CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

Annual Report for Spinach Downy Mildew Research, 2020-2021

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 mildew of spinach caused by the obligate oomycete pathogen *Peronospora effusa* is a very destructive disease on organic spinach in California. The pathogen produces an abundance of short-lived asexual airborne spores that initiate infections and also long-lived sexual oospores that reside in seeds and soil. The role of the oospores is less clear in initiating infections. Using plant isolators at the USDA location in Salinas, we conducted two experiments to study seed transmission of spinach downy mildew. Unlike the 2019-2020 winter season, we did not observe disease on spinach plants in isolators that were grown from oospore-infested spinach seed lot samples. The viability of oospores associated with each seed lot may have been reduced over time. In a separate objective, DNA-based detection assays were improved to quantify the levels of airborne inoculum of *P. effusa* using spore traps. Specifically, a multiplex assay was deployed for dual quantification of airborne *P. effusa* and *Bremia lactucae*, the cause of lettuce downy mildew. Airborne inoculum load present during two different periods of February to April and October-November in 2020 in the Salinas Valley, CA versus the Coachella Valley, CA. Similar levels of detection of both pathogens were observed February to April period but dramatically higher levels of both airborne *P. effusa* and *B. lactucae* were present in the Salinas Valley versus the Coachella Valley in October-November, 2020, suggesting that the higher temperatures and lack of hosts had eliminated the pathogens in the Coachella Valley. Further, we tested a new type of cyclone spore trap versus the impaction spore traps used previously. The results indicated that the cyclone spore traps were nearly 10-fold more sensitive in the detection of airborne *P. effusa*. We also investigated the potential role of the weed nettleleaf goosefoot (*Chenopodium murale*) in serving as a reservoir for spinach downy mildew. In repeated experiments *P. effusa* did not cause disease on nettleleaf goosefoot. A nitrogen dioxide (NO_2) fumigation treatment yielded highly encouraging results previously, as it was effective at killing *Verticillium dahliae* and *P. effusa* carried on spinach seed, without significantly affecting germination. We sought OMRI approval for use of NO_2 fumigation of seeds. Oospores of *P. effusa* have been detected in a total of 18% of the commercial seed lots tested since 2014, revealing that sexual reproduction of the pathogen is commonplace. The role of the oospores in initiating infections from seeds however was not confirmed in this reporting period. In summary, this work provides additional evidence that *P. effusa* is host specific, only infecting spinach. Effective biopesticide treatments in fields and seeds would be helpful to limit some downy mildew outbreaks, especially for organic spinach. Tracking the levels of windborne inoculum in two different growing regions provides valuable information on the conditions and locations conducive to downy mildew in California.

PROJECT TITLE: Detection, epidemiology, and control of spinach downy mildew.

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: **Yong-Biao Liu, Kelley Clark, Frank Martin, Beiquan Mou**
USDA-ARS, Salinas, CA

Allen Van Deynze, Charlie Brummer
University of California, Davis

Jim Correll
University of Arkansas

OBJECTIVES:

Objective 1: Examine *P. effusa* oospore production and seed transmission.

Objective 2: Evaluate whether a weedy plant species located near spinach fields can serve as a reservoir for spinach downy mildew

Objective 3: Determine airborne inoculum load present at two different periods of the season in the Coachella Valley, CA versus the Salinas Valley, CA.

Objective 4: Complete biopesticide testing and analyze a nitrogen dioxide seed fumigation treatment for the killing of *P. effusa* and *V. dahliae* in organic spinach.

PROCEDURES:

We obtained 20 additional commercial seed lot samples for testing in this period for the presence of *P. effusa* oospores using a wash-off method described previously [13]. Briefly, samples of 1000 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 three replicates were performed, and an average number of oospores for all three was obtained.

To investigate potential seed transmission of downy mildew, we planted six seed lots with varying levels of *P. effusa* infestation in contained isolators at the USDA-ARS Salinas facility (Table 1). The isolators received filtered air and were not opened for the duration of the experiment, ruling out windborne sporangia as a potential inoculum source. Two of the six lots planted were cultivar Viroflay as control groups since Viroflay is susceptible to all *P. effusa* isolates.

