CALIFORNIA LETTUCE RESEARCH PROGRAM

April 1, 2014 - March 31, 2015

BIOLOGY AND EPIDEMIOLOGY OF DOWNY MILDEW OF LETTUCE

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SUMMARY

Downy mildew of lettuce, caused by *Bremia lactucae*, is problematic in the cool climate of central coastal California. The disease can be controlled by fungicide applications, although it is costly to routinely apply fungicides to prevent the establishment of downy mildew. Furthermore, similar control measures are unavailable to control *B. lactucae* in organic production. Repeated use of the chemicals also can lead to fungicide resistance in the pathogen. Early detection of the pathogen, coupled with knowledge of other factors that favor downy mildew outbreaks, may enable disease forecasting for judicious timing of fungicide applications. Knowledge of pathogen availability, by airborne detection techniques, may also be useful to characterize and control for equivalent disease pressure in parallel fungicide trials. The recent availability of the genomic sequences of multiple *B. lactucae* isolates enabled comparison of mitochondrial sequences for development of *B. lactucae*-specific primers, for detection of *B. lactucae*. To assess species-specificity to *B. lactucae*, we analyzed these primers in conventional PCR for DNA amplification from the DNA obtained from twelve other *Bremia* species, and found that the primers were solely specific for *B. lactucae*. Additionally, testing of 17 isolates of *B. lactucae* obtained in the United States and other countries revealed that the *B. lactucae* PCR primers developed for the assay enabled amplification of DNA from each one of these isolates. Specificity tests revealed that the qPCR assay is specific to *B. lactucae* and not to related *Bremia* species. The next step is the development of specific probes that is in progress, coupled with the available PCR primers, for quantitative real time PCR-based (qPCR) detection and quantification of *B. lactucae* in the field. The *B. lactucae* qPCR assay is anticipated to be highly sensitive, enabling specific detection of very low levels of *B. lactucae* inoculum DNA that may be present in the field, or in other samples, such as plant tissues. For lack of disease, the objective evaluating fungicide combinations for downy mildew control could not be accomplished.

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OBJECTIVES:

A. Develop qPCR for specific detection of *Bremia lactucae* and deploy for environmental monitoring of inoculum levels.
B. Screen protectant and systemic (if available) fungicides registered to identify those efficacious against lettuce downy mildew during fall in coastal fields.

PROCEDURES:

Objective A. Develop qPCR for specific detection of *Bremia lactucae* and deploy for environmental monitoring of inoculum levels.

Conventional polymerase chain reactions (PCR) were carried out in a PTC200 DNA Engine thermocycler (MJ Research, Piscataway, NJ). The PCR amplicons were visualized in agarose gels by the use of SYBR Gold staining or ethidium bromide.

RESULTS:

The results indicate specific amplification of the mitochondrial target DNA from all of the *B. lactucae* isolates obtained from within the continental United States (Figure 1; National Isolates). The positive and negative controls, derived from *B. lactucae*-infected or non-infected lettuce leaves,

were positive and negative, respectively, when the DNA from these tissues was used as template (Figure 1).

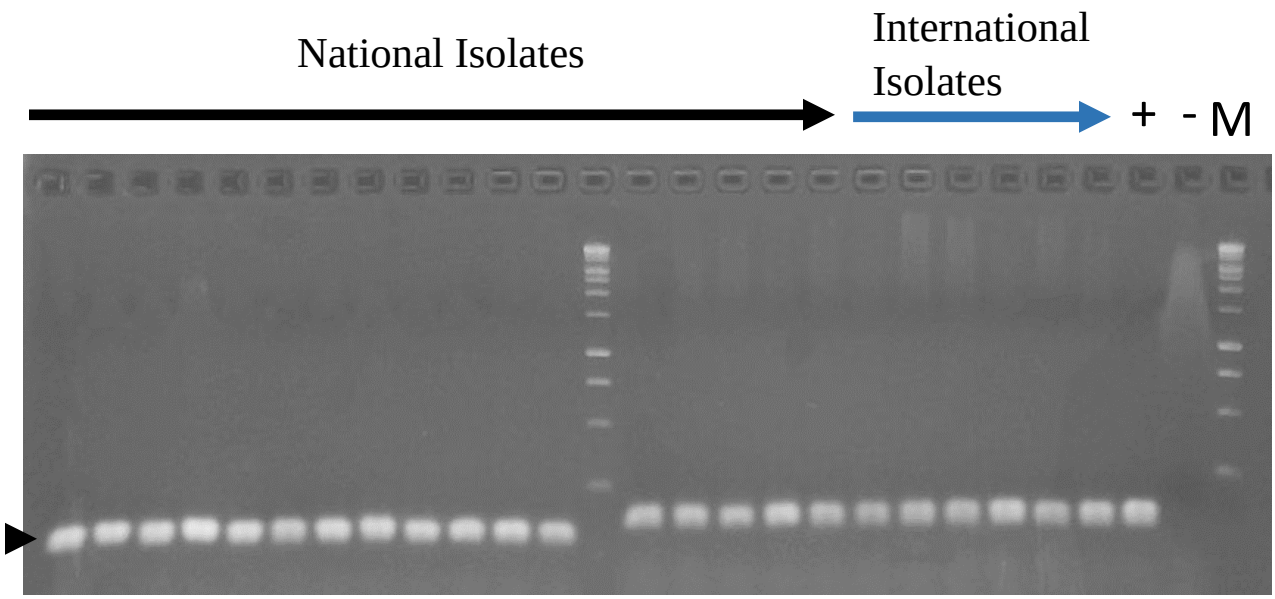

Figure 1. Polymerase chain reaction (PCR)-amplification of mitochondrial DNA of *Bremia lactuca* isolates, obtained from national and international sources. The arrowhead on the left indicates the position of the amplicon, resulting from positive amplification. The DNA template used for the positive control (+) was derived from a *B. lactucae*-infected lettuce leaf. The negative control template (-) was derived from lettuce leaf only. The DNA from the isolates of *B. lactucae* from both national and international sources were supplied by Richard Michelmore and Cayla Tsuchida (UC, Davis) and PCR amplification was carried out by Amy Anchieta (USDA ARS). M = Molecular weight marker.

