ARIZONA ICEBERG LETTUCE RESEARCH COUNCIL

FINAL REPORT

Project title: Integrated use of fungicides and lettuce genetics to manage downy mildew, 2017

Authors: Michael Matheron[1], Martin Porchas[1], Claudia Nischwitz[2], and Richard Michelmore[3]. [1]University of Arizona, Yuma Agricultural Center, 6425 W. Eighth St., Yuma, AZ 85364; [2]Utah State University, 5305 Old Main Hill, Logan, UT 84322; [3]University of California, Davis, CA 95616.

Introduction

According to a survey conducted by The Arizona Iceberg Lettuce Research Council in 2012, downy mildew is one of the top five diseases of concern to lettuce producers. This disease, caused by the fungus-like organism *Bremia lactucae*, is an obligate parasite that can only grow and reproduce on living lettuce plants. Downy mildew is a major disease in lettuce production systems worldwide. Disease epidemics can be devastating when environmental conditions are favorable for disease development.

Effectively managing downy mildew of lettuce is reliant on fungicides and planting of lettuce varieties with genetic resistance to the disease. Many races of the downy mildew pathogen exist, and "resistant" lettuce varieties are not immune to the disease and may show symptoms and damage when disease pressure is high. Actual performance of resistant varieties will depend on the races of the pathogen present in the field during a particular growing season. Fungicide applications will be needed if the particular planted lettuce variety is susceptible or if the genetic resistance package is not sufficient to control the pathogen races present in the crop.

This research project had two objectives.

Fungicide trial. The objective of the field fungicide trial was to evaluate the effectiveness of individual fungicides as well as treatment programs containing more than one fungicide, applied to seven different lettuce varieties. Fungicides tested included a biofungicide as well as conventional compounds. The goal was to determine if lettuce genetics plus fungicides can work together to provide a level of disease control superior to that provided by the plant or the fungicide product alone.

Identification of lettuce downy mildew races. At plant maturity, samples of downy mildew from untreated lettuce varieties in the field trial were sent to Dr. Michelmore for race identification. Race determination was accomplished by inoculating a series of different lettuce varieties with known resistance or susceptibility to the different races of the pathogen.

Additionally, Dr. Nischwitz collected downy mildew samples from lettuce varieties in the

field not treated with fungicides and evaluated a DNA based molecular technique that potentially could be used to rapidly identify downy mildew races in the future. RAPD primers have been used to identify races in other downy mildew pathosystems. They are short segments of randomly assembled nucleotides that will attach in the target organism genome during polymerase chain reaction (PCR) if this same sequence is found in the genome. If the sequence is found at least twice a DNA band will be present in the agarose electrophoresis gel run after PCR. Otherwise no band will appear. The size of the band and the number of bands that will be present depends on how often the RAPD sequence is found in the organism genome and how far apart they are. This banding pattern can vary from race to race and can be used to identify races. Currently there are 156 commercially available RAPD primers. One advantage of this method of pathogen race determination would be that the time from DNA extraction to obtaining results can be as short as one day.

Materials and Methods

Fungicide trial. This study was conducted at the Yuma Valley Agricultural Center. The soil was a silty clay loam (7-56-37 sand-silt-clay, pH 7.2, O.M. 0.7%). Lettuce varieties Bubba, Domingos 67, Gabilan, Havasu, Navajo, Salute, and SV773560 were seeded, then sprinkler-irrigated to germinate seed on Nov 16, 2017 on beds with 84 in. between bed centers, with each bed containing six rows of lettuce. Sprinkler irrigation was used to germinate seed and grow the crop to maturity. Treatments were replicated five times in a randomized complete block design. Each replicate consisted of a 25 ft length of row on beds containing six rows of lettuce. Plants were thinned Dec 21, 2017 at the 3-4 leaf stage to an approximate 12-inch spacing. Treatments were applied with a tractor-mounted boom sprayer that delivered 50 gal/acre at 100 psi to flat-fan nozzles spaced 12 in. apart. Foliar applications of treatments were applied 20 and 27 Feb and 7 Mar, depending on the treatment. Downy mildew (caused by *Bremia lactucae*) was first observed in plots on Feb 26, six days after the first application of products. Maximum and minimum ranges (°F) of air temperature were as follows: Nov 16 to 30, 70-91, 44-58; Dec, 60-82, 29-56; Jan, 2018, 64-85, 35-64; Feb, 62-87, 31-51; Mar 1 to 20, 69-86, 39-59. Maximum and minimum ranges (%) for relative humidity were as follows: Nov 16 to 30, 58-85, 6-22; Dec, 20-89, 6-31; Jan 2018, 27-97, 8-54; Feb, 61-95, 8-45; Mar 1 to 20, 39-83, 5-33. Monthly rainfall in inches was as follows: Nov 23 to 30, 0.00; Dec, 0.00; Jan, 0.07; Feb, 0.01; Mar 1 to 20, 0.01. Disease severity was determined Mar 16, 19, and 20 by recording the number of downy mildew lesions on 10 lettuce plants arbitrarily selected within each treatment plot. Statistical analysis of disease severity among the seven nontreated lettuce varieties revealed no significant differences; therefore, fungicide treatment data for all lettuce varieties were combined before final statistical analysis.