Experiments to assess whether the weed nettleleaf goosefoot (*Chenopodium murale*) served as a reservoir for *P. effusa* were conducted using seeds collected from *Chenopodium murale* and cultivar Viroflay. Plants were spray-inoculated with 1 x 10^5 spores from either spinach or nettleleaf goosefoot in a dew chamber maintained in an air-conditioned room at the USDA station in Salinas. The initial incubation period following initial inoculation was maintained in the range of 7.5 to 13.3ºC for 24 hr. After this initial 24 hr period, plants were moved to a mist tent maintained in a cold room for seven days before returning to the dew chamber for overnight incubation. Symptoms of chlorosis and pathogen sporulation on leaves were monitored after the final incubation.

Airborne sampling for downy mildew were conducted at locations in the Coachella Valley, CA, and in the Salinas Valley, CA using a pair of solar/battery-powered impaction spore traps (Fig. 1) obtained from Dr. Walt Mahaffee (USDA-ARS, Corvallis, OR) or from Revolution Crop Consultants (Corvallis, OR), which manufacturers impaction spore traps for a fee. The pairs of 1.1 mm x 40 mm stainless steel rods (Fig. 1B) coated with silicone vacuum grease (Dow Corning) were collected at 2 to 3-day intervals. The collected rods (that trapped downy mildew spores) were stored at 4ºC until DNA extraction using the Nucleospin Plant II kit (Machery Nagel) following the manufacturer's protocol for isolating genomic DNA from fungi.

For multiplex detection of both *P. effusa* and *B. lactucae*, the *P. effusa* TaqMan probe based on mitochondrial DNA markers (unpublished) was tested with same quantitative PCR reaction composition and temperature profile as the *Bremia lactucae* TaqMan probe [12] on a LightCycler 480 II (Roche). The standard curves showed that the efficiency of the multiplexed reaction was above 90% for each probe. The absence of cross-amplification or crosstalk of the two colored dyes was confirmed by amplifying 1 ng *P. effusa* and 1 ng *B. lactucae* with the multiplexed reaction. The *P. effusa* TaqMan probe was labeled at the 5' end with FAM and at the 3' end with BHQNova quencher while the *B. lactucae* TaqMan probe was labeled at the 5' end with Cal Fluor Gold 540 and at the 3' end with Black Hole Quencher 1. TaqMan probes were manufactured by BioSearch Technologies. Weather data were obtained from Accuweather.com.

Comparisons were also performed for airborne *P. effusa* detection using two different spore trap types near a sporulating spinach plot planted by Charlie Brummer and Allen Van Deynze (UC Davis) at the USDA ARS Spence farm in November to early December 2020. The cyclone spore trap (Fig. 1C) from Root Applied Sciences (Berkeley, California) was compared with the impaction spore trap (Fig. 1A). Using the cyclone traps, the spores of the pathogen and similar

sized particles drop through a center of a cyclone, into the tube which can be easily collected (Fig. 1D). This could potentially eliminate the need to coat steel rods as those shown in Figure 1B with grease.

