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# Analysis of *Potato Mop-Top Virus* Survival Probability in Post-Harvest Storage

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**Abstract** *Potato mop top virus* (PMTV) induced necrosis can cause tuber quality loss at harvest and storage. Stored potatoes help maintain a constant supply of potatoes to the market and processing industry. PMTV-induced necrosis continues to develop during storage and appropriate timelines are needed for growers to make marketing decisions of their potatoes before incurring any significant quality losses. Survival analysis was used to estimate the time to event occurrence (PMTV-induced necrosis) in four (red-, russet-, white-, and yellow-skinned) potato market types across six post-harvest assessments conducted over two years. At each assessment the presence and absence of PMTV-induced necrosis was recorded and probability of tuber survival was estimated. Survival curves were significantly different among the four market type potato cultivars (Log-Rank test, $P < 0.0005$). Red- and russet-market type cultivars showed low and high survival probability, respectively, demonstrating that red cultivars need priority marketing. The survival probabilities decreased with increased storage time during both years, indicating that PMTV-induced necrosis development is dependent on potato cultivar and post-harvest storage. The median (50% of tubers with symptoms) survival times were estimated as 167 and 214 days for red- and other market type potato cultivars. The information from this study could potentially help growers regulate storage times for their cultivars to minimize tuber quality loss due to PMTV-induced tuber necrosis.

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**Resumen** La necrosis inducida por el virus *mop-top* de la papa (PMTV) causa la pérdida de calidad en los tubérculos a cosecha y en almacén. El almacenamiento de tubérculos es esencial para mantener un flujo constante de papas al mercado de consumo fresco e industrial. La necrosis inducida por PMTV continua su desarrollo bajo condiciones de almacenamiento por lo que la estimación de los plazos de tiempo es requerida a fin de que los productores puedan tomar decisiones de mercadeo de su producto antes de incurrir en pérdidas significativas de calidad. El análisis de supervivencia fue empleado a fin de estimar el tiempo de ocurrencia del evento (necrosis inducida por PMTV) en cultivares de papa pertenecientes a cuatro clases comerciales (tubérculos de piel roja, arrosetada, blanca y amarilla) en seis evaluaciones post-cosecha realizadas durante dos años. En cada evaluación, la presencia o ausencia de necrosis inducida por PMTV fue evaluada y la probabilidad de supervivencia de los tubérculos fue estimada. Las curvas de supervivencia de las cuatro clases comerciales de papa variaron significativamente (Prueba Log-Rank, $P < 0.0005$). Los tubérculos de las clases comerciales piel roja y arrosetada mostraron probabilidades de supervivencia baja y alta respectivamente, indicando que los cultivares de piel roja requieren ser comerciados con prioridad. Las probabilidades de supervivencia disminuyeron con el incremento del tiempo en almacén en ambos años, indicando que el desarrollo de la necrosis inducida por PMTV es dependiente del cultivar y el almacenamiento post-cosecha. El tiempo de supervivencia de la mediana (50% de tubérculos con síntomas) se estimó en 167 días para los cultivares de piel roja y 214 días para todas las demás clases comerciales. La información presentada en este estudio puede potencialmente ayudar a productores a regular los tiempos de almacenamiento de sus cultivares a fin de minimizar las pérdidas de calidad debidas a la necrosis inducida por PMTV.

**Keywords** Survival analysis · Probability · Time-to-event · Median

## Introduction

Potato is host to several pathogens causing yield and quality loss. *Potato mop top virus* (PMTV) is mostly prevalent in cool and humid regions of Northern Europe, Northern America, China, Japan and Andes region (Kirk 2008). PMTV is vectored by the potato powdery scab-causing soilborne obligate parasite known as *Spongospora subterranea* (Arif et al. 1995; Jones and Harrison 1969; Qu et al. 2006). PMTV colonizes the cytosori (galls) produced by *S. subterranea* and survives in the soil for several years (Jones and Harrison 1972). Inter-field transmission of PMTV occurs through planting infected seed material, movement of infested soil or machinery with adhered cystosori (Domfeh and Gudmestad 2016; Sandgren 1995; Sandgren et al. 2002). PMTV symptoms appear as raised lines and rings on the exterior tissues (Carnegie et al. 2012) and brown or necrotic arcs on internal tuber tissue (Mumford et al. 2000). Also, typical virus symptoms like stunting, mottling, chevrons and yellow rings may appear on infected potato foliage (Xu et al. 2004). Since chemical treatment is not effective in the control of PMTV, planting disease-free seed into non-infested soils remains the best method of disease management (Domfeh et al. 2015b; Merz 2008; Montero-Astúa et al. 2008). Although tuber yields are not usually affected due to virus infection, infected potatoes are not acceptable for consumption, processing (Carnegie et al. 2012), or marketing as seed material.

