CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

Combined Annual Reports for Spinach and Lettuce Downy Mildew projects, 2018-2019

Risk assessment, early detection, and control of downy mildew of lettuce and spinach

Steven J. Klosterman
USDA-ARS, Salinas, CA

Alexander I. Putman
University of California, Riverside, CA

Krishna V. Subbarao
University of California Davis, Salinas, CA

ABSTRACT

Downy mildews are very destructive diseases on leafy green crops in California. Downy mildew diseases on spinach and lettuce are caused by the obligate oomycete pathogens *Peronospora effusa* (*P. effusa*), and *Bremia lactucae* (*B. lactucae*), respectively. Sporangia of both pathogens can be airborne and may also initiate disease from seed or potentially by soilborne oospores. DNA-based detection assays were previously deployed to quantify the levels of airborne inoculum of *P. effusa* and *B. lactucae* from spore traps, which may be useful in disease forecasting. Over the past year, we completed all analyses on correlations of weather parameters and decreased or increased the presence of *B. lactucae*. Though there was a clear visual graphic correlation between increased temperature and wind speed and increased detection of *B. lactucae*, statistical analyses revealed that all such correlations with weather data were not significant. Results are presented showing the number of airborne spores that were correlated with *B. lactucae* DNA detection levels of some samples. A major objective of the *P. effusa* research entailed an examination of oospore production, survival, germination, and conditions necessary for reproducible infection of spinach. Oospore production is a sign of sexual reproduction in the pathogen population, and sexual reproduction is known to increase the rate at which new races or pathotypes emerge. We did not observe symptoms of downy mildew in our plots in the desert. Since we had previously determined that *P. effusa* could be detected in leaves at least a week prior to leaf symptom development, we had continued to improve our own in-house assay and supplied material to a local biotech company to test and develop the *P. effusa* leaf detection assay further. We tested eight biopesticides to determine if each could reduce symptoms and sporulation of *P. effusa*. In two separate experiments in 2017 and 2018, we noted a statistically significant reduction in disease incidence with the biopesticide Procidic. In summary, tracking the levels of windborne inoculum of the pathogen and in-field leaf detection applications may be valuable to inform efficient spray applications for disease control, and in the case of spinach, to assess whether a field is infected (for early harvest before loss) or prior to the entire field becoming infected and symptomatic. Oospores of *P. effusa* have been detected in nearly 21% of commercial seed lots and reveal that sexual reproduction of the pathogen occurs worldwide and is commonplace. Biopesticide treatments in the field and treatments that can eliminate *P. effusa* on spinach seed may limit some outbreaks, especially for organic spinach.

PROJECT TITLE: Risk assessment, early detection, and control of downy mildew of lettuce and spinach.

INVESTIGATORS:

Steven J. Klosterman
USDA-ARS
1636 E. Alisal St
Salinas, CA 93905

Alexander I. Putman
Dept. of Plant Pathology
University of California, Riverside
Riverside, CA

Krishna V. Subbarao
Dept. of Plant Pathology
University of California, Davis,
c/o USDA-ARS Station
1636 E. Alisal St
Salinas, CA 93905

COOPERATING PERSONNEL:

Steven T. Koike
Trical Diagnostics, Hollister, CA

Shyam Kandel, Frank Martin, Beiquan Mou
USDA-ARS, Salinas, CA

Allen Van Deynze, Charlie Brummer, Juliana Osorio Marin, Bullo Mamo, Nikhilesh Dhar
University of California, Davis

OBJECTIVES (downy mildew on lettuce):

1) Conduct correlation analyses between spore trapping results from lettuce fields with weather data to determine local conditions and airborne inoculum load favorable for downy mildew to time fungicide application in a forecast system.

OBJECTIVES (downy mildew on spinach):

1: Examine *P. effusa* oospore production, survival, and conditions necessary for reproducible infection of spinach.

2: Provide the necessary materials, protocols, and advice to commercialize the previously developed leaf assay for early detection of spinach downy mildew.

3: Test biopesticides to reduce sporulation of *P. effusa* on organic spinach.

PROCEDURES:

Data were collected for detection of *Bremia lactucae* in commercial lettuce fields near Salinas, CA in three experimental plots. Solar/battery-powered impaction spore traps (Figure 1) obtained from Dr. Walt Mahaffee (USDA-ARS, Corvalis, OR) were sampled for windborne inoculum of *B. lactucae* at each location. The pairs of 1.1 mm x 40 mm stainless steel rods (Figure 1B) coated in silicone vacuum grease (Dow Corning) were collected at the 72- or 96-hour time intervals. The collected rods (with the downy mildew spores) were stored at 4^{o}C until DNA extraction using the Nucleospin Plant II kit (Machery Nagel) following the manufacturer's protocol for isolating genomic DNA from fungi. Quantitative PCR and the estimates of spore load were based on the DNA level detected was determined as described [13].