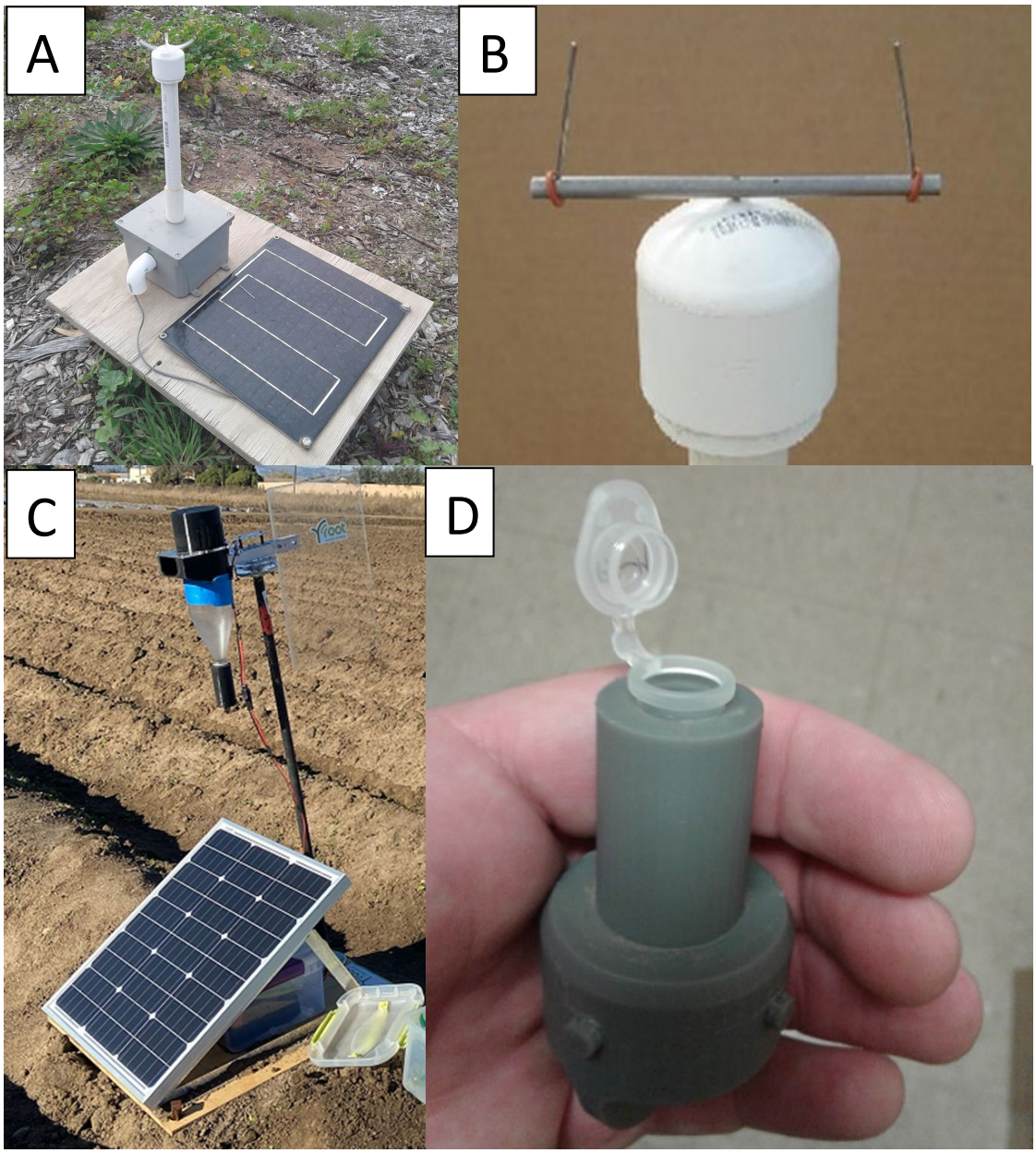

Figure 1. Two spore trap systems to detect airborne *Peronospora effusa* and *Bremia lactucae*. **A)** Example of a spore trap in operation. **B)** Impaction spore trap head with rotating arm and removable stainless-steel rods coated with grease for spore adhesion. **C)** A cyclone spore trap from Root Applied Sciences. **D)** In the cyclone spore trap, the spores of the pathogen and similar sized particles drop through a center of a cyclone, into the microfuge tube shown.

Two small field plots were established at the USDA station in Salinas, CA in 2020 for further testing of the biopesticide Procidic. Spinach cultivar Viroflay was planted in four 80" beds x 120

ft in length at the USDA-ARS station. The plots were examined weekly for downy mildew disease incidence.

We did not conduct nitrogen dioxide fumigation experiments in this reporting period, although we communicated further with the National Organic Program Standards Division in efforts to try to get this fumigation approved for use on organic spinach seed.

RESULTS:

In total, with the additional seed examinations made in this reporting period, the number of commercial seed lots infested with oospores of *P. effusa* stands at 18% (59/319 seed lots) since the initial finding by our (USDA) lab in 2014. One of the seed lot samples contained a relatively high number of oospores (376 per 1000 seeds) while six others had a range of 2-56 oospores per 1000 seeds. No oospores were detected in 13 of the 20 lots samples using the wash-off method. We had previously verified viability of the oospores from seeds by demonstrating oospore germination [9]. However, these fresh lot samples were not used in the 2020-2021 seed transmission experiments.

We investigated seed transmission of spinach downy mildew in the winter of 2020-2021 to complement the experiment in the 2018-2019 and 2019-2020 winter periods. In the effort to remove the possibility of windborne inoculum contaminating these experiments, we used the isolator system shown in Figure 2 to prevent the introduction of windborne inoculum. Six seed lot samples collected in 2017-2018 in contained isolators at the USDA-ARS Salinas facility. These lot samples had varying levels of *P. effusa* infestation (Table 1). The seed lot sample of 07542 was derived from a lot that was planted in a field that had downy mildew. The isolators were not opened for the duration of the experiment. Each lot was tested for the presence of *P. effusa* DNA via PCR and for oospores via extraction from seed and microscope observations (Table 1). Seed lots that tested PCR positive, but oospore negative could either contain *P. effusa* sporangia or mycelia or a low level of oospores we did not observe using our current detection method. The spinach plants grown from each of these seed lots were observed for a four-month time span). Over this time span no signs of the *P. effusa* pathogen were observed (Fig. 2).

