Title

Measurements of Aerial Spore Load by qPCR Facilitates Lettuce Downy Mildew Risk Advisement.

Journal

Plant Disease, 104(1)

ISSN

0191-2917

Authors

Dhar, Nikhilesh

Mamo, Bullo Erena

Subbarao, Krishna V

et al.

Publication Date

2020

DOI

10.1094/pdis-03-19-0441-re

Measurements of Aerial Spore Load by qPCR Facilitates Lettuce Downy Mildew Risk Advisement

Nikhilesh Dhar, Bullo Erena Mamo, and **Krishna V. Subbarao,** Department of Plant Pathology, University of California, Davis, c/o USDA, Agricultural Research Service Station, 1636 E. Alisal Street, Salinas 93905; **Steven T. Koike**, TriCal Diagnostics, 8100 Arroyo Circle, Gilroy CA 95020; **Alan Fox**, Fox Weather, LLC, Fortuna, 95540; **Amy Anchieta**, and **Steven J. Klosterman,** USDA ARS, 1636 E. Alisal St., Salinas, CA 93905.

Corresponding author's email: Steve.Klosterman@ARS.USDA.GOV.

Abstract

The lettuce downy mildew pathogen, *Bremia lactucae*, is an obligate oomycete that causes extensive produce losses in lettuce. Initial chlorotic symptoms that severely reduce the market value of the produce are followed by the appearance of white, downy sporulation on the abaxial side of the leaves. These spores become airborne and disseminate the pathogen. Due to the heterogeneity and quick adaptation of this pathogen in the field, containing lettuce downy mildew has relied on repeated fungicide applications to prevent outbreaks. However, in addition to direct economic costs, repeated application of fungicides leads to the development of fungicide-insensitivity in the pathogen. We therefore deployed a quantitative PCR assay-based detection method using a species-specific DNA target for *B. lactucae* coupled with a spore trap system to measure airborne *B. lactucae* spore loads within three commercial fields that each contained experimental plots. Based upon these measurements, we advised whether or not to apply fungicides on a weekly basis within the three experimental plots. This approach allowed the savings of approximately 1.7 sprays over the course of these field experiments. The reduction of

fungicide applications to manage *B. lactucae* can decrease lettuce production costs for the growers while slowing down the development of fungicide resistance in the pathogen.

Bremia lactucae Regel, the causal agent of the lettuce (*Lactuca sativa* L.) downy mildew, is an obligate oomycete phytopathogen (Raid and Datnoff, 1992; Choi et al., 2011; Wu et al., 2017). Downy mildew is an important foliar disease of lettuce worldwide (Raid and Sui, 2012). In lettuce, the disease causes light green to yellow angular spots on the leaf, along with a white, fluffy, downy (downy mat like) growth on the abaxial surface. Since downy mildew is primarily a foliar disease, these leaf symptoms reduce the marketability of lettuce while impacting the produce during transit and storage (Wu et al., 2017). Until recently, introduction of resistant cultivars, fungicide application and cultural practices were adequate to manage outbreaks of the disease in endemic areas (Kunjeti et al., 2016). Novel variation of *Bremia lactucae* has rapidly led to the emergence of numerous races over the past two decades, and thus plant breeding efforts are unable to keep pace with the emerging pathotypes (Kunjeti et al., 2016). Thus, fungicides have remained as an effective option against this pathogen and significantly improving the marketability of lettuce (Raid and Datnoff, 1989; Koike and Turini, 2017). However, emergence of fungicide-resistant races of the pathogen (Crute et al., 1987; Raid et al., 1990; Schettini et al., 1991; Brown et al., 2004; Raid and Sui, 2012), and the loss of registration of many fungicides due to concerns over environmental safety and adverse impact on human health have limited the available fungicides for the management of downy mildew disease in lettuce (Brown et al., 2004; Crute et al., 1987; Isaac, 1999; Wicks et al., 1994). Without the means to adequately control disease, there are also food safety concerns associated with downy mildew infections on lettuce, as these infections and subsequent lesions can lead to secondary infections of non-phytopathogenic enteric pathogens

such as *Escherichia coli* O157:H7 (EcO157) and *Salmonella enterica* Typhimurium *(S. Typhimurium*) (Simko et al., 2015).

The necessity for effective and alternative management practices has led to the use of currently approved fungicides before infection as a means of reducing disease outbreaks (Zijlstra et al., 2011) and is a practice currently favored by growers. In such a scenario, application of fungicides, even before the development of the disease, is as effective way to manage the disease in field or greenhouse conditions. Most available fungicides for this disease management strategy are protective rather than curative in nature, and thus requires repeated applications to prevent an outbreak of the disease. Such overuse of chemicals is not consistent with the current regulatory objectives to reduce their usage and prevent the development of fungicide insensitivity in the pathogen populations (Raid and Sui, 2012). Development of fungicide insensitivity in *B. lactucae* has been well documented for the phenylamide fungicide metalaxyl and fosetyl-Al in California and Florida (Crute et al., 1987; Raid et al., 1990; Schettini et al., 1991; Brown et al., 2004).