Under most commercial potato production conditions, harvested tubers are placed in a storage facility under controlled environmental conditions prior to marketing (Yellareddygari et al. 2016). The incidence of PMTV-induced tuber necrosis can vary during the storage period in many potato cultivars (Domfeh et al. 2015b; Nielsen and Engsbro 1992; Nielsen and Molgaard 1997). In view of this, a means by which growers could appropriately manage marketing decisions to minimize quality loss caused by the pathogen during storage would be valuable. Although previous studies have demonstrated that PMTV-induced tuber necrosis can vary during post-harvest storage, details of the dynamics of symptom development among different potato types during storage have not been elucidated. Grading PMTV-induced necrosis at harvest could lead to an erroneous conclusion because symptom development continues in tubers during storage (Davey et al. 2014; Domfeh et al. 2015b). Therefore, the focus of the current study was to quantify the risk of disease expression in tubers over a storage period and provide a window to market potatoes prior to incurring quality loss.

## Materials and Methods

The field trials were conducted in North Dakota during summer of 2011 and 2012. The experimental design was randomized complete block design with four replications. Overall, fourteen potato cultivars comprising of seven russet-skinned (Russet Burbank, Russet Norkotah, Ranger Russet, Umatilla Russet, Alpine Russet, Bannock Russet, and Dakota Trailblazer), three white-skinned (Ivory Crip, Shepody, and Kennebec), three red-skinned (Red LaSoda, Red Pontiac, and Red Norland) and one yellow-skinned (Yukon Gold) market types were planted during these trials. Fifteen seed tubers per cultivar were planted in each replication with 0.3 m spacing. Standard agronomic and cultural practices typical of the North Dakota potato production were applied during the crop growing season. Overhead sprinkler irrigation was applied as necessary for the crop. The potato vines were desiccated by means of a rotobeater and harvested tubers were bagged for transportation to potato storage facility located at NDSU for post-harvest mop-top evaluation. The tubers are maintained at $10^{o}$ C and 85% humidity storage conditions until their evaluation for PMTV symptoms.

Post-harvest tuber assessment was performed as previously described (Domfeh et al. 2015b). Tubers were visually evaluated for mop-top incidence over three different times in each year. For the 2011 trial, the evaluations were performed at 37, 117, and 204 days (T1, T2, and T3) post-harvest (DPH) and for the 2012 trial, 56, 167, and 214 DPH (T4, T5, and T6). For evaluation, a sample consisting of 100 random tubers per cultivar from each replication were obtained and one-third of the tubers were graded at each assessment (Domfeh et al. 2015b). Cleaned tubers were cut lengthwise into 1-cm thick slices and PMTV incidence was evaluated (Nielsen and Molgaard 1997). Since the survival analysis is a nonparametric test (binary data), the presence of PMTV tuber necrosis incidence was denoted by '1' and absence as '0'.

### Survival Analysis

Survival analysis is a statistical method for analyzing data to predict the expected duration of time until an event happens, in this case, the development of PMTV incidence (outcome) in tubers during storage. Briefly, survival data measures the post-harvest storage follow-up time from a set starting point to time until an event (PMTV symptom) occurs or fails to occur. Conventional statistical methods such as ordinary least regression (OLS) and logistic regression are not appropriate for time to event analysis, because the former do not account for censoring and the later fails to consider the information regarding timing differences of an event occurrence (Scherm and Ojiambo 2004). In epidemiological studies, censoring is present when there are observations where information on time is not known due to abrupt end of the study before the event occurs in all individuals or the participant is lost during the study period (Prinja et al. 2010). Information from censored and uncensored observations is extracted by survival analysis using the likelihood-based parameter estimates to produce reliable parameter estimates (Allison 1995; Le 1997; Scherm and Ojiambo 2004).