Table 1. Spinach seed lot samples selected for tests of seed transmission of downy mildew in the contained isolators at the USDA-ARS Salinas facility.

Seed lot	Oospores	PCR	Additional Notes
Viroflay1	Negative	Positive	Control (Viroflay)
Viroflay2	Negative	Negative	Control (Viroflay)
57031	Positive	Positive	Oospore germination observed (~8%)
57032	Positive	Positive	Previously got DM in isolators
52032	Positive	Positive	Previously got DM in isolators
07542	Negative	Weak positive	DM was observed in the field

Figure 2. Six seed lots were planted in plant isolators for examination of seed transmission of spinach downy mildew in November, 2020 to March, 2021. Seed samples of lots 57031, 57032, 52032, and 075542 contained variable *Peronospora effusa* oospore-infestation levels. Viroflay with no detectable oospores was used as a control. All samples were planted in duplicate.

Results from the spore trapping and quantification indicated similar amounts of airborne detection of *P. effusa* in the Salinas and Coachella Valleys during the October-November, 2020 sampling period, though there was a trend of increasing spore load in the Coachella Valley in April (Fig. 3). However, there was a substantially increased spore load of *P. effusa* in the Salinas versus the Coachella Valley during the October-November, 2020 sampling period (Fig. 3). Spore quantities derived from qPCR analysis reached detection values of hundreds of spores in the October-November sampling period in the Salinas Valley. In contrast, the values observed for the Coachella Valley in the same time frame were below 5 spores throughout this period (Fig. 3). Detection values less than 5 are considered background in this assay, and hence there was not detection of *P. effusa*.

Results from the spore trapping and quantification of *B. lactucae* indicated a gradual increase in the amounts of airborne detection in the Salinas and Coachella Valleys during the February - April, 2020 sampling period (Fig. 4). There was a marked drop in the spore load of *B. lactucae* detected in October – November in the Salinas Valley. Spore quantities derived from qPCR analysis reached detection values of thousands of spores in early October but dwindled to nearly 50 by the end of the November sampling period in the Salinas Valley. In the Coachella Valley, remarkably, there was no detection of *B. lactucae* during the October – November, 2020 sampling period (Fig. 4). Detection values less than 1 are considered background (no detection) in this assay. Average high temperatures for the months of October and November were 97 and 82°F, respectively, in the Coachella Valley. Average high temperatures for the months of October and November were 79 and 69°F, respectively, in the Salinas Valley.

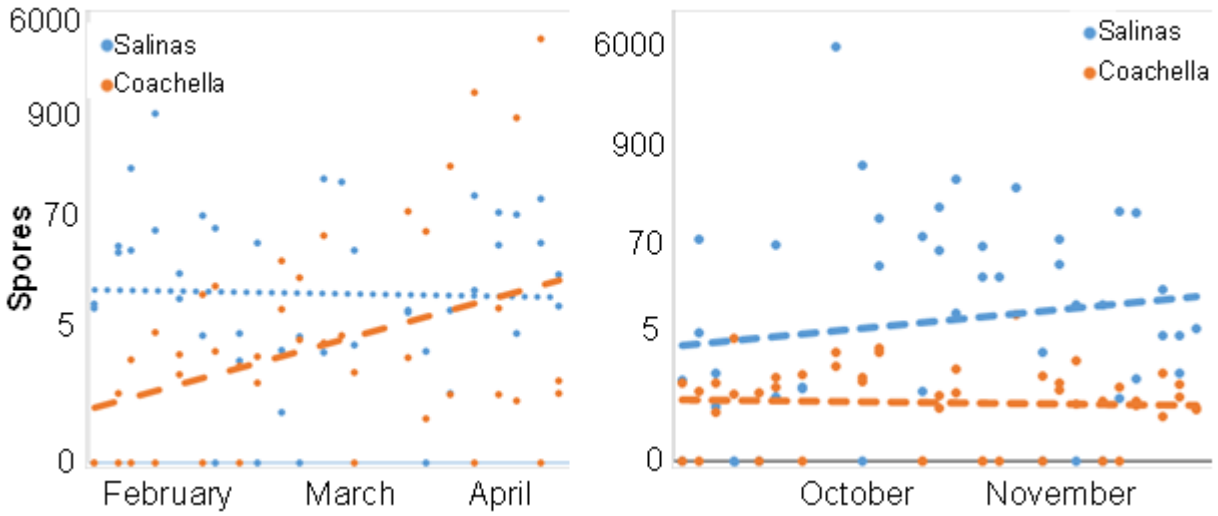

Figure 3. Impaction spore trapping and quantification of *Peronospora effusa* in the Salinas versus the Coachella Valley during the period of February – April, and October – November, 2020.