Downy mildew of lettuce is a polycyclic disease that is caused by spores that are dispersed in the air (Fall et al., 2016); once they land on the crop, germinate and infect leaves, they can produce a second generation of spores in as few as 8 days (Fall et al., 2015). Hence, the chances of disease outbreak over the growing season increases not only from downy mildew incidence from within the field but also from the inoculum from adjacent lettuce fields. Although frequent application of fungicide has proven to effective in preventing an extensive disease outbreak, it could also be unnecessary and further add to the cost of production. Thus, a detection system for outbreak of disease based on an increase in the aerial spore count could prove to be highly effective in reducing the number of fungicide applications and further delay the emergence of fungicide resistance in the pathogen.

Spore trapping and other early detection techniques for quantification of fungal and oomycete pathogens hold promise in forecasting disease outbreaks (West et al., 2008; Carisse et al., 2009; Gent et al., 2009; Úrbez-Torres et al., 2010; Zijlstra et al., 2011; Granke et al., 2013; Schena et al., 2013; West and Kimber, 2015; Mahaffee and Stoll, 2016). Previous work in our laboratory enabled quantification of the fluctuations in the amount of *B. lactucae,* and further suggested that it may be feasible to deploy the method of airborne detection of *B. lactucae* (Kunjeti et al., 2016) to formulate a spore trap-based downy mildew risk advisory to optimize the timing and number of fungicide applications necessary for disease control. Reductions in fungicide applications in turn could translate into lower production costs to the growers.

The objectives of the current work were to determine whether disease risk levels based on quantitative PCR of *B. lactucae* from spore impaction samplers on a local scale could be used to effectively time fungicide applications for disease control, and secondly to determine which, if any, weather parameters are associated with increases or decreases in the detectable DNA from the airborne sporangia.

Materials and Methods

Experimental field plots and placement of impaction spore trap sampler rods

Three different experimental plots were established within three different commercial lettuce fields near Salinas, CA, in the period between April and October in 2016. The dimensions of each experimental plot within the fields were 6.1 m x 36.6 m (Fig. 1). Each treatment and the untreated control were replicated four times in total, and each treatment area was subdivided into sections of 2.0 x 9.1 m (w x l) (Fig. 1). The three treatments were replicated and randomized and the treatments included: 1) no spray, 2) calendar spray applied about weekly, and 3) the spore-trap advised spray.

The commercial field surrounding each of the three experimental plots was sprayed with fungicides for control of downy mildew, on a schedule determined by the grower. For spore collection, two impaction spore traps were employed. One spore trap was placed on the North side, and the second trap was placed on the South side of each of the lettuce fields (Fig. 1); since the predominant wind direction in the Salinas Valley is either from the Northwest, or from the Southeast (Choudhury et al. 2017).

Impaction spore trap sample collection, DNA extraction and quantitative PCR

Impaction spore trap sample collections from impaction spore sampler rods were conducted as described previously (Klosterman et al. 2014) unless specified otherwise. Briefly, samples from the two impaction spore traps were collected twice per week, on Mondays and Thursdays, over a period of 8-10 weeks for each of three field experiments. DNA was extracted as described in Kunjeti et al. (2016).

The primers and probe used for the study were previously evaluated for species-specificity and characterized for use in qPCR (Kunjeti et al., 2016). The species-specific primers and probes were designed to amplify an 86 bp region close to the 5' end of a unique 861 bp putative open reading frame (orf286) of unknown function in *B. lactucae* that was not present in other oomycete taxa. Briefly 5' flap (lower case letters) was added to the primers; F17 (5'-aataaatcataaGTCATTGTTTGATTTAACT-3') and R18 (5'-aataaatcataaGAGCTAGATTTACCA-3'); Bl_probe, 5'-ATCAATAGAATGTCCCACTGCAAT-3' (Kunjeti et al. 2016). The 5' end of the probe was labeled with FAM (fluorescein), and the 3' end with Black Hole Quencher-1 (BHQ1; Biosearch Technologies, Inc., Novato, CA). Additional BLAST analyses since the original publication

116 (Kunjeti et al., 2016) revealed that this sequence of nucleotides is still unique to *B. lactucae* and is
117 not similar to any other sequence in the GenBank database.

118 All qPCR experiments for determining the Cq values that corresponded to a previously
119 calculated spore count (Kunjeti et al., 2016) were performed in 384 well plates using a LightCycler
120 480 II real time PCR machine (Roche Diagnostics, Basel, Switzerland). The qPCR reactions were
121 run in triplicate using one DNA extraction per spore trap. The qPCR reaction volume of 12 µl
122 contained 200 nM probe, 200 nM of each primer, 1x real master mix (5 PRIME®, Hilden,
123 Germany), and 1 µl of DNA extracted from the impaction spore trap rods. The LightCycler 480
124 software (release 1.5.0) and the Absolute Quant/Fit Point analysis were used to run all of the qPCR
125 assays for *B. lactucae* DNA quantification with a reaction profile of 10 min at 95ºC initial
126 denaturation followed by 55 cycles of 95ºC for 10 s and 56ºC for 30s.