Additionally, the PCR primers used are specific for amplification of *B. lactucae* obtained from *Lactuca serriola* or *L. sativa*, and not any of the other twelve species of *Bremia* examined (Figure 2). As shown in Figure 2, a DNA amplicon marked by the red arrow was observed in the first 10 lanes when the *B. lactucae*-infected plant tissue DNA was used as a template for PCR, but not in any other lanes corresponding to the use of DNA template from other *Bremia* species.

DISCUSSION:

The development of novel molecular markers, specific for only *Bremia lactucae*, respresents an important step forward for those studies that aim to detect and quantify the inoculum of the lettuce downy mildew pathogen in the environment. Our next step is to test the *B. lactucae*-specific primers for spore trap based detection of *B. lactucae* in the field.

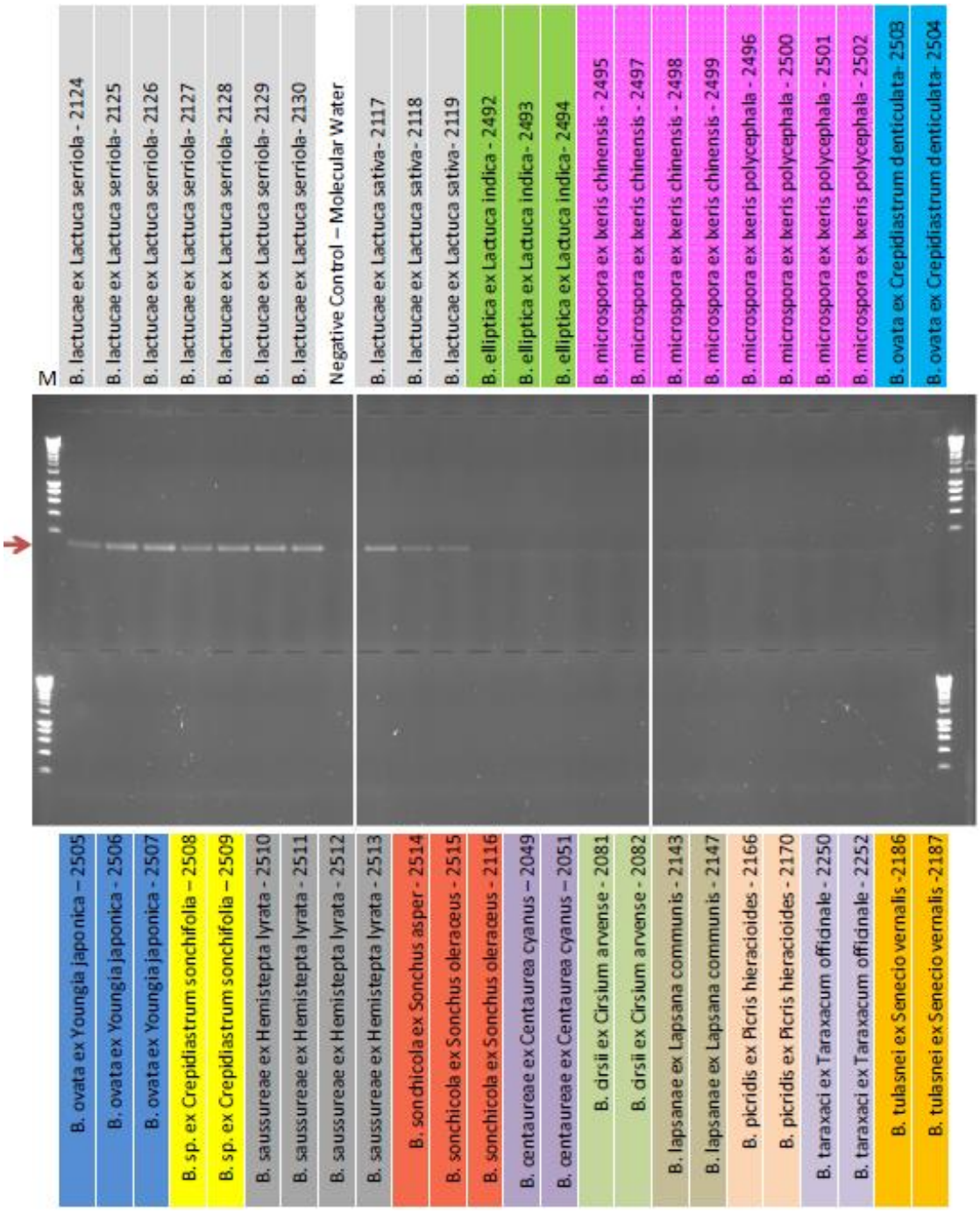

Figure 2. Species-specific polymerase chain reaction (PCR) amplification of mitochondrial DNA from the lettuce downy mildew pathogen, *Bremia lactucae*. The PCR analysis shown was conducted by Young-Joon Choi and Marco Thines, Biodiversity and Climate Research Center (BiK-F), Frankfurt, Germany. M = Molecular weight marker.