High-quality weather data from each of the trap locations were obtained from Fox Weather LLC. Logistic regression based on spore increase and decrease was used to correlate temperatures, relative humidity, and wind speed with spore load over the course of six time frames throughout the day. Summary data from weather variables such as temperature, windspeed, and relative humidity were directly correlated with spore load (indirectly determined by pathogen DNA amounts detected). Lettuce fields nearby trap sites were monitored for disease incidence. Disease incidence was measured as a percent of diseased plants in each experimental plot within the field.

Correlation analysis was conducted and shown for experiment one to determine the possible relationship between weather conditions (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 statistical analysis software (R software). Experiment two was not included in correlation analysis as only a few Cq data points were available.

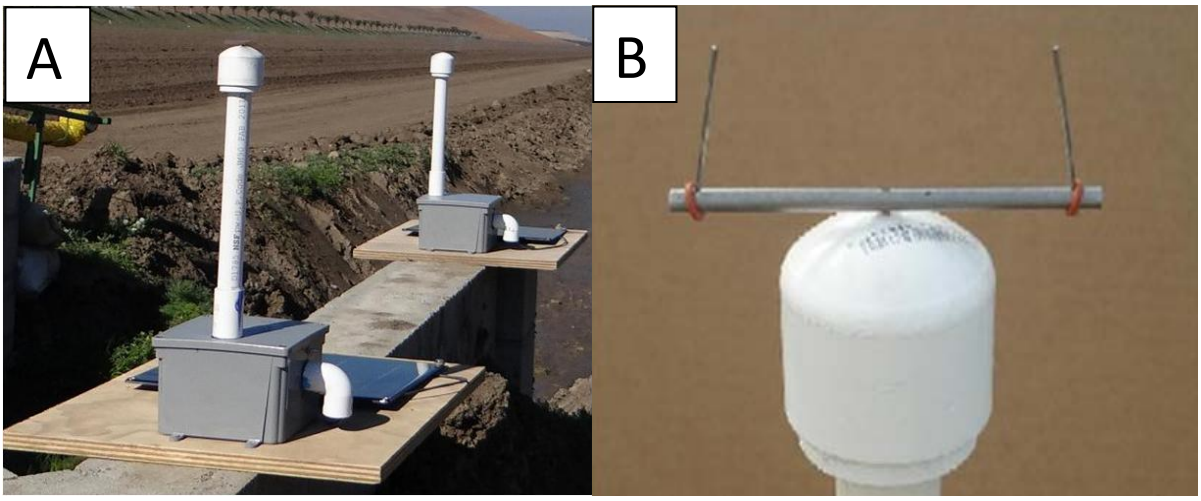

Figure 1. Spore trap system to detect *Bremia lactucae* and *Peronospora effusa*, the causal agents of lettuce and spinach downy mildew, respectively. **A)** Two spore traps in operation south of King City, CA. **B)** Spore trap head with rotating arm and removable stainless-steel rods.

Biopesticide testing was conducted using experimental plots of spinach cultivar Viroflay planted at the USDA-ARS station in Salinas in the summer and late fall of 2017 and spring of 2018. These plots in each season consisted of twelve 80" beds x 120 ft in length. The biopesticide treatments (Table 1) were applied by manual backpack sprays to the foliage and the spinach was monitored for disease incidence. Spinach plants received brief overhead irrigation two evenings per week to maintain the high humidity in the plant canopy. Watering was timed

around biopesticide applications so as not to wash them off. In addition to the untreated control, the eleven treatments included biopesticide combinations with wetting agents, the conventional fungicide-Aliette, and applied at similar or different schedules. These treatments were randomly assigned with four replications. The biopesticide dose, equipment calibration, and fungicide spray schedules were maintained according to the agreement with IR-4 Project management. The application of biopesticides was initiated 20 days after seed sowing and continued for six weeks. The downy mildew incidence was monitored twice; 22 and 32 days after seed sowing, respectively. Four samplings were done from each subplot containing the individual treatments. Each of these incidence samplings included observations of spinach plants in a 1 ft^2 area.

Table 1. Treatments and spray schedule used to test the biopesticides for the control of spinach downy mildew in the study.