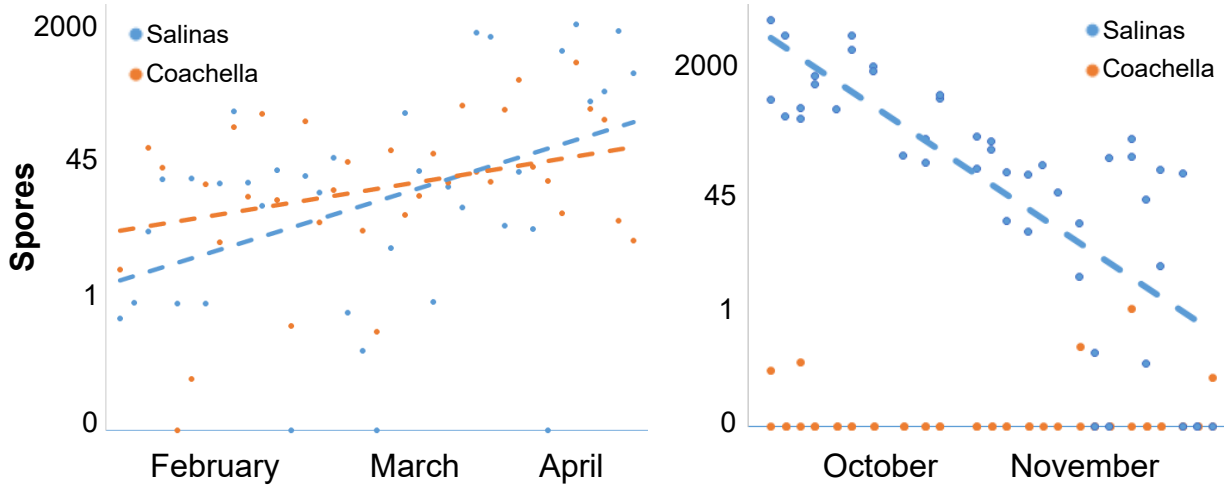

Figure 4. Impaction spore trapping and quantification of *Bremia lactucae* in the Salinas versus the Coachella Valley during the period of February – April, and October – November, 2020.

Comparison of the new cyclone type spore trap (Fig. 1C) with the old impaction type spore trap (Fig. 1A) was conducted in November – early December 2020. The results shown in Figure 5 reveal nearly a 10-fold increase in sensitivity in *P. effusa* detection with the new cyclone traps versus the old impaction spore traps. The average detection across this time period was approximately 133 picograms DNA (2000 spore equivalents) for the cyclone type traps but nearly 13 picograms DNA (200 spore equivalents) for the impaction spore traps.