This study was designed to estimate the probability (*Pr*) of PMTV-induced tuber necrosis incidence occurrence at time point *T*, where *T* is a non-negative random variable having cumulative distributive function. The survival function estimates the time *t* elapsed for the participant (cultivar) present at time 0. This can be written as $S(t) = Pr\ (T \geq t)$, where *T* is the time of event occurrence (research interest outcome). Therefore, the survival function at $t = 0$ is equal to one at the beginning of the experiment and equals to zero at time infinity. The $S(0) = 1$, indicates that the certainty of participant to survive at $t = 0$ and $S(\infty) = 0$, indicates the participant's certainty to get infected/fail at $t = \infty$.

### Statistical Analysis

The Kaplan-Meier method (PROC LIFETEST in SAS 9.3) was used for survival analysis of censored PMTV-induced necrosis data with a Bonferroni adjustment for multiple comparisons (Wilcoxon test). PROC LIFETEST also provides the Log-Rank and Wilcoxon statistics for testing the homogeneity of survival curves (Domfeh et al. 2016). The current data set summarized was right-censored because, for each grading period, the study ended prior to event (symptom appearance) occurrence in all tubers.

## Results

Bannock Russet, followed by Dakota Trailblazer, showed highest survival rates, while Red LaSoda and Red Norland recorded lowest survival across both years of the study (Table 1). The cultivars were grouped into four market types and null hypothesis of whether the survival curves were similar was tested. For both years, the Log-Rank and Wilcoxon tests of equality *P* values were less than 0.0005, indicating that survival curves for PMTV-induced tuber necrosis were significantly different among four groups of potato cultivars. Since there was a significant difference among survival curves, as a follow up multiple comparisons among treatments were demonstrated (Table 2). Except for russet vs. white, russet vs. yellow (in 2011) and white vs. yellow skin type potato cultivars (in 2011 and 2012), other pairwise comparison of treatments (cultivar groups) were significantly different.

Tuber survival probabilities estimated at time periods (T1, T2, and T3) followed decreasing trend with an increase in post-harvest duration for four market type cultivars (Fig. 1). At 37 days post-harvest storage (T1) the survival probability of potato tubers was approximately 0.92, 0.90, 0.87, 0.84 for russet-, yellow-, white-, and red- market type cultivars, respectively (Fig. 1). This means that tuber survival (having zero or no PMTV tuber necrosis incidence) at T1 were estimated as 92%, 90%, 87%, and 84% for each market type of potato cultivars. Also, lower survival curves were observed at T2 when compared to T1 and at T3 when compared to T2 and T1 (Fig. 1). Similar trends of survival curves were observed in 2012 trial (Fig. 2). For the 2011 trial, less than 50% tuber survival probabilities were observed at 204 DPH for red-market type cultivars whereas cultivars in other market types had substantially higher survival probabilities. In the 2012 trial, white, yellow- and russet- market type cultivars showed

**Table 1** Survival rates of potato cultivars tested for PMTV-induced tuber necrosis symptom expression across both years

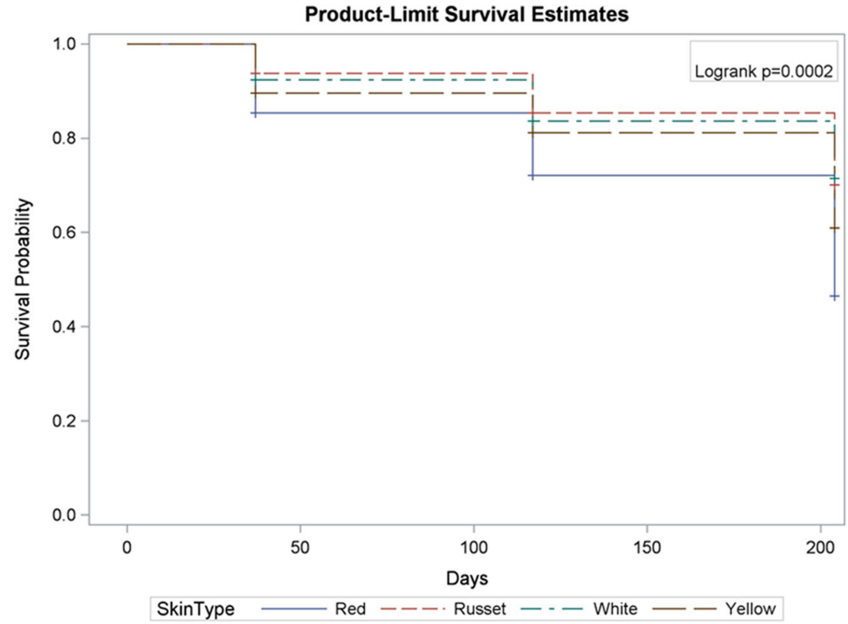
| Cultivar | Market Type | Disease free samples (%) |
|---|---|---|
| Bannock Russet | Russet | 91.67 |
| Dakota Trailblazer | Russet | 86.46 |
| Umatilla Russet | Russet | 76.04 |
| Ivory Crisp | White | 66.67 |
| Russet Burbank | Russet | 59.38 |
| Yukon Gold | Yellow | 56.25 |
| Ranger Russet | Russet | 54.17 |
| Russet Norkotah | Russet | 54.17 |
| Shepody | White | 54.17 |
| Kennebec | White | 47.92 |
| Red Pontiac | Red | 42.71 |
| Alpine Russet | Russet | 40.63 |
| Red LaSoda | Red | 34.38 |
| Red Norland | Red | 29.17 |

