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INTEGRATING NEXT-GENERATION TECHNOLOGIES FOR BLACKLEG AND SOFT ROT MANAGEMENT IN POTATO

NON-TECHNICAL SUMMARY: A recent outbreak of an aggressive soft rot bacterium (Dickeya dianthicola) in U.S. potatoes affects an industry that struggles to control the spread of endemic bacterial pathogens and manage the diseases they cause.Potatoes are the most important non-grain food crop grown in the US and worldwide. Soft rot bacteria cause potato plants to wilt and potato tubers to rot. They are among the most common and damaging potato pathogens in the United States. In 2015, D. dianthicola was reported for the first time in U.S. potatoes and it caused millions of dollars in losses in at least 10 states and seriously impacted trade in seed potatoes. Dickeya spread rapidly to 13 additional states in 2016 and, as of early 2017, is still being found in a high percentage of seed lots in some states. In 2017,seed in at least one additional state was found infested with Dickeya. It appears that Dickeya is endemic and that it will likely be a long-term and major threat to the U.S. potato industry. Without an intensive national effort, the impact of this outbreak will grow and persist in impact. Because of the dearth of knowledge about its biology and management, the Dickeya outbreak has created an urgent need for research and engagement with affected producers and the potato industry. Industry stakeholders contributed to project development and research goals from the beginning of this outbreak in the summer of 2015, and they participated in all aspects of proposal development. An advisory panel is assembled to address the SCRI goal of developing a strategy that can be implemented for this specialty crop. Because Dickeya and Pectobacterium cause nearly identical symptoms and often occur together, our project must address both pathogens. Our project goals are to provide the potato industry with: (1) improved pathogen detection tools for Dickeya and the closely related pathogen Pectobacterium, (2) information required to develop resistant or tolerant potato varieties, and (3) epidemiological information and economic analysis tools to aid growers in disease management decisions. Our multi-disciplinary team has implemented and will continue national extension efforts to educate seed and commercial potato growers about management of blackleg and soft rot. Together, these outputs will significantly improve management of potato bacterial diseases. Our project addresses SCRI goals, including:

"efforts to identify and address threats from diseases; new innovations and technology; efforts to improve production efficiency, handling and processing, productivity, and profitability: and research in other methods to improve disease management."

OBJECTIVES: There is a critical need to develop management tools for the on-going and costly blackleg epidemic in potato. This epidemic was first recognized in late 2014/early 2015 and has grown every crop year since then. Based on seed potato testing results in affected states, it will cause significant losses again in 2017. Since bacterial pathogens are difficult to eliminate from seed potato systems, blackleg will be a serious problem for many years, and will spread to all areas where potatoes are grown.The long-term goal of this project is to provide farmers with improved management strategies, and economic analysis tools to guide them in implementation of these strategies. Since both Dickeya and Pectobacterium have broad host ranges, knowledge gained through this work will impact numerous specialty crops.Objective 1 - The pathogen: We will develop efficient detection tools for Dickeya and Pectobacterium to reduce pathogen spread. Extension will include protocols for diagnostic assays and training on testing and test result interpretation.Objective 2 - The environment: We will identify key environmental components to potato colonization and disease development caused by Dickeya and Pectobacterium and will evaluate management approaches. We will determine appropriate thresholds for Dickeya and Pectobacterium in seed potato lots. Extension will include quantitative information on expectations for crop performance with different Dickeya or Pectobacterium incidence in seed lots and information on management of likely inoculum sources.Objective 3 - The host: We will use modern 'omics approaches to identify and validate naturally-occurring potato molecules that provide resistance to Dickeya and will use this information to determine the relative susceptibility of commonly grown commercial potato varieties and elite breeding lines to Dickeya and Pectobacterium species common in the United States. Extension will include providing growers and plant breeders with information on relative susceptibility of commercial varieties to Dickeya and Pectobacterium, and data to support marker development for Dickeya resistance genes.Objective 4 - Economics: We will develop appropriate thresholds for seed potato certification for Dickeya and Pectobacterium and determine the economic impacts of various disease management options. Extension will include quantitative information provided to seed potato certification agencies and economic analysis tools developed for potato farmers to aid them in deciding which interventions are appropriate for their farms.Outreach will occur at regional and national farmer education meetings, online via a project website (http://agsci.colostate.edu/potato-blackleg/) and Focus on Potato webcasts (http://www.plantmanagementnetwork.org), and extension publications. The majority of our project co-PDs and co-PIs are regular speakers at industry meetings and they will continue to provide outreach of knowledge generated by this project.