Treatment designation	**Treatment details**	**Spray schedule (applied on the days listed, beginning 20 days after sowing)**
A=T1	Untreated control	Not applicable
B=T2	MBI-110	0, 7, 14, 21, 28 and 35
C=T3	Oxidate 2.0	0, 7, 14, 21, 28 and 35
	TerraGrow	0, 7, 14, 21, 28 and 35
D=T4	OS0 5%	0, 7, 14, 21, 28 and 35
	Cueva	4, 10, 17, 24 and 31
E=T5	Oxidate 2.0	0, 7, 14, 21, 28 and 35
F=T6	LifeGuard	0, 7, 14, 21, 28 and 35
G=T7	Zonix	0, 7, 14, 21, 28 and 35
H=T8	Oxidate 2.0	0, 7, 14, 21, 28 and 35
	Zonix	0, 7, 14, 21, 28 and 35
I=T9	Lifeguard	0, 7, 14, 21, 28 and 35
	Cueva	4, 10, 17, 24 and 31
J=T10	Procidic	0, 7, 14, 21, 28 and 35
K=T11	Sil-Matrix	0, 7, 14, 21, 28 and 35
L=T12	Aliette	0, 7, 14, 21, 28 and 35

We (USDA, Salinas) previously acquired a dew chamber and purchased a wall-mounted air conditioner to develop a reliable system to infect spinach with *P. effsua*. These items were installed in a room dedicated for this purpose to maintain the necessary cold temperatures. Using this new equipment/facility, the conditions for infections within the growth chamber and the humidity tent were as described previously [11]. The exception was that the dew chamber was maintained at cooler temperatures, in the range of 7.5 to 13.3^{o}C. Twenty-four hours following the initial inoculation, the plants were moved to a humidity tent maintained in an improvised cold room for seven days before returning overnight to the dew chamber.

To analyze seeds for the presence of *P. effusa*, 1000 or 500 seeds from seed lots were washed with water for 10 min by vigorous vortex mixing, the debris was pelleted by gentle centrifugation for 5 min, and then the pelleted debris was analyzed under light microscopy. At

least two or three replicates were performed, and an average number of oospores for all three was reported. Viability tests were as described [7,14].

In this reporting period, we established a field trial in a grower field in the Coachella Valley. The susceptible cultivar Viroflay, which was confirmed to be free of *P. effusa* oospores, and a commercial cultivar, in which *P. effusa* oospores were detected, were each planted on Nov. 29 and December 20, 2018. Seed was planted to achieve 2.3 million seed per acre with two passes from a small-plot planter with 12 lines on a 2 in. spacing. The trial consisted of 80" to the center beds, and plots measured 10 ft. in length. There were three replicate plots per treatment, and each plot was separated by a bare bed or a bare plot on either side. The trial was sprinkler irrigated and with the exception of fungicide applications generally maintained according to commercial standards for the Coachella Valley. Following emergence, the plots were scouted approximately weekly for symptoms of downy mildew. The trial was terminated in early April.

USDA-ARS, Salinas supplied genomic DNA of downy mildew pathogens to AgBiotech, Monterey, CA to begin efforts commercialize an early detection *P. effusa* assay. The method of the assay is based on competitive allele-specific PCR (KASP) assays and takes advantage of a single nucleotide polymorphism previously identified in the rDNA sequence of *P. effusa* and *P. schachtii* [12].

RESULTS:

Bremia lactucae

We had previously been successful in devising an assay for quantifying the levels of airborne spores (through DNA quantification) of *Bremia lactucae* [12]. To assess the amount of airborne inoculum of *B. lactucae* in relation to weather patterns near Salinas, spore traps (Fig. 1) were placed at either end of three different commercial lettuce fields (each planted at different times) and spore trap samples were processed twice weekly.

The results for field experiment 1 are shown in tabular form in Table 1 spore count is shown relative to the Cq values. The Cq values that are lower correspond to the higher levels of airborne pathogen detectable. Thus, the average Cq value on 5/19/16 of 14.86 is associated with a count of 394,351 sporangia.

The results from Table 1 indicate the Cq values obtained by quantification of the pathogen DNA over the course of the third experiment. In the rightmost column of Table 1, the estimated numbers of sporangia of *B. lactucae* are indicated, revealing high numbers of sporangia present during the sampling periods. Because we had observed downy mildew symptoms on several plants within the commercial field when the Cq value fell below the value of 24 on both spore traps, this was set as the threshold value at which fungicide sprays were initiated in a plot within the commercial field. Airborne sporangia detection values ranged from nearly 18 on 4/28/19 when we could not detect symptomatic plants in the field to nearly 394,000 sporangia on 5/19/16 when there were indeed many plants with active disease in the field.

