side of the leaf (Kennelly et al. 2005). The third is yellow spots that have an oily appearance on the adaxial surface, and this symptom is spatially connected to the abaxial sporulation. This article will focus on the occurrence of sporulation. The most common way to screen for sporulation in genetic studies has been to use a leaf disc assay in which a leaf disc is extracted from a leaf using a cork borer and plated on a medium before inoculation with a single *P. viticola* isolate or multiple *P. viticola* isolates, after which a visual rating using a scale of sporulation area is taken (Bellin et al. 2009).

Grapevine species are diverse with respect to their leaf trichome morphology (Ma et al. 2016). Their hydrophobic leaf trichomes have been hypothesized to play a role in resistance to downy mildew by preventing germination of zoospores and preventing germinated sporangia from reaching stomata (Kortekamp and Zyprian 1999). However, determining causality between leaf trichome density and downy mildew resistance requires an experiment without the confounding effect between ancestry and leaf trichome density. Leaf trichomes have traditionally been measured using International Organization of Vine and Wine descriptors (Loughner et al. 2008; Paolucci et al. 2014). Due to the possible causal link between leaf trichome density and downy mildew resistance, accurately phenotyping leaf trichome density is important for downstream analyses in distinguishing effects of genomic variants due to morphological and nonmorphological traits.

In the breeding of crops, one desires to decrease the time and money spent evaluating cross progeny while increasing the number of cross progeny being screened without sacrificing phenotype quality. A common downy mildew screening method in grapevine breeding programs is to visually screen grapevines in the field following natural infection periods (Eibach 2000). Although inexpensive, the method can give inaccurate ratings for genotypes, especially in years with mild disease pressure. In quantitative trait loci (QTL) studies, increasing the number of individuals that are phenotyped increases the statistical power of analyses designed to locate significant QTL. High-throughput plant phenotyping strategies would help to accomplish these goals in a cost-effective manner (Pauli et al. 2016). Numerous methods to use computer vision to detect and quantify plant diseases and their symptoms have been developed (Barbedo 2013). The benefits of using computer vision rather than manual ratings to assess disease include greater precision and accuracy, decreased time needed for ratings, and reduced labor costs (Bock et al. 2010).

Two computer vision methods using the leaf disc assay have been developed for rating downy mildew sporulation; however, they are limited in their use. In one method (Peressotti et al. 2011), a single

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image is required for every leaf disc that is being phenotyped, which is a time-consuming step if there are thousands of leaf discs to phenotype. In the other method (Kim Khiook et al. 2013), the leaf discs need to be removed from their original medium and the images need to be taken on a photographic reproduction bench under artificial lighting, increasing the time and cost associated with the phenotyping. Additionally, proprietary software is used to do the analysis, which further increases the cost of the method. We present a method of using images taken from a smartphone camera that capture inoculated leaf discs in bulk, and open source scripts are then used to quantify percent area of downy mildew sporulation. We show that the same method can be used to quantify the percent leaf trichome area.

MATERIALS AND METHODS

Plant material. Two F_1 grapevine families, *V. rupestris* B38 $\times$ 'Horizon' (*Vitis* sp. interspecific hybrid) (RH) and Horizon $\times$ *V. cinerea* B9 (HC), were grown unreplicated in a vineyard in Geneva, NY (Hyma et al. 2015). *V. cinerea* B9 and the HC family had white trichomes on the abaxial side of their leaves, whereas leaves of Horizon, *V. rupestris* B38, and the RH family were glabrous. In 2015, 163 and 152 F_1 genotypes from the RH and HC families, respectively, were phenotyped for downy mildew sporulation, with three experiments conducted with the RH family and two with the HC family. In 2016, only 157 and 145 genotypes from the respective families were phenotyped, with two experiments conducted with both families; some genotypes were not available due to cold injury. The starting dates of the experiments for the RH family were 22 and 29 June 2015, 13 July 2015, and 5 and 27 July 2016; and those for the HC family were 15 June 2015, 6 July 2015, 27 June 2016, and 12 July 2016.

