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Short Communication
Novel Approach for Identification of Potato Seed Lots Suspected to be Effected by *Dickeya*

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Abstract

Background and Objective: *Dickeya* spp. was first reported as a potato pathogen from the Netherlands in the early 1970's. *Dickeya* epidemics can initiate from very low inoculum levels, spread quickly and aggressively can be latent in plant material and may be multiply in subsequent generations. The objective of this study was to identify seed lots with high potential for losses owing to *Dickeya* when planted. **Materials and Methods:** Descriptive statistics were collected from emergence data from 2010-2015 potato seed winter grow-out tests. From this, an algorithm was developed to identify seed lots that exhibited poor emergence. One-tailed t-test created a conservative comparison value for identifying seed lots. **Results:** These seed lots with poor emergence have high potential for *Dickeya* contamination. Additionally, seed lots that precursed these high potential lots were identified with the algorithm. The algorithm was applied to 2017 emergence data from winter grow-out tests. Candidate potato seed lots were identified for removal from the Maine potato seed system using the algorithm applied to emergence data from post-harvest grow-out tests in 2015, with some of the data used to generate it. The algorithm was applied to 2017 emergence data from winter grow-out tests and identified seed lots to be purged. The application of the algorithm allows potato seed growers to identify seed lots that should not be planted, either on their or anyone else's farm. This proactive approach has the potential to reduce the stand losses owing to *Dickeya*. **Conclusion:** This study presented a method to identify potato seed lots that have a high potential for *Dickeya* losses before they were planted. This proactive approach is highly beneficial to potato growers as no method exists to do so.

Key words: Potato seed, *Dickeya*, no emergence, winter grow-out test, screening, algorithm

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dickeya spp. was first reported as a potato (*Solanum tuberosum*) pathogen from the Netherlands in the early 1970's[1,2]. In the following years, similar detections were made in Denmark, Finland, France, Hungary, Poland, Slovenia, Spain and Switzerland[1]. *Dickeya* spp., cause soft rots and wilts of many plants and is responsible for wilting and tuber rot of potato[3]. *Dickeya* spp. (formerly *Pectobacterium chrysanthemi, Erwinia chrysanthemi*) has emerged as an aggressive potato pathogen in Europe[4,5].

Symptoms of *Dickeya* spp. were first identified as present in Maine during 2014[6]. Symptoms included no emergence, blackened stems, plant wilting and severe stand losses[6]. A dramatic increase in the symptoms occurred in 2015[6]. Samples were again collected and identified as *Dickeya* and then further confirmed as *Dickeya dianthicola*[4]. The increase in *Dickeya* symptoms in 2015 was not unexpected with *Dickeya*, a seed-transmitted pathogen, as has been documented previously[7]. Areas particularly hard-hit with *Dickeya* epidemics were those areas receiving seed potatoes from Maine. These losses have been traced back to a limited number of seed lots and seed growers. Not all seed lots and not all varieties caused *Dickeya* epidemics. A few varieties dominate the list of suspect lots. These same lots produced poor stands, poor yields and *Dickeya dianthicola* positive samples. *Dickeya* epidemics are particularly troubling as they can initiate from very low inoculum levels, spread quickly and aggressively. The pathogen can be present latently in plant material and infected plant material may be multiplied while the pathogen remains unnoticed[3]. The aggressive nature of the pathogen and the rampant spread from Maine seed potatoes dictated the need for a new approach to identify suspect seed lots in the Maine potato seed system.

Subsequent generations produced from infected seed stock increase seed-borne bacterial pathogens such as *Dickeya*[6]. In Maine, potato seed starts as tissue culture in labs[6]. Tissue culture plantlets are transferred to and grown in greenhouses and finally in field soil. Generally, the first two years grown in field soil are conducted at a potato seed facility specifically designated for the production of nuclear seed. Seed then leaves the facility and is subsequently increased by potato seed growers[8]. The final step in potato seed certification in Maine is a winter grow-out test where virus levels are verified to be within tolerances[9].

A notable symptom present in recent *Dickeya* epidemics is no emergence of the planted seed. The no emergence from winter grow-out tests are proposed to be used as a screening mechanism to identify seed lots that may initiate severe *Dickeya* epidemics. This approach has the potential to add a new aspect to *Dickeya* control and regulation, as well help reduce the *Dickeya* levels in seed stocks. This proactive method will provide a process to identify potential problem potato seed lots before they are planted.

The purpose of this study was to identify candidate potato seed lots for removal from the Maine potato seed system using emergence data from post-harvest grow-out tests. The challenge was two-fold. First, identify seed lots with high potential for *Dickeya* contamination so growers can purge them from the seed system. These are the poorest performing lots when planted. Second, identify seed lots that precursed those lots with potentially high levels of *Dickeya*. These are the lots that produced the poorest seed lots in subsequent generations. Purging only the lots with high potential for *Dickeya* contamination without purging the lots that precursed them will only serve to maintain the pathogen in the seed production system.