Sample represents all tuber samples graded per each cultivar (4 replications +3 disease assessments)

**Table 2** Pairwise comparison of survival curves for red-, russet-, white-, and yellow-skinned potato cultivars

| Year | Market-type | Market-type | *P* value (Bonferroni adjusted) |
|---|---|---|---|
| 2011 | Red | Russet | 0.0012 |
| 2011 | Red | White | 0.0144 |
| 2011 | Red | Yellow | 0.0186 |
| 2011 | Russet | White | 1 |
| 2011 | Russet | Yellow | 0.1294 |
| 2011 | White | Yellow | 1 |
| 2012 | Red | Russet | <.0001 |
| 2012 | Red | White | 0.0008 |
| 2012 | Red | Yellow | <.0001 |
| 2012 | Russet | White | 0.0008 |
| 2012 | Russet | Yellow | <.0001 |
| 2012 | White | Yellow | 1 |

**Fig. 1** Estimates of the Kaplan-Meier curves describing the times for PMTV symptom expression in four market-type potato cultivars during storage (2011 trial)

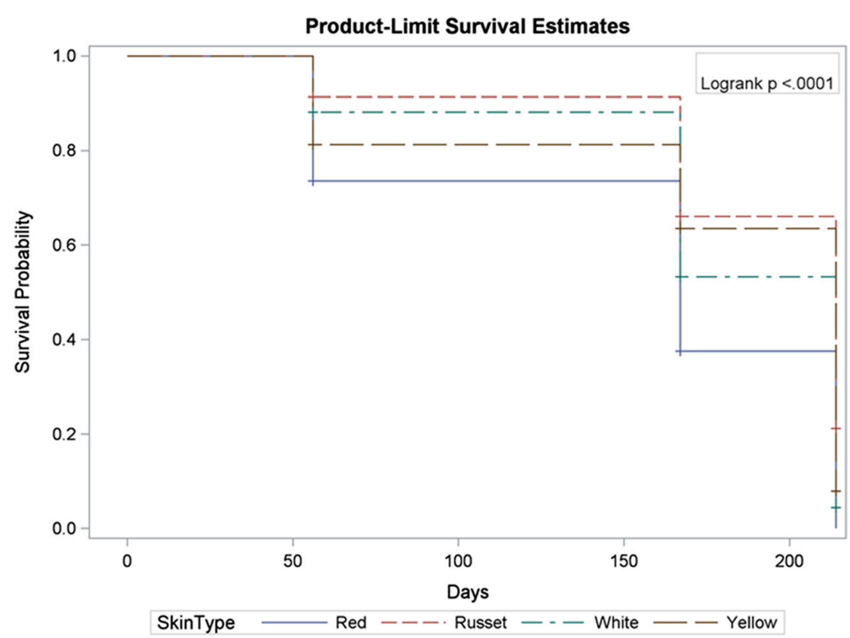



less than 50% tuber survival at 214 days storage and red market type cultivars at 167 days storage (Fig. 2).

## Discussion

This is the first attempt to estimate PMTV-induced tuber necrosis survival probability as a research outcome among potato cultivars during post-harvest storage. The results from this study will assist growers in regulating the tuber storage period and timely marketing of various potato cultivar market types. From a management standpoint, this is important because PMTV necrosis symptom development during storage can lead to rejection of crops intended for the processing industry or for the pre-packed super market trade (Mumford et al. 2000). Since PMTV incidence changes over the storage period, we thought it would be appropriate to measure the time-to-event outcome in potato cultivars representing four important market classes. Changes in PMTV-induced necrosis have been observed among different potato cultivars (Domfeh et al. 2015b; Kurppa 1989; Graversen 1992; Nielsen and Engsbro 1992; Nielsen and Molgaard 1997; Ryden et al. 1989). Our results showed that PMTV incidence variation (survival curves) during post-harvest storage were significantly different among four market class cultivars. This indicates that an increase in PMTV-induced tuber necrosis during

**Fig. 2** Estimates of the Kaplan-Meier curves describing the times for PMTV symptom expression in four market-type potato cultivars during storage (2012 trial)



storage is dependent on both potato cultivars and post-harvest storage duration. From this, it can be inferred that research should prioritize the identification of cultivar resistance and recognizing post-harvest timelines for minimizing losses.