APPROACH: Objective 1: To develop and validate Dickeya and Pectobacterium detection assay. This objective is building upon on-going work in development of detection methods. Wewill analyze genome sequences from several hundred Enterobacteriaceae for primer development and will develop isothermal detection methods based on these analyses. We will also use direct full-genome sequencing methods to gather information on strains/species present in stem and tuber samples to assist with detection method development. This objective also includes technology transfer of methods to diagnostic labs.Objective 2. Disease epidemiology - To determine losses likely to occur with varying incidence of Dickeya or Pectobacterium in seed, to identify important inoculum sources for these pathogens, and to test chemical management methods. A survey of inoculum sources will be performed primarily in commercial fields in Maine, Michigan, North Dakota, and Oregon.PCR or RPA detection methods will be used to determine if Dickeya

or Pectobacterium are present on or in the plants. Additionally, the presence or absence of Dickeya and Pectobacterium will be determined in samples of surface/irrigation water from each of the fields and swabs from farm machinery and warehouses used in association with the sampled fields. If symptomatic crop plants or weeds are observed, disease incidence will be recorded and the plants will be sampled.To determine if blackleg occurrence of incidents relates to pathogen prevalence in potato seed lots, we will obtain samples of 30 to 50 seed lots per year per location (Maine, Michigan, North Dakota, and the Pacific Northwest) and assay the stem end and periderm of 400 tubers per seed lot (16 composites of 25 tubers each) for Dickeya and Pectobacterium. The seed lot samples will be planted in field plots and blackleg incidence will be recorded and compared to pre-plant estimates of pathogen prevalence.We anticipate that certain Dickeya and Pectobacterium combinations will be more likely to result in disease than others and that prevalence of the genera will change over the course of the growing season. Anecdotally, we have found in some locations that it is easier to find Dickeya early in the season and Pectobacterium later in the season. To examine this, a subset of samples planted in one location (Michigan) will be tested with genomic DNA analysisprior to planting and during the growing season.To examine D. dianthicola survival in agricultural environments, potato tubers either naturally infested with D. dianthicola or artificially inoculated will be used. The tubers will be assayed for D. dianthicola at harvest or after storage(5treatments measuring length of storage and 4 measuring temperature). Bacterial inoculum will also be applied to potato periderm and cut surfaces, dry metal surfaces, concrete, and other surfaces commonly found in potato warehouses to allow measurement of survival at varying times and temperatures.We will conduct replicated field trials in disparate environments to quantify bacterial spread during routine field operations. The first of these plots will be planted prior to the start of this grant, but if funded, we will conduct an additional two years of replicated field trials as described below.In each trial, blackleg symptoms will be rated weekly and symptomatic stems will be tested for the presence of Dickeya with a PCR assay. These data will help quantify spread and movement of Dickeya during seed cutting, handling, planting and crop growth.We will conduct both dose-response studies and field-based yield studies to evaluate potential losses to Dickeya and Pectobacterium in potato in Maine, Michigan, and Oregon.The data from field experiments will be used to estimate yield loss in terms of size and grade as appropriate for the specific marketing channel associated with incidence of bacterial soft rot pathogens. Information on per acre costs and yield consequences will be used to assist growers by developing cost effective risk assessment tools to manage disease impacts on the farm. In conjunction with data collected by plant scientists different control options will be examined economically, along with the probabilities of their success.We will test sanitizers already used by potato growers or in greenhouses, but not labeled for use in seed, for efficacy with soft rot pathogens.At the request of growers, allied industry and advisory panel, chemical and biological products with potential activity against Dickeya and Pectobacterium will be evaluated in controlled replicated field trials in North Dakota, Maine, and Oregon. The trials will include application at post-cutting, seed treatment and in-furrow. The products used will include a chlorine dioxide gas (Fruitgard), Certis biological products Double Nickel and Lifeguard, and other products that may be identified by our advisory panel.Objective 3 - Host resistance: To identify transcripts, proteins, or metabolites correlated with stem or tuber resistance to Dickeya and Pectobacterium.For this project, we will use laboratory-based assays to screen tubers and stems of commercial varieties for relative tolerance to the Dickeya and Pectobacterium. We will also monitor global transcriptional changes in stems of potatoes upon inoculation with D. dianthicola using RNA-Seq. We will identify antimicrobial metabolites and/or proteins produced by resistant and susceptible potato lines before or after infection with D. dianthicola. We will perform metabolomics and proteomics on a set of six resistant and susceptible lines from the S. tuberosum X S.

chacoense cross described above and on commercial potato cultivars with varying resistance.Objective 4. Assessing the economic impacts of Dickeya and Pectobacterium on commercial and seed potato production. Based on field trials from objective 2, yield and grade of harvested potatoes will be evaluated using fresh market and processed market pricing systems. This information allows for accurate evaluation of the crop resulting from the planting of infected seed. In conjunction with data collected by plant scientists, economic costs and benefits from different control options will be evaluated, along with the probabilities of their success. This includes both potential field based controls as well as managing tubers going into storage. This approach will allow for a per-acre or per cwt (100 pounds) Monte Carlo assessment of the costs and probability of success for various control scenarios. Risk modeling will allow for the assessment of the probabilities of success or failure of treatment options and timing. Economic models of the seed potato production and marketing, along with information on the prevalence and spread of Dickeya and Pectobacterium during the growing season will be used to compare costs of probable disease losses with costs of disease mitigation measures, such as additional seed screenings. Using a series of these comparisons, an assessment will be made for reasonable economic thresholds of the pathogens in certified seed potatoes.