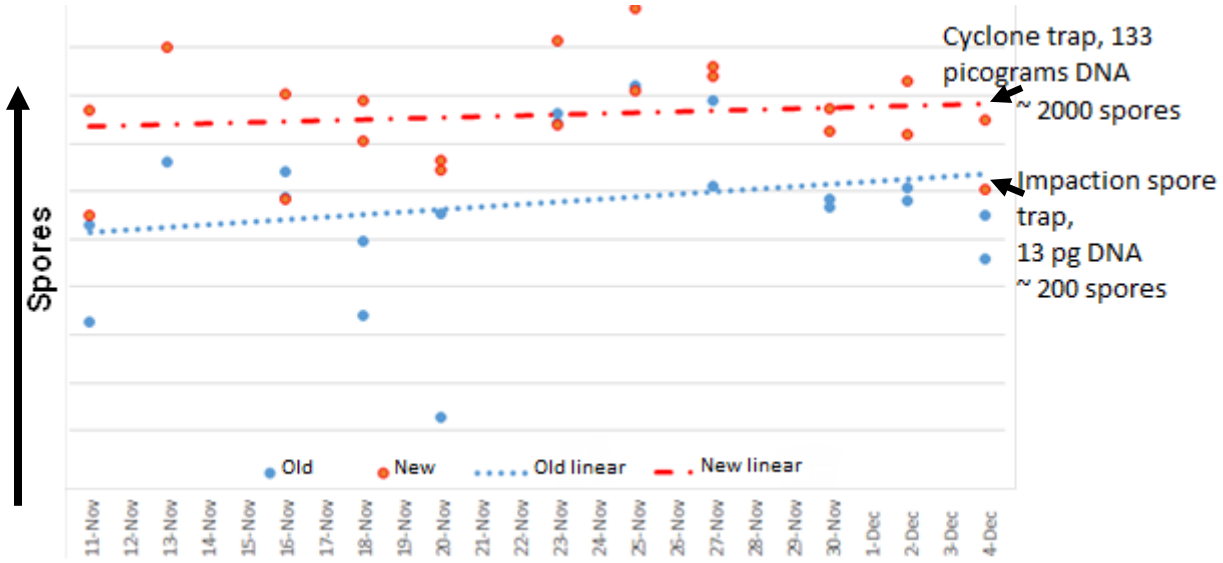

Figure 5. Comparison of spore trap-based detection and quantification of *Peronospora effusa* DNA by quantitative PCR (qPCR) in samples collected from two different types of spore trap devices, the "new" cyclone spore trap from Root Applied Sciences (Red, with trendline) versus the "old" impaction spore trap (Blue, with trendline). The comparison was conducted on sampling dates of November 11 through December 4, 2020.

Cross-inoculation experiments were conducted twice to assess whether the weed goosefoot nettle serves as a reservoir for *P. effusa*. The results indicated that downy mildew pathogen from goosefoot nettle does not infect spinach, nor does *P. effusa* infect goosefoot nettle.

Two field plots were also established in 2020 to examine the biopesticide Procidic in 2020, but these plots did not yield adequate disease for testing.

DISCUSSION:

We previously published the work on the finding of oospores in modern spinach seed lots and examined additional seed lots for oospores using the wash-off tests previously published [13]. Combined with those 20 lots which we examined for oospores in this reporting period, 18% of the 319 commercial seed lots contained oospores. The presence of oospores in spinach seed lots suggests long term survival of the pathogen on seed [6,8,10,13], and frequent mating between different isolates since *P. effusa* is heterothallic [1,7]. But seed transmission of spinach downy mildew has not been reported in the literature since 1983 [6].

We undertook seed transmission experiments for the third straight year using the plant isolators at the USDA ARS station in Salinas. Unlike the immediate past reporting period, we did not find seed transmission of downy mildew in plant isolators at the USDA station in the 2020-2021 winter season. Potentially the oospores present in the seed lots used in these experiments for the past three years lost viability over time. Also, the amount of seeds sampled is still relatively small, and thus we may have not selected samples for planting with ample numbers of oospores. In the previous reporting period we did observe a low level of sporulating plants in the isolators, which suggested a confirmation of seed transmission. But we also observed that negative control of Viroflay seeds (without **detectable** oospores) yielded spinach plants that were positive for downy mildew development in these isolator experiments. The most

likely explanation is that the Viroflay seed lot contained oospores at a low level undetectable in seed wash-offs as we observed DNA amplification of *P. effusa* by PCR in lots that were negative in seed wash-off tests. Regardless, it is important to determine optimal conditions under which oospores of *P. effusa* germinate and infect spinach, for routine lab procedures and as beneficial knowledge for the field. To further address seed transmission of spinach downy mildew in additional experiments, we will use the recently collected seed lot samples that contain oospores, and thus these should be fully viable.

In our previous work, we have applied spore trapping and qPCR for quantification of the downy mildew pathogens of lettuce and spinach [2,3,11,12]. 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 [2,3,12]. In the 2020-2021 reporting period, we completed the development of a new assay for simultaneous detection of *P. effusa* and *B. lactucae* from spore trap samples. The assay uses species-specific mitochondrial TaqMan probes and combines those probes for *P. effusa* in unpublished work (with cooperators Dr. Allen Van Deynze and Dr. Frank Martin) and the probe previously published for *B. lactucae* [12].