***P. viticola* isolation and maintenance.** In October 2014, a clonal isolate of *P. viticola* was obtained by single sporangiphore isolation, as previously described (Cadle-Davidson 2008), from a leaf of an organically grown 'Frontenac Gris' (Luby and Hemstad 2006) vine at Cornell Orchards in Ithaca, NY. The isolate was maintained by weekly transfers to surface-sterilized, susceptible leaves plated abaxial side up on a 1% agar Petri dish. The new leaf was misted with sterile water, and the sporulating leaf was inverted briefly onto the new leaf to touch-transfer sporangia. The susceptible genotypes of leaves used for maintenance were *V. vinifera* 'Chardonnay', 'Cabernet Sauvignon', and 'Riesling' and the interspecific hybrid 'Delaware'.

Leaf disc assay. For each experiment, the fifth leaf from the growing tip of four shoots of each F_1 genotype of a family and susceptible and resistant controls was harvested and put in a flexible plastic compact disc holder with holes punched out (Cadle-Davidson 2008). Leaves were maintained at 4 to 8°C until the following day, when they were surface sterilized in 0.5% NaOCl for 2 min and rinsed in sterile, double-distilled water thrice for 2 min/rinse. Two leaf discs per leaf were punched out using a 1-cm cork borer and plated abaxial side up on 30-by-20-cm Pyrex dishes filled with sterile 1% agar. Each leaf disc replicate (eight in total for each experiment) was placed in a separate dish, such that every genotype was represented on every dish. Genotypes were randomized in blocks of 20 in order to control for possible dish location effects while still allowing for rapid plating of leaf discs. Susceptible and resistant controls were included to ensure inoculum quality. For the RH family, Cabernet Sauvignon was the susceptible control for the first two experiments in 2015, whereas Chardonnay was the susceptible control for all other experiments, and *V. rupestris* B38 was the resistant control. For the HC family, Chardonnay was the susceptible control and *V. cinerea* B9 was the resistant control. On the day after plating, each leaf disc was inoculated with 50 μ l containing the *P. viticola* single-sporangial isolate at 5×10^4 sporangia/ml using a HandyStep S repeating pipette (BrandTech Scientific, Essex, CT), and the Pyrex dishes were sealed with plastic wrap and placed in a temperature-controlled room at $23 \pm 1^\circ\text{C}$. The inoculation droplets were absorbed the following day

with tissue paper (Kimwipes). Sometimes the inoculation droplet failed to adhere to the leaf disc, and phenotypes from these leaf discs were not included in the analyses. Sporulation was evaluated between 3 and 6 days postinoculation (dpi) for the RH family in all experiments, except the second one in 2016, where it was evaluated between 4 and 7 dpi due to a delay in sporulation. Sporulation was evaluated between 4 and 7 dpi for the HC family in all experiments. Evaluation was done using both manual and computer vision methods. Leaf trichomes were quantified with the computer vision method at 2 dpi when no sporulation was visible.

Manual and computer vision rating methods. The manual rating method followed an established protocol, whereby sporulation area on leaf discs was rated on a 1-to-5 ordinal scale, with 1 being no sporulation area, 2 being small sporulation area, 3 being moderate sporulation area, 4 being moderately high sporulation area, and 5 being high sporulation area (Kono et al. 2015). Because all of the leaf discs were approximately the same size, the manual rating is implicitly a percent area of sporulation phenotype. For the computer vision rating method, digital images of leaf discs were taken using the native camera application (default settings, without flash) of a handheld Apple iPhone 5s with an image resolution of 3,264 by 2,448 pixels. Each image contained, at most, 20 leaf discs in four rows and five columns. Images were taken on a black laboratory bench within 0.75 m of a window with the fluorescent room lights on. No other supplemental lighting was used. All images for a particular time point of an experiment were analyzed simultaneously via a pipeline of four Python (Python Software Foundation) scripts developed using OpenCV, an open-source computer vision library (Bradski 2000). Of the four scripts, crop.py is used to crop the initial images and values.py is used to find the percent area of sporulation per leaf disc. The other two scripts, circles.py and lines.py, are used to find the correct function parameters that are then used in the values.py script. The script circles.py is used to find the threshold and Hough circle transform algorithm parameters and lines.py is used to find the Hough line transform algorithm parameters. All of the scripts are parallelized, such that, when run, they automatically use all available CPU cores for faster image processing. The scripts and a guide for the scripts can be found at <https://github.com/kdivilov/downymildew-CV>.