127 **Pesticide application based on spore trap advisory**

128 Preventative fungicides Manzate (UPI), Reason (Bayer) and Revus (Syngenta) were applied
129 in succession at a rate of 0.95 kg, 0.23 L and 0.23 L, respectively, in a total spray volume of
130 542.51L/hectare with the surfactant Syl-tac at a final concentration of 0.05% (v/v) manually with
131 a back-pack sprayer. For the plots designated for calendar spray, the first two calendar sprays were
132 done with Manzate followed by Reason and Revus for the third and final sprays, respectively
133 (Table 1). For the plots designated for spore trap-based advisory sprays, only Revus was applied.
134 The application schedule is provided in the supplementary section/data (Table 1).

135 The spore trap-based advisory to determine whether or not to apply fungicides was based on a
136 Cq value of 24 from the qPCR analyses of the spore trap sample coinciding with the first detection
137 of plants with downy mildew symptoms. Based on extensive replicated analyses of standard curves
138 using known amounts of sporangia of *B. lactucae* (Kunjeti et al. 2016), we were able to interpolate

the approximate levels of airborne sporangia collected within the 72 or 96-hour sample collection intervals.

Collection of disease incidence data

Field plots were visually examined each week for downy mildew symptoms and sporulation. Plants within plots were counted as positive for disease when there was one or more symptomatic leaf. Disease incidence was calculated based on the number of plants that displayed symptoms, divided by the total number of plants within a replicated treatment area of 2.03 m x 9.1 m. The area under disease progress curve (AUDPC) was calculated based on days after planting (dap).

Collection of Weather Data

Weather data for all experimental plot sites were generated by Fox Weather, LLC using the MtnRT® custom software described by Fox (2011). For the basic initial data, we used the North American Regional Reanalysis (NARR), obtained from NOAA at 32 km grid spacing and 3-hour time step. From the NARR data, we produced sequential nested WRF runs (4 km grid) to provide input for MtnRT. Using MtnRT, running at a 1.5 km grid, we produced a continuous record of hourly data, including temperature, relative humidity, leaf wetness, wind direction and speed at 2 m above ground. The location coordinates for the weather data calculation, within 100 m of each experimental plot, were 36.6896N, 121.5909, and elevation of 45 m.

The MtnRT processing included the interpolation to 1-hour increments for the surface weather data, including wind direction and speed (Mesinger et al., 2006). This included consideration of MtnRT's calculation of wind vector variations resulting from development of nocturnal inversions. Wind speed was calculated at 2 m height above ground instead of the standard 10 m height. This was done to better represent wind speeds more closely approximating the canopy level for lettuce. The calculation of wind speed at 2 m was based on a logarithmic relationship of wind speed versus

distance above ground. A simplified version of the theoretical logarithmic relationship is U2 = U1 (ln (h2/z0)/ln (h1/z0)), where U2 is wind speed to be calculated (in this case at h2 = 2 m), while U1 is the speed at the reference height (h1). The value of roughness length (z0) for most purposes would be 0.1 to 0.2 for the experimental location. Implementation for near-surface wind profiles in MtnRT provides inputs to operational forecasts in many different types of terrain and roughness conditions. For this project, we used the standard logarithmic wind profile in our operational version of MtnRT. All wind calculations are referenced above the plant canopy.

When necessary, supplemental weather data were collected from the nearby Salinas CIMIS weather station (Salinas South II, Station #214) and the Salinas Municipal Airport weather station (KSNS). Weather data collected from the CIMIS station included hourly measurements of temperature, relative humidity, wind speed, and wind direction.

Correlation analysis was conducted to determine the possible relationship between weather variables such as temperature, wind speed and relative humidity and the detectable DNA from the airborne sporangia (i.e., the Cq values from the qPCR) using R. Experiment 2 was not included in correlation analysis as fewer Cq data points were available.

Results

Flow cytometry-based *B. lactucae* sporangial counts and C_q values using DNA extracted from sporangia had previously revealed a very high correlation (Kunjeti et al., 2016). The Cq values collected across the three experiments by qPCR (Fig. 2) were used to determine the approximate corresponding number of sporangia for all sample rods from the field experiments. In the 72 to 96 hr spore trap sample collection intervals employed in the current study, the corresponding sporangia counts per impaction spore trap ranged from a low of 7 sporangia detected on April 28,

2016 (Cq = 31.99 ± 0.47), to a peak of 605,306 sporangia on May 19, 2016 (Cq = 13.91 ± 0.1). The average Cq values on these dates from both impaction spore traps that were placed on the North and South ends of the field were 30.88 on April 28, 2016 and 14.86 on May 19, 2016 (Fig. 2), reflecting similar values on both spore traps. In May 2016, we observed downy mildew symptoms and sporulation on three plants in the field surrounding experimental plot 1. Detection of *B. lactucae* DNA at the nearby impaction spore trap samplers corresponded to the qPCR-based average Cq value of 23.3. Subsequently, fungicide applications to the field were advised when the Cq value obtained from the impaction spore trap samplers fell below the threshold of 24 (~ 1136 sporangia equivalents).

