EVALUATION OF THE RELIABILITY OF DETERMINING SOFT ROT RESISTANCE IN POTATOES BY THE TUBER SLICE METHOD

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Abstract

Soft rot resistance in potato (*Solanum tuberosum* L.) tubers can be determined by inoculating tuber slices with soft rot *Erwinia* species. Questions have been raised in the literature concerning the reliability of the tuber slice method. The objectives of this study were to 1) examine the statistical assumptions underlying the analysis of variance for different response variables as measures of soft rot resistance using the tuber slice method of evaluation; 2) estimate the sample sizes necessary to detect specified differences with power 0.83; and, 3) choose the "best" variable for measuring resistance to soft rot based on valid statistical analysis and minimal sample size. Slices from fifteen tubers from each of three cultivars (Atlantic, Norchip, Superior) were inoculated with *Erwinia carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora* or *E. chrysanthemi* and incubated at two different temperatures (20 C and 30 C) for 48 hrs. The test was conducted on two dates. Tuber samples were sliced and weighed prior to inoculation and after the macerated tissue was removed following a 48 hr incubation period. The maximum diameter of macerated tissue, actual weight loss, four measures of proportional weight loss, and various transformations of these variables were analyzed. The "best" response variables for measuring resistance to soft rot by the tuber slice method were the diameter of the macerated tissue and the square root transformation of one of the measures of proportional weight loss. No differences were found among the cultivars nor the *Erwinia* subsp. for either of these response variables, and the cultivar x *Erwinia* subsp., cultivar x temperature and cultivar x *Erwinia* subsp. x temperature interactions were not significant. However, there were significant differences between the incubation temperatures, and the *Erwinia* subsp. x temperature interaction was significant for both response variables. Estimates of sample sizes necessary to find a 20% difference in main effect and interaction effect means were calculated.

Resumen

Se puede determinar la resistencia a la pudrición blanda en tubérculos de papa (*Solanum tuberosum* L.) mediante la inoculación de rodajas de tubérculo con especies de *Erwinia* causantes de esta enfermedad. Han surgido interro-

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gantes en la literatura en cuanto a la confiabilidad de este método. Los objetivos de este estudio fueron: 1) examinar los supuestos estadísticos que subyacen el análisis de la variancia para las diferentes variables como medidas de la resistencia a la pudrición blanda empleando el método de evaluación en las rodajas del tubérculo de papa; 2) estimar los tamaños de muestras necesarios para detectar diferencias específicas con poder 0.83; y 3) escoger la variable "mejor" para medir la resistencia a la pudrición blanda basándose en los análisis estadísticos válidos y en el tamaño mínimo de la muestra. Las rodajas de quince tubérculos de cada uno de los tres cultivares analizados (Atlantic, Norchip, Superior) fueron inoculadas con *Erwinia carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora* o *E. chrysanthemi* e incubados a dos temperaturas (20 C y 30 C) durante 48 horas en dos fechas diferentes. Los tubérculos fueron rebanados y pesados antes de la inoculación y luego de que el tejido macerado fuera extraído después de un período de incubación de 48 horas. Se analizaron el máximo diámetro del tejido macerado, la pérdida real de peso, las cuatro medidos de la pérdida proporcional de peso y diversas transformaciones de estas variables. Las "mejores" variables para la medición de la resistencia a la pudrición blanda mediante el método de rodajas de tubérculo fueron el diámetro del tejido macerado y la transformación de la raíz cuadrada de una de las medidas de la pérdida proporcional de peso. No se encontraron diferencias entre los cultivares o subespecies de *Erwinia* para ninguna de estas variables y las interacciones de cultivar x *Erwinia* subsp., cultivar x temperatura y cultivar x *Erwinia* subsp. x temperatura no fueron significativas. Sin embargo, hubo diferencias significativas entre las temperaturas de incubación, y la interacción *Erwinia* subsp. x temperatura fue significativa para ambas variables. Se calcularon estimados de los tamaños de muestras necesarios para hallar un 20% de diferencia en el efecto principal y el medio del efecto de interacción.

Introduction

Potato tuber soft rot and blackleg, caused by *Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye (Eca), *E. carotovora* subsp. *carotovora* (Jones) Bergey *et al.* (Ecc) and *E. chrysanthemi* Burkholder, McFadden and Dimock (Ech), cause losses during growth through reduced emergence after planting, reduced yield and smaller tubers (Lapwood and Gans, 1984), and post-harvest losses through tuber decay in storage and transit (Lapwood *et al.*, 1984; Perombelon and Kelman, 1980). Eca and Ecc are the prevailing soft rot species in cooler potato growing areas (DeBoer and Kelman, 1978; Powelson and Apple, 1986), whereas, Ech is wide-spread in the warmer potato growing areas of the tropics (Hidalgo and Echandi, 1983; Perombelon and Kelman, 1980).