MATERIALS AND METHODS

The winter grow-out test consists of planting 400 tubers (fewer if the seed lot is small). The growing plants are evaluated for the expression of virus diseases. Emergence data are also collected during the virus evaluation. Data from 2012-2015 were used to develop an algorithm to identify seed lots with high potential for *Dickeya* contamination.

Identifying seed lots with high potential for *Dickeya* contamination: The winter grow-out test consisted of a 400-tuber sample of each seed lot (larger than 1 acre in size). These were planted and evaluated for virus levels to ensure the levels present are within established certification tolerances. Plant emergence was also recorded. It is these plant emergence data that have potential for determining problem seed lots.

Trace back in 2015 confirmed that seed lots with 25% or less emergence (100 or fewer emerged out of 400 planted) in the winter grow-out test have performed poorly when planted commercially. While no emergence is not a symptom limited to *Dickeya*-infected seed, there is the high association with *Dickeya* presence in fields planted to these poor-performing seed lots. A 25%, or less, emergence is proposed for identification of seed lots with potentially high levels of *Dickeya* contamination.

Identifying seed lots that precursed those lots with potentially high levels of *Dickeya* contamination: Where more than half of the seed lots within a variety are over 75% germination (300 out of 400 plants emerged), 50 is subtracted from the mean number of emerged seed. Seed lots with emergence values less than the calculated mean minus 50 should be flushed from the seed program as there is a serious emergence problem with the lots.

Where less than half of the seed lots within a variety are less than 75% germination (300 out of 400 plants emerged) but more than half of the seed lots are over 50% emergence (200 out of 400 plants), 25 is subtracted from the mean number of emerged seed. Seed lots with emergence values less than the calculated mean minus 25 should be flushed from the seed program, as there is a serious emergence problem with the lots.

Similar to above, if less than half of the seed lots within a variety have less than 50% germination (200 out of 400 plants emerged), subtract 12.5 from the mean number of emerged seed to identify seed lots that should be flushed from the seed program.

Statistical analysis: Descriptive statistics of emergence data from 203 non-*Dickeya*-affected lots in 2010-2011 and 2012-2013 yielded a mean emergence of 358 (out of 400 planted) with a standard deviation of 25. Doubling this standard deviation value to 50 and using it for a one-tailed t-test created a conservative comparison value for identifying seed lots that produce seed lots with potentially high levels of *Dickeya*. This approach was proposed for identification of seed lots that precursed those lots with potentially high levels of *Dickeya* contamination[6].

RESULTS

Current and past winter grow-out reports showed a buildup of poor emergence in some varieties. The variety Reba had serious emergence problems in 2015 and extensive tracking confirmed the presence of *Dickeya* in fields where poor performing lots were planted. This tracking was repeated with several other varieties.

The approach applied *ex post facto* identified the poor-performing seed lots as a serious concern in 2015. This was repeated in 2017 *ex ante facto*. Seed lots, identified as suspect lots, when planted showed poor stands and typical *Dickeya* symptoms.

Many of the seed lots of the variety Reba were identified as problem lots in 2015. The precursors to these problem lots from the previous year were also identified with the algorithm (Fig. 1).

Identifying seed lots with high potential for *Dickeya* contamination: The presence of *Dickeya* not with standing, a seed lot with 25% or less germination should be a concern. When these seed lots were put into commercial use, a similar germination rate to the winter grow-out test occurred. These seed lots have been followed in field plantings and poor emergence were also present. Any lot with less than 25% emergence should be flushed from the seed program. These seed lots potentially have high levels of *Dickeya*. Diverting these seed lots to alternative markets is their best use.

Identifying seed lots that precursed those lots with potentially high levels of *Dickeya* contamination: Historical emergence data from winter grow-out tests predating the widespread presence of *Dickeya* were used to identify seed lots that, if planted, may produce subsequent seed lots that may perform poorly in the field. Data from 2015 were included with the development of the approach. With the failure of the 2015-2016 winter grow-out test, 2017 was the first data set available to challenge the approach.

This analysis, developed with data from 2011-2015 was applied to 2017 data. About 3% of the current seed lots were identified as having a high potential for *Dickeya* contamination (25% or less germination) and 13% of the seed lots are identified as those that precursed those lots with potentially high levels of *Dickeya* contamination.

The cut-off value of 50 is twice the calculated standard deviation of the mean emergence that typifies healthy seed lots in the winter grow-out test. This conservative approach was used to compensate for potential emergence problems that may occur from field variability. Only within-variety values were used to compensate for the potential emergence problems that may occur from variety variability.

A noticeable increase in the *Dickeya* no emergence symptoms have been occurring since 2013 (Table 1). In retrospect, with *Dickeya* as a seed-borne pathogen, this is an expected event. The approach did not identify any lots to be flushed from the seed program before 2010. With that, it is proposed that the pathogen has been present for a number of years and spreading within and between seed lots.