We had observed some sporulation of *B. lactucae* on lettuce within the same commercial field on May 9, 2016, and within two weeks after that initial outbreak, the highest incidence of downy mildew symptoms was recorded. Observations of these graphical data as presented in the last report revealed that these lower values (higher pathogen) correlated with periods of highest average wind speeds and temperatures during the experiment but not relative humidity. However, additional statistical analyses revealed that correlations between wind speed and temperature and inoculum load were not significant.

Table 1. Lettuce downy mildew of spore load near a commercial lettuce field. Spore load was determined indirectly by monitoring *Bremia lactucae* DNA levels as previously described and correlating DNA levels with sporangia that were counted [13] to arrive at the spore counts shown.

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

Peronospora effusa

Results for two experiments on the testing of multiple biopesticides for organic spinach for protection against downy mildew are shown in Figures 2 and 3. The first experiment was conducted in 2017, and results are shown in Figure 2. Because of rain delays, we moved the second experiment to the current reporting period. We completed the second experiment in early 2018 (Figure 3). In both the first and second experiments, there was a significant reduction in disease incidence for the treatment Procidic compared with the untreated control (Figs. 2 and 3). However, the performance was variable between different treatments, as mean comparisons indicated that biopesticides MBI-110, and the combination of OSO 5% and Cueva reduced overall reported disease incidence (Fig. 2), though these differences were not statistically significant.

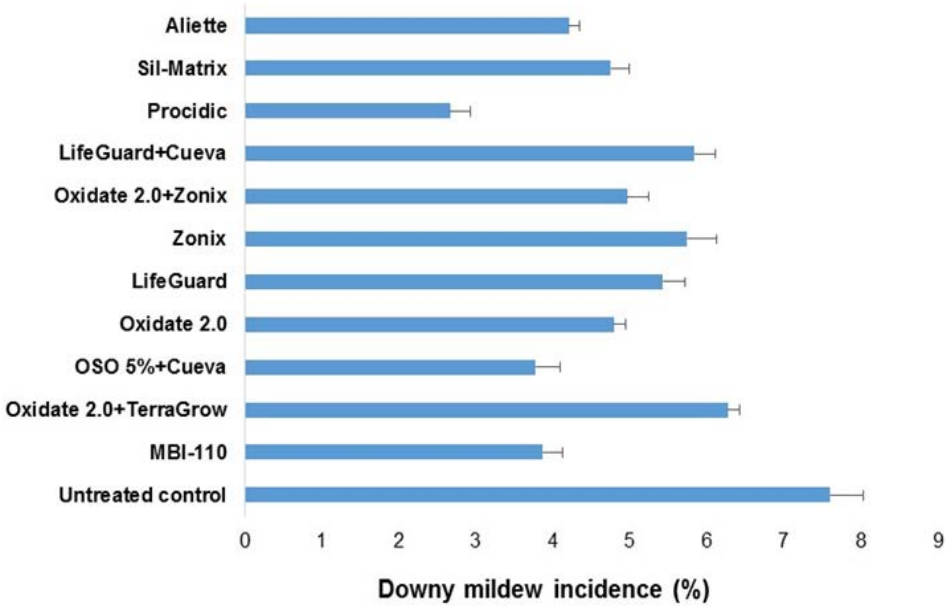

Figure 2. 2018 Biopesticide trial. Disease incidence was recorded three days after treatment.