Since PMTV-induced tuber necrosis development can vary over time, time-to-event analysis was used to measure the research outcome. This analysis is statistically appropriate because it includes the independent variables whose values change over the course of study (Scherm and Ojiambo 2004). Different assessment times were included to incorporate disease variability from harvest to post-harvest storage of tubers. Cultivar sensitivity classification has traditionally been based on PMTV-induced tuber necrosis assessments at harvest and after three months of storage (Nielsen and Molgaard 1997). During both years (at three post-harvest assessments), the results suggest that red- and russet-skinned potato cultivars were most and least affected, respectively. This clearly demonstrates that some cultivars are more sensitive to PMTV symptom development over storage compared to other cultivars. It has been reported that internal PMTV necrotic symptoms develop slowly during storage and fluctuating storage temperatures can hasten symptom development (Carnegie et al. 2010). Symptom development in four market type potato cultivars was slow and took more than 150 days for 50% of the tubers to have PMTV tuber necrosis. This may be due to the constant storage temperature ($10^{o}$ C) maintained during storage. Further studies are needed to study the role of temperature on symptom development during storage for prolonged periods.

Results of the current study indicate that time-to event appearance (incidence) of PMTV symptoms among cultivars increased during storage. The increased frequency of tubers with PMTV-induced tuber necrosis over time indicates that marketing potatoes immediately after harvest would be advantageous, particularly in cultivars highly sensitive to PMTV-induced tuber necrosis, since the incidence of symptom development and associated monetary losses would be lower. However, storage is needed for maintaining potatoes in an edible and marketable condition and to provide constant flow to market and processing plants through the year (Eltawil et al. 2006). Therefore, understanding the storability of PMTV infested tubers among cultivars within each market segment is important. Among the cultivars tested, red-skinned cultivars were affected more at 37 to 56 days storage and would require priority marketing compared to other cultivars studied. Delayed marketing (T2-T3 and T5-T6) of red-skinned cultivars may compromise tuber quality if infected with PMTV. Russet- and white-skinned cultivars are least affected and more than 80% of tubers (without symptoms) were unaffected at 117 days of storage. However, the survival probability of the same market type cultivars dropped to 60% and 55% at 167 days storage. This indicates that a 50 day delay in marketing may result in 20–25% more tubers showing PMTV-induced necrotic symptoms during storage. At 214 DPH, the survival curves show that tubers without symptoms ranged from 40% to 10% indicating that prolonged storage (beyond T2 and T5 storage duration) is not ideal for all market type cultivars.

The information from survival analysis can be useful in the identification of cultivar sensitivity to PMTV-induced tuber necrosis. For example, PMTV disease rating of tubers immediately after harvest and after prolonged storage may result in false conclusions of cultivar sensitivity. To overcome this, multiple disease rating of tubers over the storage duration were conducted and the aggregated data was used for determining cultivar sensitivity (Domfeh et al. 2015a, b). This approach of disease rating is useful because the number of tubers with symptom development changes during storage. Additionally, it is impractical to continue grading until all the tubers show symptoms. Therefore, median survival times (where 50% of the tubers show symptoms) were used as the standard to determine the appropriate time for PMTV disease evaluations. The median survival times for red-skinned and other market type cultivars are 167 and 214 days storage, respectively. The use of median survival time is also appropriate from a statistical point of view because the distribution of survival data is often skewed and median survival time is an important measurement (Seppa and Hakulinen 2009). Based on median survival times, we recommend that multiple disease ratings be scheduled before 50% of the tubers show symptoms. From this study, we conclude that a minimum of two ratings are appropriate for identifying cultivar sensitivity to PMTV-induced tuber necrosis and those ratings should be planned within 4–5 months post-harvest storage. For further studies, more cultivars and additional disease assessments should be used to test the survival times of PMTV tuber necrosis in storage.

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