For the computer vision system, the images were initially cropped so that only leaf discs that were fully contained in an image were kept (Fig. 1). The cropped images were then converted to Lab color space, which, unlike RGB color space, includes all colors visible to the human eye, with all of the layers thresholded using user-specified values and masked over the original image to filter the background which, in our case, was agar. The blue layer of the original images, which highlights sporulation better than the other layers, was then selected and filtered using a Wallis filter (Pratt 2007; Wallis 1976) to account for unequal lighting conditions during image capture. A threshold was then applied to the grayscale Wallis-filtered images to keep only the brightest pixels (i.e., only pixels with a value of 255 were kept, with the rest set to 0). Leaf veins that were present in the Wallis-filtered images were removed using the Hough line transform algorithm. Leaf discs were detected as circles using the Hough circle transform algorithm. The phenotype obtained at the end was the number of white pixels within a leaf disc divided by the area of the disc in pixels. This corresponded to the percent area of sporulation for a leaf disc in the RH family whereas, in the HC family, it corresponded to the percent area of sporulation and leaf trichomes for a leaf disc. We did not use the white pixel quantity as the phenotype because images were taken at slightly different distances from the leaf discs. Thus, the area of a leaf disc acted as a scaling factor to allow phenotype comparisons between images.

Statistical analysis. The manual and computer vision phenotype distributions for both families were obtained using phenotypes averaged across leaf discs and compared using violin plots made using the ggplot2 (Wickham 2009) and RColorBrewer (Neuwirth 2014) packages in R (R Core Team). Normality of the distributions was evaluated using the Shapiro-Wilk test in R, where the null

hypothesis is that phenotypes are normally distributed. Significance of the test statistic was determined by a z test with a Bonferroni-corrected α value of 0.0025 and 0.0031 for the RH and HC families, respectively, obtained by dividing 0.05 by the number of experimental time points across years and experiments. Because the computer vision phenotype for the HC family measured the percent area of sporulation and leaf trichomes, the effect of leaf trichomes was removed from that phenotype. To do this, a simple linear model was fitted, with the explanatory variable being the averaged computer vision phenotype for an experiment at 2 dpi, representing the percent area of leaf trichomes, and the response variable being the averaged computer vision phenotype for an experiment at a particular day postinoculation. The residuals plus the intercept from the model added on were the new phenotypes that were used for the comparison. The addition of the intercept did not alter the distribution because it is a constant and was done so that the new phenotypes can have a more intuitive interpretation (viz., the percent area of sporulation when the correlation between the averaged computer vision phenotype and the averaged leaf trichome phenotype is zero). Because of this modification, the lower bound on the computer vision phenotype was no longer zero.

Comparisons of manual and computer vision phenotypes for each day postinoculation within an experiment were conducted in R (Seongho 2015) using Spearman's rank correlation coefficients for the RH family and semi-partial Spearman's rank correlation coefficients for the HC family, with the significance of the correlations tested using a t test with a Bonferroni-corrected α value of 0.0025 and 0.0031 for the RH and HC families, respectively. The covariate for the computer vision phenotype for the semi-partial Spearman's rank correlation coefficient was the leaf trichome phenotype. Spearman's rank correlation coefficient rather than Pearson's correlation coefficient was used because the association between the manual and computer vision phenotypes was nonlinear. Comparisons were made using averaged and unaveraged phenotypes.

To test whether the computer vision phenotype at 2 dpi in each experiment was capturing the leaf trichome phenotype, QTL analyses were performed as in Hyma et al. (2015) using a previously created de novo curated genetic map from genotyping-by-sequencing single-nucleotide polymorphism (SNP) linkage data of the HC family (Hyma et al. 2015), except that the genotyping error rate used to calculate conditional genotype probabilities was set to 0.001. An approximate Bayes credible interval was used to find the region in

Fig. 1. Computer vision phenotyping system for grapevine downy mildew sporulation caused by *Plasmopara viticola*. **A**, **B**, and **C**, *Vitis rupestris* B38 × Horizon family and **D**, **E**, and **F**, Horizon × *V. cinerea* B9 family. **A** and **D**, After the initial images have been cropped; **B** and **E**, after they have been passed through the Wallis filter; and **C** and **F**, the same as **B** and **E**, with the exception that the detected leaf discs and their centers and the detected leaf veins have been highlighted.

which a QTL resides with probability 0.95. Physical locations of the SNPs were obtained using the 12X.2 version (URGI 2014) of the grapevine reference genome PN40024 (Jaillon et al. 2007).