Examination of weather parameters relative to increases or decreases in detectable levels of *B. lactucae* DNA was carried out at intervals of 12:00 to 6:00 am and 6:00 to 10:00 am. Sporangia are produced during periods of high humidity and lower wind speed, conditions prevalent during the hours of 12:00 to 6:00 am during the lettuce growing seasons of the Salinas Valley, while increases in wind speed and temperature during the 6:00 to 10:00 am interval are critical for dispersal of *B. lactucae* (Wu et al., 2001). For both field experiments 1 and 3, we observed correlations between changes in wind speed and/or temperature with corresponding changes in detectable levels of *B. lactucae* DNA. This is illustrated in Figures 3 and 4, where the detection interval preceding the two lowest Cq values (between the hashed red lines) is also marked by sharp increases in wind speed and temperature. A similar trend was observed during experiment 3 (Figs. 5 and 6), where the two lowest Cq values were associated with increases in temperature and wind speed over the detection interval (between the hashed red lines). This also was evidenced by negative correlation coefficients between temperature and Cq values during the third experiment at both intervals of 12:00 to 6:00 am (r = -0.41, $p > 0.05$) and 6:00 to 10:00 am (r = -0.37, p >

208 0.05). In experiment 1, temperature was positively correlated with Cq values ($r = 0.25$) during the
209 detection intervals indicated above. Wind speed and Cq values were positively correlated during
210 both experiments 1 ($r = 0.25$) and 3 ($r = 0.52$ to 0.78) over the indicated detection intervals.
211 Between relative humidity and Cq values, positive correlations were detected during experiment
212 3 ($r = 0.29$ to 0.34). During experiment 1, the correlation between relative humidity and Cq values
213 was weak ($r = 0.04$, $p > 0.05$). In field experiment 2, a visual association was not observed between
214 the two lowest Cq values and changes in wind speed and temperature (Supplemental Figs 1 and
215 2). As there were fewer data points for Cq values in this experiment, statistical correlation analysis
216 was not feasible.

217 For all three field experiments, an average qPCR threshold Cq of 24 was used to advise
218 fungicide sprays. That is, when the average level of *B. lactucae* DNA detection from the impaction
219 spore traps was recorded with a Cq value of ≤ 24, fungicide was applied on replicate 3 shown in
220 Figure 1. For comparative purposes, fungicides were applied roughly once per week on regular
221 calendar-based fungicide spray intervals (Fig. 1, replicate 2). Thus, relative to the regular spray
222 routine, spore trap-advised sprays enabled the savings of one fungicide application in experiments
223 1 and 2, since the Cq values were recorded as ≤ 24 early in experiments 1 and 2 (Table 1). For
224 experiment 3, however, Cq values of ≤ 24 were not recorded until about 1 month after the initial
225 spore trap readings (Table 1), thus saving three fungicide sprays. In total, across all three
226 experiments, there was an average savings of 1.7 fungicide applications per crop.

227 The records of disease incidence in experiment 3 (Table 3) enabled calculations of the
228 effectiveness of withholding fungicides applications (Table 2), relative to the levels of disease
229 control whether regular calendar-based sprays were applied, or whether the spore trap advised
230 sprays were made. The untreated replicates had the highest AUDPC of 387.3% in dap unit as

231 compared to 203.6% for calendar and 369.65% for advisory sprays (Table 3); disease incidence
232 increased chronologically over the three different dates in September 2016. At the final
233 observation date (28 September), 32.6% of the plants in the untreated replicates developed downy
234 mildew, while 6.9% of the plants in the regular calendar-based sprays had symptoms, and for the
235 spore trap advised sprays 16% of the plants were symptomatic (Table 3). Therefore, there was an
236 effective disease control of 79% in the regular calendar-based spray routine as compared to the
237 untreated control replicates, and nearly 50% disease control for the spore trap-based fungicide
238 spray advisory.
239