We applied the new dual detection assay for quantification of both *P. effusa* and *B. lactucae* DNA from impaction spore trap samples to examine the relative spore load at both the Salinas and Coachella Valleys in California. The results indicated similar amounts of airborne detection of *P. effusa* and *B. lactucae* in the Salinas and Coachella Valleys during the October-November 2020 sampling period, though there was a trend of increasing spore load of *P. effusa* and *B. lactucae* in the Coachella Valley in April. Strikingly, the spore load of both *P. effusa* and *B. lactucae* decreased dramatically to essentially undetectable levels in the Coachella Valley during the October - November 2020 sampling period. The airborne spores of *P. effusa* survive only hours to a couple days at best under hot, dry conditions [4]. Because the average high temperatures in the Coachella Valley for the months of October and November were 97 and 82^{o}F, respectively, we speculate that both the lack of spinach and lettuce in October and the increased temperature destroyed airborne inoculum of both pathogens in the Coachella Valley. Average high temperatures in the Salinas Valley for the months of October and November were 79 and 69^{o}F, respectively, which would be more hospitable for downy mildew survival. In the long-term, we anticipate that correlation of the values of inoculum load with temperature will provide more accurate disease forecasting, though temperature itself is not the only driver of inoculum load, as was evident in the Salinas Valley data in this reporting period. Even though the average temperature in the Salinas Valley was 79 ^{o}F in October (with many days at > 90^{o}F) and 69^{o}F in November, the levels of *B. lactucae* estimated spore load were dramatically higher in October versus November. Thus, as much of the lettuce is harvested in November, perhaps a primary driver of inoculum load is the amount of crop present in the Salinas Valley.

The results show that the new cyclone spore traps (example shown in Fig. 1C) are 10-fold more sensitive than the impaction spore traps (Fig. 1A) in detection of airborne *P. effusa*. Though this experiment showing the increased sensitivity requires repetition, the new cyclone spore traps are much easier and cheaper to operate over the long term to handle. While both the cyclone and impaction spore traps require solar power and batteries, we have found the cyclone trap batteries have not run down so quickly like the 12 V batteries on the impaction traps. The cyclone spore traps also do not require disposable stainless-steel rods and vacuum grease for spore adherence. Rather, the samples are easily collected in a microfuge tube at the base of the trap. Therefore, the operation of the cyclone spore traps is easier and less expensive.

Additionally, Root Applied Sciences continues to improve on the cyclone design and is seeking to automate the design for field use.

We were unable to complete the biopesticide testing of Procidic (active ingredient citric acid) of a lack of disease in one planted field plot. However, we did publish the results of the 2017 and 2018 field tests of biopesticides [9]. In both studies, the level of disease incidence was not high in the untreated control plots (~13%), and thus we aimed to repeat the trial for Procidic. Because of the problems with the field plot trials in not getting adequate disease in the past two years, we will move the Procidic testing indoors for the next reporting period. Additionally, we (USDA) have agreed to test another product known as AgroPro (AgroMagen) which is registered for organic use in California.

Previously published research indicates that NO_2 produced by the combination of nitric oxide and oxygen is highly effective at killing the fungus *Aspergillus flavus* [14,15], which produces aflatoxins harmful to humans commonly found in contaminated peanuts and corn seeds. Our data in the previous report revealed that NO_2 is effective at killing two problematic plant pathogens *V. dahliae* and *P. effusa*, both present in/on spinach seeds [5,13]. In the current report, we further petitioned representatives of the National Organic Program to proceed with a new petition to the Organic Materials Review Institute (OMRI) to get approval to use NO_2 as a seed treatment for organic seeds. From these discussions, we (USDA, Salinas) realized that we may need to apply for approval of NO_2 on seed as a nonfood item. Considering seeds of spinach are not eaten directly, this may be a suitable approach. Dr. Klosterman added the further testing of NO_2 as a seed treatment for organic seeds to his 5-year USDA ARS appropriated project plan with cooperator Dr. Liu.

In summary, we completed the development of a multiplex assay for dual detection of both *P. effusa* and *B. lactucae* on spore traps. Spore trapping experiments examining airborne spore loads of *P. effusa* and *B. lactucae* yielded remarkable differences in the Salinas and Coachella Valleys over the same two-month period of October - November 2020. These differences can be associated with crop production or weather events to strengthen disease forecasting. Previous experiments supported seed transmission of spinach downy mildew. We were unable to duplicate seed transmission of spinach downy mildew in isolator experiments in this reporting period, but considering its importance, we are conducting additional experiments to assess the conditions oospores may transmit the disease as primary inoculum. Experiments to assess whether the weed goosefoot nettle serves as a reservoir for *P. effusa* indicated that *P. effusa* does not cross-infect this weed species. Field plots did not yield adequate disease for the testing of the biopesticide Procidic. Procidic is under further investigation indoors under controlled conditions.