RESULTS

A majority of progeny and susceptible control samples had visual signs of sporulation within 7 dpi. The average inoculation failure rate (i.e., the rate at which the inoculation droplet failed to adhere to the leaf disc) was 8.9% across all experiments. The average manual ratings for the susceptible control for the experiments with the RH family at the final time point measured were 4.2, 5.0, and 3.9 in 2015 and 2.8 and 5.0 in 2016. The average manual ratings for the resistant control for the experiments with the RH family at the final time point measured were 1.4, 1.4, and 1.3 in 2015 and 2.3 and 1.5 in 2016. The average manual ratings for the susceptible control for the experiments with the HC family at the final time point measured were 5.0 and 4.9 in 2015 and 5.0 and 5.0 in 2016. The average manual ratings for the resistant control for the experiments with the HC family at the final time point measured were 1.0 and 1.0 in 2015 and 1.3 and 1.0 in 2016.

Using the manual visual rating method required 180 min for an experienced rater to phenotype 1,336 leaf discs on eight Pyrex dishes and 30 min to input the phenotypes into a spreadsheet on a computer. Using the computer vision system from raw images required 3 min for an experienced rater to capture 72 images spanning these same samples and 15 min to phenotype the leaf discs by executing four scripts. Using all four logical cores on a 3.10 GHz Intel Core i3-2100 Processor, crop.py, circles.py, lines.py, and values.py

took an average of 1 min 38 s, 1 min 28 s, 2 min 45 s, and 2 min 16 s, respectively, when run on the 72 images. Across four consecutive days of data collection and analysis, manual rating time totaled 840 min while the computer vision pipeline required 72 min, an estimated time savings of 12.8 h (91%) per experiment and 115.2 h across all nine experiments.

The computer vision phenotypes for the RH family were more often normally distributed than the manual phenotypes, which were often skewed (Fig. 2A and B). For the HC family, the computer vision phenotypes were close to being normally distributed but, at times, were skewed, whereas the manual phenotypes were never normally distributed (Fig. 2C and D). Spearman's rank correlation coefficients for the RH family were between 0.10 and 0.81 for timepoints from 4 to 7 dpi for the unaveraged phenotypes (Fig. 3A). Correlations at 3 dpi, and at 4 dpi in the third experiment in 2015, were low, and these days postinoculation corresponded to time points when sporulation was sparse (Fig. 2B). Semi-partial Spearman's rank correlation coefficients for the HC family were between -0.04 and 0.33 for the unaveraged scores (Fig. 3C). For the unaveraged phenotypes, all correlations were significant, with the exception of the correlations at 3 dpi for the second experiment in 2015 for the RH family and at 4 dpi for the second experiment in 2015 for the HC family, as well as the correlations at all time points for the second experiment in 2016 for the HC family. Averaging the eight scores for each individual gave higher Spearman's rank correlation coefficients for all time points in the RH family, except at 3 dpi in the second experiment in 2015 and at 3 and 4 dpi in the third experiment in 2015 (Fig. 3B). Averaging also gave higher

Fig. 2. Violin plots showing the **A**, computer vision and **B**, manual phenotype distributions of the *Vitis rupestris* B38 × *Horizon* (RH) family for all experiments and the **C**, computer vision and **D**, manual phenotype distributions of the *Horizon* × *V. cinerea* B9 (HC) family for all experiments. The computer vision phenotypes for the HC family were processed to account for the variation in leaf trichome density. The computer vision phenotype measures the percent area of sporulation by *Plasmopara viticola* whereas the manual phenotype measures the area of sporulation using a 1-to-5 ordinal scale. Distributions with dashed lines indicate those significantly different than normal tested using a Shapiro-Wilk test of normality with a Bonferroni-corrected α value.

semi-partial Spearman's rank correlation for all time points in the HC family, except at 4 and 5 dpi in the second experiment in 2016 (Fig. 3D). For the averaged phenotypes in the RH family, all correlations were significant, with the exception of the correlations at 3 dpi for the first and second experiments in 2015 and at 3 and 4 dpi for the third experiment in 2016. For the averaged phenotypes in the HC family, all correlations were significant, with the exception of the correlations at 4 and 5 dpi for the first experiment in 2015 and at 4, 5, and 7 dpi for the second experiment in 2015, as well as the correlations at all time points for the second experiment in 2016.