240 ## Discussion

241 The aims of this study were to assess whether impaction spore trap samplers could be used to
242 time fungicide applications based on the load of detectable airborne *B. lactucae*, and to further
243 assess environmental parameters that favor airborne detection and pathogen dispersal. Spore trap
244 advised fungicide applications were made based upon a numerical Cq value of 24 from qPCR that
245 was obtained in week 1, of experiment 1, when sporulation was detected on a few plants on the
246 North end of the field surrounding the experimental plot. This current work builds upon our
247 previous work where we characterized a highly sensitive TaqMan assay for *B. lactucae* DNA
248 derived impaction spore trap samplers and extended the application of this technique to advise if
249 and when to apply fungicides for control of lettuce downy mildew.
250 In plots 1 and 2, no disease incidence was recorded within the experimental plots although the
251 increases in detectable *B. lactucae* DNA were nevertheless used in our in-house downy mildew
252 risk advisory, to advise fungicide application at Cq values > 24. Some variables in these
253 experiments set up in commercial fields were beyond our control, such as the extent of genetic

resistance in fields planted with resistant lettuce cultivars. Thus, disease incidence may be reduced or absent if the pathotype population present in the area is unable to infect resistant lettuce genotypes. Different races of *Bremia lactucae* are known. Additionally, the weather parameters may not have been conducive for disease development following periods of higher disease risk, even when sporangia counts were higher than the spray-threshold of 24.

By the third trial under field conditions, fungicide application based on the spore trap-based spray advisory reduced the disease incidence by ~50% with respect to untreated plots. Overall an average of 1.7 sprays/growing season was conserved with our spore trap method of advance detection of the pathogen, with a maximum saving of three fungicide sprays out of four regular sprays during the third trial where greater efficacy was achieved.

Temperature, relative humidity, and wind speed have been shown to play a direct role on the sporulation mechanism in *B. lactucae* (Su et al., 2004; Su et al., 2009). Lettuce cultivation has been heavily limited to geographical regions with cool to moderate temperatures, and high humidity, environmental factors which also are the conditions that predispose the crop to *B. lactucae* infection (Scherm et al., 1995). To this effect, we included these weather parameters in our study to determine if a relationship exists between these factors and the detectable pathogen DNA levels. Associations between weather data and Cq values in this study indicated a pattern that the period of increased detection of *B. lactucae* DNA at the impaction spore trap samplers was marked by conditions that typically favor spore production and dispersal in *B. lactucae*. Specifically, peak periods of DNA detection, indicative of spore dispersal, were observed at higher levels in field plots 1 and 3, in association with higher temperature, increased wind speed, and a drop in relative humidity between 6:00 and 10:00 a.m. *B. lactucae* spore dispersal has been tightly correlated with the late morning hours in other studies, when there are decreases in relative

277 humidity and increasing temperature (Wu et al., 2001; Wu et al., 2005). The results of our study
278 shed some light on the timing of dispersal, and further supports those earlier findings with actual
279 impaction spore trap data. However, further research using our methodology of impaction spore
280 trap should analyze DNA from the airborne sporangia at multiple time points; sufficient enough
281 to permit the establishment of the observed relationship between weather parameters and the
282 detectable DNA amount.

283 The fungicide applications in this study were advised based on the Cq values from the amount
284 of DNA detected from spores captured by the rods. The fungicide applications were not based on
285 combining advanced prediction of weather patterns. However, a close analysis of the relationships
286 between the Cq values for detection of spores and weather parameters indicated a weather pattern
287 reported in other studies that is favorable for production and dispersal of *B. lactucae* spores. Hence,
288 we propose that the use of impaction spore traps for advanced detection in conjunction with
289 advanced weather pattern prediction will provide an added layer of protection to crops and a
290 simultaneous reduction in application of fungicides.

291

292 ## Acknowledgment

293 We acknowledge funding from the California Leafy Greens Research Program and the
294 California Department of Food and Agriculture (Grant SCB1043). We thank L. Ochoa (USDA-
295 ARS, Salinas, CA) and Daniel Marchebout for collecting impaction spore trap samples. The
296 authors appreciate the assistance of Raquel Gearheart with processing of the data and software
297 support, and Leonard Montenegro in obtaining the NARR data and running the WRF 4 km
298 simulations.

299 The mentioning of trade names or commercial products in this publication is solely for the
300 purpose of providing specific information and does not imply recommendation or endorsement by

301 the U.S. Department of Agriculture (USDA). USDA is an equal opportunity provider and
302 employer.
303
304 ## Literature Cited
305
306 Brown, S., Koike, S. T., Ochoa, O. E., Laemmlen, F., and Michelmore, R. W. 2004. Insensitivity
307 to the fungicide fosetyl-aluminum in California isolates of the lettuce downy mildew pathogen,
308 *Bremia lactucae*. Plant Dis. 88:502-508.
309
310 Carisse, O., Tremblay, D. M., Lévesque, C. A., Gindro, K., Ward, P., and Houde, A. 2009.
311 Development of a TaqMan real-time PCR assay for quantification of airborne conidia of *Botrytis*
312 *squamosa* and management of Botrytis leaf blight of onion. Phytopathology 99:1273-1280.
313
314 Choi, Y.-J., Thines, M., Runge, F., Hong, S.-B., Telle, S., and Shin, H.-D. 2011. Evidence for high
315 degrees of specialization, evolutionary diversity, and morphological distinctiveness in the genus
316 *Bremia*. Fungal Biology 115:102-111.
317
318 Choudhury, R. A., Koike, S. T., Fox, A., Anchieta, A. G., Subbarao, K. V., Klosterman, S. J.,
319 McRoberts, N. 2017. Spatiotemporal patterns in the airborne dispersal of spinach downy mildew.
320 Phytopathology 107:50-58.
321
322 Crute, I. R., Norwood, J. M., and Gordon, P. L. 1987. The occurrence, characteristics and
323 distribution in the United Kingdom of resistance to phenylamide fungicides in *Bremia lactucae*
324 (lettuce downy mildew). Plant Pathol. 36:297-315.