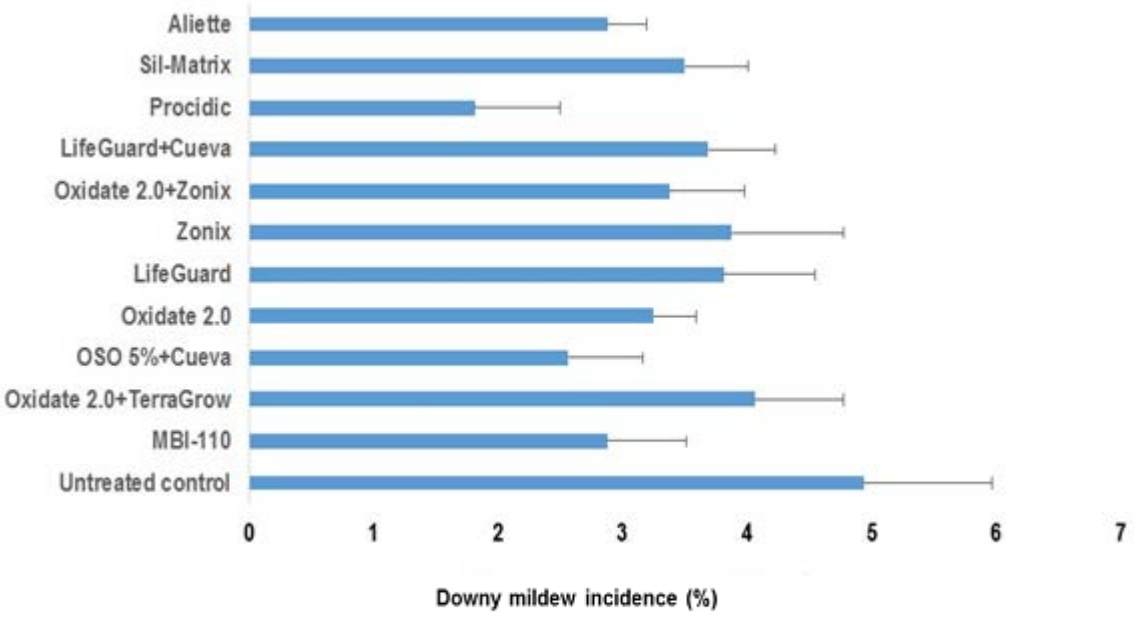

Figure 3. 2018 Biopesticide trial. Disease incidence was recorded three days after treatment.

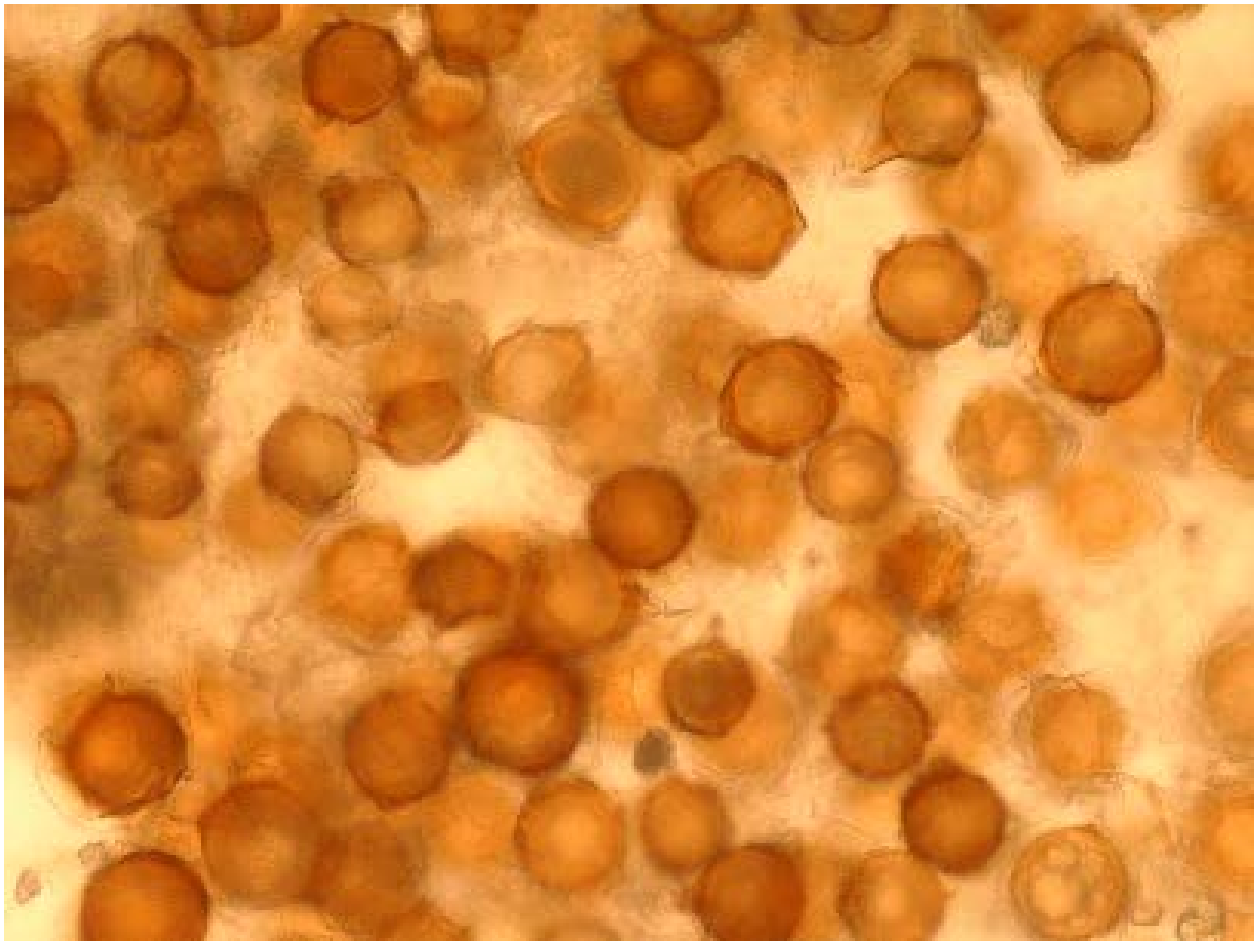

Figure 4. Oospores detected on the inner surface of a pericarp from a *Peronospora effusa* infested seed -- the area examined is approx. 0.5 mm^2. The oospores are approx. 30 micrometers in diameter, the approximate value determined previously for average oospore diameter [2,14].