QTL analyses using the 2-dpi phenotype in each experiment of the HC family found two QTL in each experiment, one each on chromosomes 5 and 8 from Horizon. The 95% approximate Bayes credible interval for each of the two QTL overlapped across experiments. The percent phenotypic variance explained by the QTL on chromosomes 5 and 8 ranged from 10.3 to 21.6 and 13.0 to 18.1%, respectively.

DISCUSSION

Key challenges in phenotyping are selecting the response that reflects the treatment effect and accurately measuring that response at the right time (Cadde-Davidson et al. 2016). In our case, multiple factors from penetration to colonization to sporulation may affect grapevine downy mildew resistance (Boso and Kassemeyer 2008). One approach is to use destructive assays such as quantitative polymerase chain reaction analysis of *P. viticola* DNA concentration (Valesia et al. 2005), microscopy of stained samples to estimate the total amount of hyphae and spores (Boso and Kassemeyer 2008), or

spore counts to quantify sporulation (Kono et al. 2015). Furthermore, one could concentrate on the hypersensitive response elicited by the leaf disc in response to *P. viticola* (Bellin et al. 2009). Alternatively, if one is interested in the area of sporulation, one can use either the computer vision or manual visual rating methods. The biochemical basis for all of these phenotypes is likely not the same but correlations among them likely exist, as previously shown for manual visual ratings and spore counts (Kono et al. 2015). Thus, deciding what phenotype to use is at least partially subjective but not trivial.

Here, we compared nondestructive methods to quantify sporulation using both a computer vision and a manual visual rating method, and showed that computer vision can reduce the time to obtain the phenotypes by >90%. Additionally, the computer vision system allowed one the option to capture all the images first and then analyze them in bulk for all the experiments in a year, as was done for the experiments in this article, if phenotypes are not needed immediately. The images also act as a form of documentation of an experiment that one could reexamine if necessary. Though not tested here, a camera other than the one on the iPhone 5s could be used, provided it has enough resolution to capture sporulation, and phenotypes from two different cameras could be compared because the phenotypes are scaled by the leaf disc area. Another benefit of the computer vision rating system over the manual visual scale is that the phenotypes are quantitative. The computer vision ratings are less frequently skewed toward the most susceptible or resistant ratings compared with the manual visual ratings (Fig. 2). Although we cannot assume that the expected phenotype distribution should be normal, the extreme skew suggests that the manual rating scale does not work well in differentiating sporulation area when sporulation area is high or low.

Fig. 3. Correlations between computer vision and manual visual ratings at all experimental time points, shown as **A**, Spearman's rank correlation coefficients for the *Vitis rupestris* B38 × Horizon (RH) family unaveraged phenotypes; **B**, Spearman's rank correlation coefficients for the RH family averaged phenotypes; **C**, semi-partial Spearman's rank correlation coefficients for the Horizon × *V. cinerea* B9 (HC) family unaveraged phenotypes; and **D**, semi-partial Spearman's rank correlation coefficients for the HC family averaged phenotypes. The computer vision phenotype measures the percent area of sporulation by *Plasmopara viticola* whereas the manual phenotype measures the area of sporulation using a 1-to-5 ordinal scale.

Although we do not have precise measurements of percent area of sporulation for comparison with our computer vision measurements, because we had multiple replications of the leaf discs, the law of large numbers (Bertsekas and Tsitsiklis 2008) tells us that we will approximately obtain the expected value of the manual or computer vision phenotype of a particular genotype. Because both the manual and computer vision ratings measured the same phenotype, although on different scales, given enough replication, the average of the manual and computer vision ratings for a particular genotype should be highly correlated. In actuality, because we had only eight replications per genotype, we may not obtain a high correlation. However, the high correlation between the averaged manual and computer vision ratings in the RH family show that a computer vision system is a suitable replacement for the manual visual rating method. When trichomes are present, the computer vision ratings will approximate the expected value of the combined sporulation area and trichome phenotype, which is not the phenotype of interest. Additional testing of the computer vision system with genotypes with trichomes is needed to determine whether standardization of illumination can remove the influence of light on the calculated phenotype in order to increase the correlation between averaged manual and computer vision ratings when the effect of leaf trichomes is removed from each computer vision rating. Standardization of illumination may also produce more precise ratings for glabrous genotypes.