Fall, M. L., Van der Heyden, H., Beaulieu, C., and Carisse, O. 2015. *Bremia lactucae* infection efficiency in lettuce is modulated by temperature and leaf wetness duration under Quebec field conditions. Plant Dis. 99:1010-1019

Fall, M.L., van der Heyden, H., Carisse, O. 2016. A quantitative dynamic simulation of *Bremia lactucae* airborne conidia concentration above a lettuce canopy. PLoS ONE 11(3): e0144573.

Fox, A. D. 2011. MtnRT White Paper: Summary of the MtnRT system. Providing weather inputs for plant disease models. Online publication. www.foxweather2.com

Gent, D. H., Nelson, M. E., Farnsworth, J. L., and Grove, G. G. 2009. PCR detection of *Pseudoperonospora humuli* in air samples from hop yards. Plant Pathol. 58:1081-1091.

Granke, L. L., Morrice, J. J., and Hausbeck, M. K. 2013. Relationships between airborne *Pseudoperonospora cubensis* sporangia, environmental conditions, and cucumber downy mildew severity. Plant Dis. 98:674-681.

Klosterman, S. J., Anchieta, A., McRoberts, N., Koike, S. T., Subbarao, K. V., Voglmayr, H., Choi, Y. J., Thines, M., and Martin, F. N. 2014. Coupling spore traps and quantitative PCR assays for detection of the downy mildew pathogens of spinach (*Peronospora effusa*) and beet (*P. schachtii*). Phytopathology 104:1349-1359.

348 Koike, S. T., and Turini, T. 2017. University of California, IPM Pest Management Guidelines:
349 Lettuce. UC ANR Publication 3450. http://ipm.ucanr.edu/PMG/r441100411.html.
350
351 Kunjeti, S. G., Anchieta, A., Martin, F. N., Choi, Y. J., Thines, M., Michelmore, R. W., Koike, S.
352 T., Tsuchida, C., Mahaffee, W., Subbarao, K. V., Klosterman, S. J. 2016. Detection and
353 quantification of *Bremia lactucae* by spore trapping and quantitative PCR. Phytopathology
354 106:1426-1437.
355
356 Lebeda, A., Pink, D. A. C., and Astley, D. 2002. Aspects of the interactions between wild *Lactuca*
357 spp. and related genera and lettuce downy mildew (*Bremia lactucae*). Pages 85-117 in: Advances
358 in Downy Mildew Research. P. T. N. Spencer-Phillips, U. Gisi and A. Lebeda, eds. Springer
359 Netherlands.
360
361 Mahaffee, W. F., and Stoll, R. 2016. The ebb and flow of airborne pathogens: monitoring and use
362 in disease management decisions. Phytopathology 106:420-431.
363
364 Mesinger, F., DiMego, G., Kalnay, E., Mitchell, K., Shafran, P. C., Ebisuzaki, W., Jovic, D.,
365 Woollen, J., Rogers, E., Berbery, E. H., Ek, M. B., Fan, Y., Grumbine, R., Higgins, W., Li, H.,
366 Lin, Y., Manikin, G., Parrish, D., and Shi, W. 2006. North American Regional Reanalysis: A
367 long-term, consistent, high-resolution climate dataset for the North American domain, as a major
368 improvement upon the earlier global reanalysis datasets in both resolution and accuracy. Bull. Am.
369 Meteorol. Soc. 87:343-360. https://doi.org/10.1175/BAMS-87-3-343.
370

371 Raid, R. N., and Datnoff, L. E. 1992. Downy Mildew of Lettuce - EDIS - University of Florida
372 https://edis.ifas.ufl.edu/pdffiles/VH/VH04400.pdf
373
374 Raid, R. N., and Datnoff, L. E. 1989. Efficacy of fosetyl-Al foliar application in controlling downy
375 mildew of lettuce. Page 362 in: Proceedings of the Florida State Horticultural Society.
376
377 Raid, R. N., and Datnoff, L. E. 1990. Loss of the EBDC fungicides: impact on control of downy
378 mildew of lettuce. Plant Dis. 74:829.
379
380 Raid, R. N., and Sui, D. D. 2012. Management of Lettuce Downy Mildew with Fungicides. Sui.
381 Proc. Fla. State Hort. Soc. 125:218–221.
382
383 Schena, L., Li Destri Nicosia, M. G., Sanzani, S. M., Faedda, R., Ippolito, A., and Cacciola, S. O.
384 2013. Development of quantitative PCR detection methods for phytopathogenic fungi and
385 oomycetes. J. Plant Pathol. 95:7-24.
386
387 Schettini, T. M, Legg, E. J., and Michelmore, R. W. 1991. Insensitivity to metalaxyl in California
388 populations of *Bremia lactucae* and resistance of lettuce cultivars to downy
389 mildew. Phytopathology 81:64-70.
390
391 Simko, I., Zhou, Y., and Brandl, M. 2015. Downy mildew disease promotes the colonization of
392 romaine lettuce by *Escherichia coli* O157:H7 and *Salmonella enterica*. BMC Microbiol. 15:1-9.
393