Reproduction of infected seed lots has led to an increase in *Dickeya* symptoms. Additionally, the past two seasons have been warmer than average, leading to widespread symptoms in Maine. Identifying and removing infected seed lots will reduce the occurrence in *Dickeya* symptoms in Maine and elsewhere.

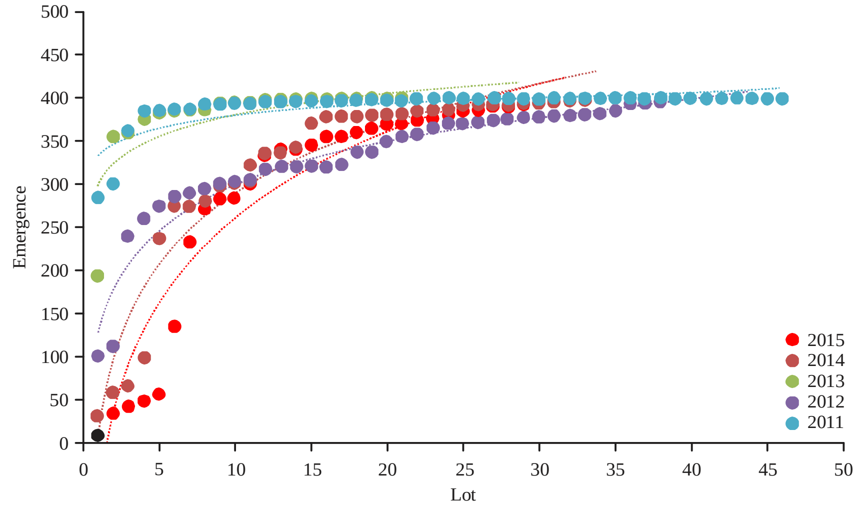

Fig. 1: Emergence (2011-2015) for potato cultivar Reba emergence from the winter grow-out test 400 tubers planted)

Table 1: Emerged potato plants from 400 seed pieces planted in the winter grow-out test

	Years				
Emergence (Out of 400)	2012-2013	2013-2014	2014-2015	2015-2016*	2016-2017
<100	0	47	125	-	25
>100 and <200	6	30	86	-	99
Number of samples	1132	1094	1001	-	747

*A flood destroyed the field and no data were gathered 2015-2016

DISCUSSION

The aggressive nature of the pathogen and the rampant spread from Maine seed potatoes dictated the need for a new approach to identify suspect seed lots in the Maine potato seed system. Presented is a novel approach to aid potato seed growers in identifying seed lots that should be culled from their seed system. The presented algorithm can be used to predict future performance. The approach presented uses a sample data set to extrapolate to a larger population. This approach is used widely in non-agriculture situations. Campbell *et al.*[10] used early measurements of young children to predict an individual's adjustment in middle childhood. Their efforts provided new insights into predictors of children's adjustment in middle childhood for children on other aggression trajectories. Children identified as exhibiting at-risk behavior continued on a pathway toward more antisocial behavior and poor peer relationships. Their work highlighted the implications for early screening and prevention as well as the need for early intervention. Alloway and Alloway[11] used a screening test to identify the strengths and weaknesses of a student's working memory profile. From this, effective approaches could be employed to bolster learning.

The health field has used a similar approach with screening to predict future development of disease[12] and future health conditions[13] based on access and availability. Fisher *et al.*[14] used screening of mothers' beverage choices to predict young girls' calcium adequacy. Their work offers novel approaches targeting both mothers and daughters.

The use of early data to predict future performance is common in finance. Behn and Riley[15] evaluated the association between nonfinancial information and financial performance in the U.S. domestic airline industry. Abarbanell and Lehavy[16] investigated how current price data affects analysts' *ex post facto* forecast errors. This effort parallels their use of existing data to predict future responses of analysts.

Social science, medicine and economics have been successful using predictive data as a screening effort. This is an agricultural application. The presented algorithm identifies seed lots for flushing from the system. Determining where the pathogen enters into the potato seed system needs to be further investigated. With the high proportion of infected seed stock, it is evident the pathogen is present very early in the seed scheme. Nuclear seed stock as a source of the pathogen can't be dismissed. Additionally, more studies are needed to address the question of variety susceptibility to *Dickeya dianthicola*.

The algorithm presented is simple, straight forward and easy to apply. This approach was developed from the Maine potato seed system but its applications are not limited to it.

CONCLUSION

This study presents a novel approach for identifying potato seed lots suspected to be affected by *Dickeya* before they were planted. Application of the presented algorithm will identify seed lots that should be removed from the seed system. This will help to reduce the loss of crops and markets from *Dickeya* seed issues.

SIGNIFICANCE STATEMENT

This study discovers a novel approach that can be beneficial for potato seed growers and others. Application of the presented algorithm will help identify seed lots for further study, something researchers have been unable to do previously. Thus, this application of this algorithm can provide information not previously available.

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