In total, with the additional seed examinations made in this reporting period, the number of modern seed lots infested with oospores of *P. effusa* stands at 21% (since the initial finding by our lab in 2014). We had previously observed oospore germination from leaf sample origin and from those washed off of seeds. In this reporting period localization of the oospores in seed was examined [10]. Oospores were present on the inner surface of the thick outer pericarp present on spinach seeds. Figure 4 indicates about 90 oospores found in an area of 0.5 mm^2, suggesting that 2268 oospores can reside in one seed. This calculation is based on an inner pericarp surface of 12.6 mm squared.

We did not observe symptoms of downy mildew within our plots in the Coachella Valley at any time during the study. Although outbreaks of spinach downy mildew occurred in the Imperial Valley following a few periods of favorable conditions, conditions were generally unfavorable overall, and downy mildew was not observed in the Coachella Valley to our knowledge.

We investigated seed transmission of spinach downy mildew further in the winter of 2018-2019. In the effort to remove the possibility of windborne inoculum contaminating the experiments, we used the isolator system shown in Figure 5A to prevent the introduction of windborne inoculum. We had previously run an experiment in 2018 in this system in which we observed very low disease incidence (> 1%), possibly due to low leaf wetness. Therefore, in this reporting period, we installed sprinklers in each of six different isolator chambers to increase leaf wetness. The possibility of soilborne inoculum was also excluded because soil in the isolators was fumigated prior to the experiments. We planted multiple oospore infested seed lots within the different sections of the isolator, and a seed lot sample from which we detected no oospores. In one of the commercial seed lot samples planted that contained *P. effusa* oospores, we observed sporulation on several leaves (see the red arrow; Fig. 5B). However, unexpectedly, we also observed sporulation on the negative control seeds in the experiment which did not have detectable oospores (Viroflay).

Figure 5. A) Plant isolators at the USDA-ARS station in Salinas, CA for planting *Peronospora effusa* oospore-infested seeds or those that were non-infested. **B)** Downy mildew chlorotic lesions on a spinach leaf (at red arrow) associated with sporulation on a plant grown within the isolator using *Peronospora effusa* oospore-infested seeds.

We have established a system to obtain routine infections in a dew chamber and humidity tents, both maintained in an air-conditioned room at the USDA station in Salinas. This system employs a dew chamber maintained in the range of 7.5 to 13.3ºC for 24 hr following initial inoculation. After this initial 24 hr period, plants are moved to a humidity tent maintained in the improvised cold room for seven days before returning to the dew chamber for overnight incubation. Under these conditions, we continued to repeat inoculations that led to routine infections resulting in characteristic symptoms (Fig. 6) in this period, and therefore this objective is complete.

Figure 6. Symptomatic differential set of spinach plants following inoculation with the downy mildew *Peronospora effusa*, at the USDA-ARS station, Salinas, CA. Note the distinct patches of chlorosis on leaves.

The assay shown in Figure 7 was conducted using DNA provided by the USDA-ARS, Salinas for two different *Peronospora* pathogens that differ by a single nucleotide in a region of DNA previously established [11], enabling the differentiation and successful identification of *P. effusa*. The drawback of this approach requires the input of DNA derived from both pathogens, *P. effusa* and *P. schachtii* (Swiss chard pathogen). In this reporting period, we exhausted the limited supply of *P. schachtii* DNA for use by AgBiotech in further assay development. Therefore, we examined the fields of organic Swiss chard, and recently obtained more *P. schachtii* and extracted the DNA for further assay development.

DISCUSSION:

In our previous work, we have applied spore trapping and qPCR for quantification of the downy mildew pathogens of lettuce and spinach [4,5,10,11,12,13]. Tracking the levels of windborne inoculum of the pathogen has yielded insights on the prevalence of the downy mildew pathogens of lettuce and spinach and increases of both pathogens have been detected in the Salinas Valley during their respective growing seasons [10,11]. In the previous report, we showed the correlation between the increased wind speed and increased temperature and increased inoculum load of *B. lactucae* in the late morning hours. This is consistent with the historical research (conducted without the aid of spore traps). However, we demonstrated that this correlation was not statistically significant, probably because we did not have enough spore trap samples in the comparison over time. When additional spore trap experiments are undertaken in the future, additional spore trap collection time points would be helpful to increase data significance.