394 Su, H., van Bruggen, A. H. C., Subbarao, K. V., and Scherm, H. 2004. Sporulation of *Bremia*
395 *lactucae* affected by temperature, relative humidity, and wind in controlled conditions.
396 Phytopathology 94:396-401.
397
398 Su, H., van Bruggen, A.H.C., and Subbarao, K.V. 2009. Spore release of *Bremia lactucae* on
399 lettuce Is affected by timing of light initiation and decrease in relative
400 humidity. Phytopathology 99:67-71.
401
402 Úrbez-Torres, J. R., Battany, M., Bettiga, L. J., Gispert, C., McGourty, G., Roncoroni, J., Smith,
403 R. J., Verdegaal, P., and Gubler, W. D. 2010. Botryosphaeriaceae species spore-trapping studies
404 in California vineyards. Plant Dis. 94:717-724.
405
406 West, J. S., and Kimber, R. B. E. 2015. Innovations in air sampling to detect plant pathogens. Ann.
407 Appl. Biol. 166:4-17.
408
409 West, J. S., Atkins, S. D., Emberlin, J., and Fitt, B. D. L. 2008. PCR to predict risk of airborne
410 disease. Trends Microbiol. 16:380-387.
411
412 Wicks, T. G., Hall, B., and Pezzaniti, P. 1994. Fungicidal control of metalaxyl-insensitive strains
413 of *Bremia lactucae* on lettuce. Crop Prot. 13:617-623.
414

Wu, B. M., A. H. C. van Bruggen, and K. V. Subbarao. Downy Mildew. Pages 32-35 in: Compendium of Lettuce Diseases and Disorders. K. V. Subbarao, R. M. Davis, R. L. Gilbertson, and R. N. Raid, eds. APS Press, St. Paul, MN.

Wu, B. M., van Bruggen, A. H. C., Subbarao, K. V., and Pennings, G.G.H. 2001. Spatial analysis of lettuce downy mildew using geostatistics and geographic information systems. Phytopathology 91:134-142.

Wu, B. M., Subbarao, K. V., and van Bruggen, A. H. C. 2005. Analyses of the relationships between lettuce downy mildew and weather variables using geographic information system techniques. Plant Dis. 89:90-96.

Zijlstra, C., Lund, I., Justesen, A. F., Nicolaisen, M., Jensen, P. K., Bianciotto, V., Posta, K., Balestrini, R., Przetakiewicz, A., Czembor, E., and van de Zande, J. 2011. Combining novel monitoring tools and precision application technologies for integrated high-tech crop protection in the future (a discussion document). Pest Manage. Sci. 67:616-625.

Figure legends

Figure 1: Design and dimension of the plots for setting up the spore trap in the field experiments near Salinas, CA. Three beds (36.576 m x 2.01 m) were set up side by side and the treatments were randomized for no spray (control), regular/calendar spray, and spore trap advisory spray. Each of the beds were sub-divided into four plots of 9.144 m x 2.029 m for the above purpose. Two solar

438 powered impaction spore traps were set up at the either end of the field and were sampled twice
439 every week on Monday and Thursday, respectively, for the entire period of cultivation.
440
441 **Figure 2:** The average quantification cycle (Cq) values *Bremia lactucae* from quantitative PCR
442 from DNA isolated from two solar powered impaction spore traps using a *B. lactucae*-specific
443 primer and probe set (Kunjeti et al. 2016). The traps were placed at the opposite end of the
444 experimental lettuce plot for all the three field trials shown in chronological order in (a), (b) and
445 (c). Spore traps were sampled twice per week on Mondays and Thursdays.
446
447 **Figure 3:** Average wind speed, temperature and relative humidity for each day over a period of
448 six weeks during the first field trial for impaction spore trap based advisory spray of an
449 experimental lettuce field plot. Each point on the graph corresponds to a single date representing
450 the average of five time points between a 6:00 am and 10:00 am block.
451
452 **Figure 4:** Average wind speed, temperature and relative humidity for each day over a period of
453 six weeks during the first field trial for impaction spore trap based advisory spray in a lettuce field
454 plot. Each point on the graph corresponds to a single date representing the average of five time
455 points between a 12:00 am and 6:00 am block. The environmental factors shown here for this
456 block of time has been reported by various studies to be critical for infection of the lettuce by
457 *Bremia lactucae*.
458
459 **Figure 5:** Average wind speed, temperature and relative humidity for each day during the third
460 field trial for impaction spore trap based advisory spray of an experimental lettuce field plot. Each

point on the graph corresponds to a single date representing the average of five time points between a 6:00 am and 10:00 am block.