Identification of lettuce downy mildew races. Thirteen samples of downy mildew from untreated lettuce varieties in the field trial were sent to Dr. Michelmore for race identification. As described earlier, race determination was accomplished by inoculating a series of different lettuce varieties with known resistance or susceptibility to the different races of the pathogen. Dr. Nischwitz selected 20 RAPD primers that had been used by other scientists for downy mildews and fungi. Downy mildew (*Bremia lactucae*) was collected from the lettuce variety trial at the

Yuma Agricultural Center in March 2018. DNA was extracted from each sample using a commercially available DNA extraction kit. PCR was conducted with each RAPD primer and downy mildew samples.

Results and Discussion

Fungicide trial. The data in the Table show the degree of disease control obtained by application of the various treatments in this trial. All treatments significantly reduced the final severity of downy mildew compared to nontreated plants. The two best treatments, V-10365 alternated with Zampro and Revus + Orondis Ultra alternated with Zampro, reduced the number of downy mildew lesions by 76% and 54%, respectively, compared to nontreated plants. Phytotoxicity symptoms were not noted for any treatments.

Identification of lettuce downy mildew races. As noted earlier, statistical analysis of disease severity among the nontreated lettuce varieties used in this trial (Bubba, Domingos 67, Gabilan, Havasu, Navajo, Salute, and SV773560) revealed no significant differences; suggesting that these varieties did not differ in their susceptibility to the races of the downy mildew pathogen present in this field trial. Dr. Michelmore found a new race of the downy mildew pathogen *Bremia lactucae*, identified as Bl: 9US, in six of 13 samples and rarer isolates with novel characteristics that have not yet been denominated as races in seven of the 13 disease samples.

Dr. Nischwitz found one primer OPB-7 that gives bands for lettuce downy mildew. Some samples had *Cladosporium* and *Alternaria alternata* on the leaves as well. Despite careful collection we could not rule out the possibility that we had a few *Alternaria* or *Cladosporium* spores as well as minute amounts of lettuce tissue. We ran the OPB-7 primer on iceberg and Romaine lettuce as well as *Alternaria* and *Cladosporium*. No bands were obtained from the lettuce or the two fungi indicating that the bands we had were from the downy mildew. It is assumed that the downy mildew in the variety trial was the same race for all varieties. In future research, the next step would be to collect downy mildew races from lettuce varieties with known resistance to specific races and test these isolates to determine if the banding pattern varies with OPB-7. We will also continue testing other RAPD primers.

Downy Mildew Management Conclusions

- The two best treatments in this fungicide trial were V-10365 alternated with Zampro and Revus + Orondis Ultra alternated with Zampro, which reduced the number of downy mildew lesions by 76% and 54%, respectively, compared to nontreated plants. .
- The *Bremia lactucae* downy mildew pathogen recovered from this lettuce planting included a new race, Bl: 9US, as well as rarer isolates with novel characteristics that have not yet been denominated as races. The race composition of the downy mildew pathogen population can differ among different lettuce growing areas or from year to year, and resistant lettuce can still sustain damage if disease pressure is high; therefore, an integrated disease management approach including use of fungicides and genetically resistant lettuce varieties when available, offers the best means of maximizing control of downy mildew on lettuce.

Table. Downy mildew severity on lettuce varieties Bubba, Domingos 67, Gabilan, Havasu, Navajo, Salute, and SV773560 treated with fungicides.

Treatment *	Rate of product per acre	Treatment dates	Downy mildew lesions per leaf
V-10365 + Kinetic	10.3 fl oz + 4.5 fl oz	Feb 20, Mar 7	4.4 f
Zampro + Kinetic	14.0 fl oz + 4.5 fl oz	Feb 27	
Revus + Orondis Ultra A + Kinetic	8.0 fl oz + 1.64 fl oz + 4.5 fl oz	Feb 20, Mar 7	8.5 e
Zampro + Kinetic	14.0 fl oz + 4.5 fl oz	Feb 27	
Ranman	2.75 fl oz	Feb 20, 27, Mar 7	9.6 de
Dithane	1.6 qt	Feb 20, 27, Mar 7	9.6 de
Zampro + Kinetic	14.0 fl oz + 4.5 fl oz	Feb 27	10.0 d
Zampro + Kinetic	14.0 fl oz + 8.0 fl oz	Feb 20, 27, Mar 7	10.1 d
Ranman	2.75 fl oz	Feb 20, Mar 7	10.3 d
Stargus	2.0 qt	Feb 27	
Ranman	2.75 fl oz	Feb 20, Mar 7	12.8 c
Stargus	4.0 qt	Feb 27	
Ranman	2.75 fl oz	Feb 20, Mar 7	14.1 b
Nontreated control	-------	-------	18.4 a
LSD (*P* = 0.05) **			1.2

* Treatments were replicated five times in a randomized complete block design. Each replicate consisted of a 25 ft length of row on beds containing six rows of lettuce.

** Statistical analysis of disease severity among the seven nontreated lettuce varieties revealed no significant differences; therefore, fungicide treatment data for all lettuce varieties were combined before final statistical analysis. Disease severity values followed by a different letter are significantly different according to Fishers Least Significant Difference Test (*P* = 0.05)