The current implementation of our computer vision system suffers from minor precision issues, as seen in the violin plots for the second experiment for the RH family in 2015 at 3 and 4 dpi (Fig. 2A). The plot for 4 dpi is shifted down toward lower values compared with the one at 3 dpi, though percent area of sporulation cannot decrease over time. Thus, the computer vision phenotypes between days postinoculation within an experiment cannot be directly compared due to the presence of noise, which is normally distributed (data not shown), and different lighting conditions. However, manual visual ratings are not without precision issues. For example, in the case of phenotyping northern leaf blight on maize leaves, intrarater precision, measured by Pearson's correlation coefficient, was, on average, 0.76 and 0.60, respectively, when using a quantitative or ordinal scale, whereas interrater correlations ranged from 0.65 to 0.93 and 0.58 to 0.82, respectively, when using a quantitative or ordinal scale (Poland and Nelson 2011). Similar precision issues were found in other pathosystems (Bock et al. 2008; Nita et al. 2003).

The leaf trichomes in the HC family presented a specific challenge for both manual ratings and computer vision but we still expected moderate correlation coefficients between the computer vision and manual visual ratings, which was not the case for the second experiment in 2016, even when phenotypes were averaged (Fig. 3D). We found that, if we used the 4-dpi computer vision phenotype as a covariate instead of the 2-dpi computer vision phenotype for the second experiment in 2016, the semi-partial Spearman correlations were 0.04, 0.08, and 0.18 between the manual and computer vision ratings at 5, 6, and 7 dpi, respectively, for unaveraged phenotypes and 0.19, 0.26, and 0.29, respectively, for averaged phenotypes. The low correlation may have been due to the inability to obtain a sufficiently unbiased estimate of the leaf trichome phenotype for each day's unique lighting conditions. Yet this is surprising given that we detected two QTL contributing to percent area of trichomes in the 2-dpi computer vision phenotype for the second experiment in 2016. Our results are consistent with a previous report on trichome density QTL in *Vitis* spp. because each QTL's 95% approximate Bayes credible interval physically overlapped with previously reported QTL (Barba 2015). However, only 34.6% of the phenotypic variance was captured by the two QTL in the second experiment in 2016, leaving the possibility that variation other than that due to the leaf trichomes affected the 2-dpi computer vision phenotype.

In our experiments, we found that approximately 9% of the inoculation droplets did not stay on the leaf discs and, thus, ratings

could not be obtained. It is possible that spraying inoculum onto leaf discs, as done by Bellin et al. (2009), might be a better inoculation method. Although our method had the advantage of placing roughly an equal amount of inoculum on each leaf disc, a comparison needs to be conducted to see whether such precision has an effect on reducing the variance of ratings within genotypes, which is important if one has only a few leaf disc replicates per genotype. Additionally, our method was slow due to the need to individually pipette inoculum onto each leaf disc, which does not scale well when a single person inoculates large numbers of leaf discs.

The current state of the art in image recognition is a convolutional neural network (Krizhevsky et al. 2012). This technique requires large amounts of labeled training images and those images, as well as the test images, would have to be single leaf discs, which take longer to obtain than images of leaf discs in bulk. However, as shown by our work, a Hough circle transform algorithm would be an efficient way to isolate single leaf discs from an image. Our computer vision system, on the other hand, phenotypes the leaf discs in an unsupervised manner, so that previously phenotyped images are not needed in order to phenotype test images. Our computer vision system is also more suited to researchers having less technical knowledge in computer vision and machine learning, two topics that are seldom formally taught to plant pathologists and geneticists. We anticipate that, with minor tweaking of parameters, most biologists could use this tool for pathosystems or traits where responses can be imaged with sufficient contrast from background.

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