Figure 6: Average wind speed, temperature and relative humidity for each day during the third field trial for impaction spore trap based advisory spray in a lettuce field. Each point on the graph corresponds to a single date representing the average of five time points between a 12:00 am and 6:00 am block.

Figure 7: Average wind speed, temperature and relative humidity for each day during the second field trial for impaction spore trap based advisory spray in a lettuce field. Each point on the graph corresponds to a single date representing the average of five time points between a 6:00 am and 10:00 am block.

Figure 8: Average wind speed, temperature and relative humidity for each day during the second field trial for impaction spore trap based advisory spray in a lettuce field. Each point on the graph corresponds to a single date representing the average of five time points between a 6:00 am and 10:00 am block.

Supplementary Figure 1: Average wind speed, temperature and relative humidity for each day during all the three field trials for impaction spore trap based advisory spray in a lettuce field plot over the six-month period from April-Sept 2016. Each point on the graph corresponds to a single date representing the average of five time points between a 12:00 am and 6:00 am block.

484 **Table 1.** Average Cq values from the quantitative PCR from DNA isolated from the impaction

485 spore traps over the course of all the three field trials on the respective dates

Date	C_q Trap #1	C_q Trap #2	Ave. C_q	Spore Count*
SET-1				
4/22/16	25.12	28.04	26.58	306.52
4/25/16	23.12	25.22	24.17	1174.91
4/28/16	31.99	29.78	30.88	17.67
5/2/16	25.48	27.31	26.39	277.40
5/5/16	28.79	24.42	26.61	435.77
5/9/16	23.30	19.59	21.45	9333.61
5/12/16	X	24.88	24.88	615.35
5/16/16	25.91	27.28	26.60	228.45
5/19/16	13.91	15.81	14.86	394350.57
5/23/16	15.53	18.23	16.88	129377.92
5/26/16	18.20	20.71	19.46	24635.23
6/2/16	22.48	22.45	22.47	2798.90
6/6/16	19.52	21.86	20.69	10954.10
6/8/16	21.28	25.24	23.26	3183.64
SET-2				
7/4/16	X	28.71	28.71	55.27
7/6/16	25.05	26.18	25.62	411.18
7/11/16	25.61	X	25.61	387.77
7/14/16	24.29	30.67	27.48	452.51
7/25/16	20.07	24.86	22.47	6614.84
7/28/16	19.64	23.50	21.57	8990.49
8/1/16	16.93	22.91	19.92	46417.06
SET-3				
8/25/16	29.11	27.89	28.50	67.75
8/29/16	26.50	26.97	26.74	193.30
9/1/16	28.16	27.99	28.08	82.49
9/6/16	28.87	X	28.87	49.98
9/8/16	25.65	25.93	25.79	347.63
9/12/16	24.87	28.99	26.93	331.86
9/15/16	26.30	25.82	26.06	295.57
9/19/16	23.94	23.80	23.87	1158.53
9/22/16	27.04	25.30	26.17	314.52
9/26/16	23.78	23.34	23.56	1419.83
9/29/16	22.13	23.81	22.97	2328.26

*Corresponding spore count calculated using formula as described in our previous work (Kunjeti et al., 2016).

Table 2. Schedule of pesticide application in the third field trial

Untreated	Regular Calendar Spray Schedule*	Spore-trap Advisory Spray Schedule
No Spray	**22 Aug. 2016** **Manzate**	No Spray
No Spray	**2 Sept. 2016** **Manzate**	No Spray
No Spray	**9 Sept. 2016** **Reason**	No Spray
No Spray	**19 Sept. 2016** **Revus**	**19 Sept. 2016** **Revus**
Harvested - 3 Oct. 2016		

*Applications of fungicides Manzate, Reason or Revus fungicides were applied on the dates listed with a backpack sprayer as described in the methods.

496 **Table 3.** Three randomized plots visually monitored for disease symptoms in the third field trial

497

8 Sept 2016	No spray	Calendar spray	Advisory spray
# Infected plants[a]	34	24	45
% Plants infected[b]	5.2%	3.7%	6.9%
21 Sept 2016			
# Infected Plants	81	48	76
% Plants infected	12.5%	7.4%	11.7%
28 Sept 2016			
# Infected plants	211	45	104
% Plants infected	32.6%	6.9%	16.0%
AUDPC[c]	387.3	203.6	369.65

510

511 [a]The number of plants appearing diseased/infected in each plot

512 [b]The percentage of total plants per plot appearing infected

513 [c]The area under disease progress curve in % days after planting unit (dap)