Acknowledgments: We thank the California Leafy Greens Research Program for funding, and the California Department of Food and Agriculture, grant number 20-0001-047-SF. We are thankful for the assistance of Amy Anchieta (USDA-ARS, Salinas) in conducting all qPCR experiments and Lorena Ochoa, Denise Soto (USDA-ARS) and Hannah Ayala (UC Riverside) for collecting spore trap samples. We appreciate the provision of spinach seed samples from the spinach seed production and distribution companies and access to fields for the spore trap studies.

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.

References:

1) Choi, Y.-J., Hong, S.-B., Shin, H.-D. 2007. Re-consideration of *Peronospora farinosa* infecting *Spinacia oleracea* as distinct species, *Peronospora effusa.* Mycological Research 111: 381-391.

2) Choudhury, R.A., Koike, S.T., Fox, A., Anchieta, A., Subbarao, K.V., Klosterman, S.J., and McRoberts, N. 2017. Spatiotemporal patterns in the airborne dispersal of spinach downy mildew. Phytopathology 107:50-58.

3) Choudhury, R.A., Koike, S.T., Fox, A. D., Anchieta, A., Subbarao, K. V., Klosterman, S.J., and McRoberts, N. 2016. Season-long dynamics of spinach downy mildew determined by spore trapping and disease incidence. Phytopathology 106:1311-1318.

4) Dhillon, B., Feng, C., Villarroel-Zeballos, M.I., Castroagudin, V.L., Bhattarai, G., Klosterman, S.J., Correll, J. 2020. Sporangiospore viability and oospore production in the spinach downy mildew pathogen, *Peronospora effusa.* Plant Disease. https://doi.org/10.1094/PDIS-02-20-0334-RE.

5) Duressa, D.O., Rauscher, G.M., Koike, S.T., Mou, B., Hayes, R.J., Maruthachalam, K., Subbarao, K.V., Klosterman, S.J. 2012. A real-time PCR assay for detection and quantification of *Verticillium dahliae* in spinach seed. Phytopathology. 102:443-451.

6) Inaba, T., Takahashi, K., Morinaka, T. 1983. Seed transmission of spinach downy mildew. Plant Disease. 67:1139-1141.

7) Inaba, T. Morinaka, T. 1984. Heterothallism in *Peronospora effusa*. Phytopathology. 74:214-216.

8) Kandel, S.L., Mou, B., Shishkoff, N., Shi, A., Subbarao, K.V., and Klosterman, S.J. 2019. Spinach downy mildew: Advances in our understanding of the disease cycle and prospects for disease management. Plant Disease (https://doi.org/10.1094/PDIS-10-18-1720-FE).

9) Kandel, S.L., Subbarao, K.V., Shi, A., Mou, B., Klosterman, S.J. 2019. Evaluation of biopesticides for managing downy mildew of spinach in organic production systems in 2017 and 2018. Plant Disease Management Reports. 13:V171.

10) Klosterman, S.J., 2016. Spinach downy mildew – Threat, prevention and control. Progressive Crop Consultant 1:12-15.

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

12) Kunjeti, S. G., Anchieta, A.,Martin, F. N., Choi, Y.-J., Thines, M., Michelmore, R. W., Koike, S. T., Tsuchida, C., Mahaffee, W., Subbarao, K. V., and Klosterman, S. J. 2016. Detection and quantification of *Bremia lactucae* by spore trapping and quantitative PCR. Phytopathology 106:1426-1437.

13) Kunjeti, S.G., Anchieta, A.G., Subbarao, K.V., Koike, S.T., Klosterman, S.J. 2016. Plasmolysis and vital staining reveal viable oospores of *Peronospora effusa* in spinach seed lots. Plant Disease. 100:59-65.

14) Liu, Y.-B., Oh, S., Jurick, W. (2019). Response of *Aspergillus flavus* spores to nitric oxide fumigations in atmospheres with different oxygen concentrations. Journal of Stored Products Research. 83. 78-83. 10.1016/j.jspr.2019.06.001.

15) OH, S. and Liu, Y.B., 2020. Effectiveness of nitrogen dioxide fumigation for microbial control on stored almonds. Journal of Food Protection 83(4), 599-604.