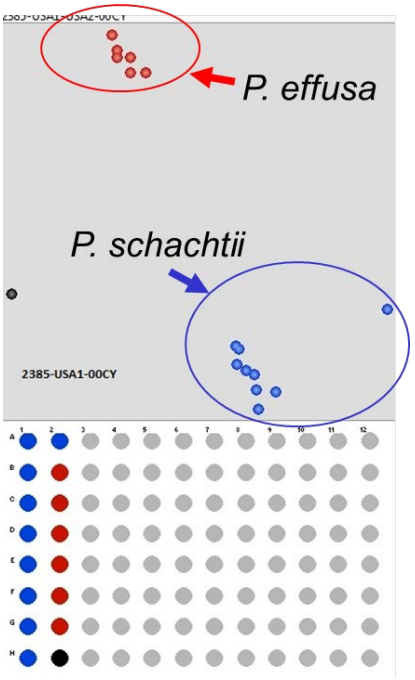

Figure 7. Differentiation of *P. effusa* (spinach pathogen) and *P. schachtii* (beet pathogen) by a single nucleotide polymorphism (SNP) assay. Image and assay courtesy of Mark Massoudi, AgBiotech, Monterey, CA. The successful assay will ultimately have to be compatible with the sampling of leaves directly. The results shown are those from using purified genomic DNA of both pathogens.

We speculate that additional trap dates will improve correlations with weather variables. In the currently analyzed experimental data for airborne detection of *B. lactucae*, we purposefully tried to use fewer trap test dates, so to test the practicality of the trap system at a field or ranch level. Though it would likely not be practical to install and routinely sample spore traps around lettuce ranches because of labor costs in addition to trap maintenance costs, we have shown that using the spore trap detection values directly with a set threshold for spray application (Cq = 24) resulted in a savings of 1.7 fungicide sprays. But more importantly, the conditions necessary for disease development can be traced back to these threshold levels, providing additional data to

spinach growers to decide when is the optimal time to apply fungicides. A full manuscript describing these findings has been submitted and is under peer review.

We previously published the work on the finding of oospores in modern spinach seed lots, and examined additional seed lots for oospores, in addition to the 82 characterized in the original publication [11]. Thus, with current, up-to-date counts, we know that about 21% of the ~ 210 commercial seed lots that have been tested contain oospores. It has been about thirty-five years since the initial report of *Peronospora effusa* on spinach seed lots. That study was conducted in Japan and a study that also provided evidence for seed transmission [8].

Since *P. effusa* is heterothallic [9], two strains of different mating type are required to form the long-lived (1-2 years) sexual oospores. The presence of oospores on 21% of spinach seed lots suggests long term survival of the pathogen on seed. New pathotypes or races of the pathogen, as well as both mating types, can be dispersed on seeds. Also, the mating of different strains of *P. effusa* after bringing the mating types together in a new region has implications of quickly increasing the genetic diversity within populations, contributing to the appearance of new "races" or pathotypes. Oospores on modern spinach seeds [8, 14] likely initiate disease outbreaks in current and new production areas. Given the importance of seed transmission in other downy mildew systems, treatments to reduce viable *P. effusa* oospores on spinach seed may slow disease spread and pathogen diversification. In this current report, we demonstrated that oospores of *P. effusa* are present in much higher numbers per seed, under the seed pericarp. This finding explains why we must shake the seed samples so vigorously to obtain countable numbers of oospores under microscopy. Further, given previous evidence of seed transmission [8], this further advances the importance of oospores arriving on spinach seed in initiating disease in current and new spinach production areas.

Seed transmission studies are still ongoing. In the previous experiment conducted in early 2018, the door to the isolators was opened once during a nearly eight-week period, prior to observation, and hence we could not rule out the possibility of airborne contamination in that experiment. Nevertheless, because the doors remained closed in this experiment finished in early 2019, and disease still developed, this suggests a reconfirmation of experiments done in the early 1980s in Japan showing seed transmission of spinach downy mildew [8]. But as pointed out in the results, the negative control of Viroflay in the experiment, supposedly without oospores, was positive for *P. effusa* symptoms and sporulation. The finding of infection and sporulation in the Viroflay used in the experiment could be explained by one of several possibilities: 1) the Viroflay seeds were positive for oospores but were not detectable using the typical seed wash-off tests; 2) hyphae of *P. effusa* were present on seed but not observed, and these could have transmitted the disease; and 3) there was soil contamination of *P. effusa* that was not adequately removed during fumigation one year earlier. Of these possibilities, the first seems most reasonable since the wash-offs from seed are usually negative, but DNA detection is positive, meaning that we likely missed oospores in counting only the wash-off of two or three thousand seeds. The latter two are not plausible since hyphae of the pathogen are thought to be short-lived, and fumigation would be anticipated to kill whatever small number of oospores that could have been present in the soil from planting spinach one year earlier. Thus overall, the data from the current seed transmission experiment supports the previous finding of seed transmission but requires another repetition and DNA testing of a sample of the seed lot used.

The majority of *Peronospora* spp. causing downy mildews that have been examined for seed transmission have been confirmed as seed transmitted, and thus the findings of seed transmission of spinach downy mildew are anticipated. In addition, questions concerning the levels of

production of oospores in spinach fields in California, and length of time that the pathogen can survive as oospores in seed and soil remain to be fully explored. We routinely detect oospores in leaves of cultivar Viroflay grown in the field at the USDA-ARS station in Salinas. However, oospores have not yet been detected in leaf tissue collected from field samples of cultivars other than Viroflay in California. The appearance of oospores in non-Viroflay samples may be a rare occurrence since the appropriate mating types of *P. effusa* must occur in the same plant and some levels of resistance may preclude one or the other mating type.

The lack of downy mildew development in plots planted with infested seed in the Coachella Valley could be explained by the overall unfavorable conditions for downy mildew early in the winter of 2018-2019, which could have precluded oospore germination and subsequent infection. Irrigation was performed according to standard practices until about mid-February when we attempted to increase the irrigation frequency. However, the conditions required for seedborne transmission are not known, and it is unknown if there is a limited time window for this type of transmission to occur. An additional possibility is that the size of our plots reduced the chances of observing a transmission event. Even though our calculations herein demonstrate that 2268 oospores can reside in one seed (in seeds from one cultivar examined this way), if the rate of actual disease transmission is small, the number of seeds in each plot in our trial may be too small of a sample size for the rare event to occur.

Biopesticide testing was completed for the second trial in 2018 to evaluate the effectiveness of some commonly used biopesticides for protection from spinach downy mildew. In both studies, the level of disease incidence was not very high in the untreated control plots (~13%), and thus increased disease pressure will also be helpful in future biopesticide evaluations. In both years, however, the spray application of the biopesticide Procidic resulted in statistically significant less disease. For this reason, Procidic is under further investigation to limit downy mildew symptoms on spinach.

We (USDA-ARS) have previously shown detection of *P. effusa* DNA seven days before the appearance of symptoms in spinach leaves [15]. The previously developed approach would not allow quick turnaround time on the results to growers because it relies on conventional PCR using DNA from the smashed leaf samples [15]. The results shown herein indicate that *P. effusa* can be identified by the SNP assay (AgBiotech, Monterey, CA), and this assay takes approximately 2 hours (personal communication, Mark Massoudi). However, this assay requires the use of *Peronospora schachtii* (cause of beet downy mildew) DNA each time to differentiate the two pathogens that differ in the sequence examined by a single nucleotide used for detection. One of the major problems that has hampered the further development of this assay has been the ability to obtain sufficient quantities of *P. schachtii* DNA recently (within the past year). *P. schachtii* does not infect spinach, and thus spinach cannot be used to increase the amount of available inoculum.

In this reporting period, we continued development of a new *P. effusa*-specific assay with collaborator Dr. Allen Van Deynze based on mitochondrial sequences of *P. effusa* and analyzed by Dr. F. Martin. We have made progress in determining that the new markers are indeed specific for *P. effusa*. DNA samples from various related downy mildew species (*Peronospora* spp.) were obtained from Drs. M. Thines and Y-J. Choi (Frankfurt, Germany and Seoul, South Korea, respectively) to test by PCR to ensure assay specificity, and this species-specificity was confirmed in this reporting period. The previous assay (like the SNP assay described above) had the drawback of requiring *P. effusa* and *P. schachtii* DNA [3], and the detection value of *P. schachtii* had to be subtracted because of the low-level nonspecific amplification of the *P.*

schachtii DNA, especially when analyzing spore trap samples. The new assay will eliminate the need for dual PCR. We are using a new target mitochondrial sequence for the development of leaf assays that may be used either directly by the grower or PCA in the field, such as recombinase-polymerase DNA amplification (RPA) assays.

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Mention of trade names or commercial products in this research report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture or the University of California Davis. USDA is an equal opportunity provider and employer.

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