It has been suggested that screening potato cultivars for resistance to tuber soft rot could be accomplished using just one of the three *Erwinia* pathogens (Lyon, 1989; Wolters and Collins, 1994). Lapwood *et al.* (1984) found that cultivars were similarly ranked when inoculated with either Eca or Ecc. Austin *et*

al. (1988) reported similar results for Eca, Ecc, and Ech with *Solanum tuberosum* clones and *S. tuberosum-S. brevidens* somatic hybrids. Wolters and Collins (1994) also reported similar results for Eca, Ecc and Ech with a *S. phureja-S. stenotomum* hybrid population.

Variations on two different inoculation methods have been used to evaluate tuber resistance to soft rot: point titration and tuber slice. The point titration method (Hidalgo and Echandi, 1982; Hidalgo and Echandi, 1983; Lojkowska and Kelman, 1994; Maher and Kelman, 1983) consists of using a syringe to inject a suspension of *Erwinia* culture to a selected depth in surface sterilized tubers and incubating the tubers under anaerobic conditions. The diameter of the macerated tissue then is used as a measure of relative resistance. The tuber slice method (Dobias, 1976; Krause *et al.*, 1982; Lapwood *et al.*, 1984) consists of inoculating tuber slices with bacterial suspensions on filter paper disks. As in the point titration method, the diameter of the macerated tissue again is used as a relative measure of resistance. Koppel (1993) and Lojkowska and Kelman (1994) compared a point titration method with a standard slice inoculation method. Lojkowska and Kelman (1994) concluded that the great variability of results obtained with the standard tuber slice method raised serious questions as to its reliability. However, Koppel (1993) concluded that the different methods assess different components of general resistance. The anaerobic conditions under which the point titration method are conducted favors a progressive rot (DeBoer and Kelman, 1975; Maher and Kelman, 1983) and inhibit the formation of wound periderm and suberin (Kolattukudy, 1981). The aerobic conditions under which the tuber slice method is conducted measures the reaction of a cultivar to bacterial challenge at the wound site and its capacity for and speed of wound-healing (DeBoer and Kelman, 1978; Lapwood *et al.*, 1984).

Recently, Goth *et al.* (1994) proposed using proportional weight loss of inoculated tuber slices as a relative measure of resistance to the soft rot *Erwinias*. No comparison has been made between measurements of the diameter of the macerated tissue and the proportional weight loss in tuber slices for evaluating relative resistance to the soft rot *Erwinia* species. Such comparisons among different response variables should be made by examining the statistical assumptions underlying the standard analysis of variance and determining an adequate sample size for testing.

Sample size is very important in hypothesis testing because both type I and type II errors can be reduced by increasing sample size (Snedecor and Cochran, 1967). A realistic rule-of-thumb proposed by Mead (1988) to determine the appropriate number of replications needed in an experiment is to require that the number of replications be sufficient to make the standard error of the difference between two treatment means no bigger than d/3, where d equals the difference to be detected. This is equivalent to 0.83 power (β) at a significance level (α) of 0.05 (Mead, 1988).

The objectives of this study were to 1) examine the statistical assumptions underlying the analysis of variance on different response variables as measures of soft rot resistance using the tuber slice method of evaluation; 2) estimate the sample sizes necessary to detect specified differences with power 0.83; and, 3) choose the "best" variable for measuring resistance to soft rot based on valid statistical analysis and minimal sample size.

Materials and Methods

Three potato cultivars (Atlantic, Norchip and Superior) were grown at Aroostook State Farm, Presque Isle, Maine in 1994 on a Caribou silt loam soil (fine-loamy, mixed, frigid Typic Haplorthods). These three cultivars were randomly selected from a cultivar collection maintained on Aroostook State Farm and are representative of the commercial cultivar germplasm base. Forty-five kg. of tubers from each of these three cultivars were harvested in October with a single row digger and picked up by hand. Tubers were stored at 5-12 C until shipped to Beltsville, Maryland in December of 1994, where they were stored at 4 C and 95% relative humidity. One week prior to the initiation of the test, tubers were removed from storage. Undamaged tubers were placed in the dark at ambient temperature (18-23 C) for 72 hr, washed, surface sterilized by a 10 minute immersion in a 0.05% w/v chlorine sodium hypochlorite solution and allowed to air dry. Two slices, approximately 13 mm thick, were cut from the center of each tuber at right angles to a line from the apical to the stem end of the tuber. The weight of each slice was recorded to the nearest 0.01 g (WT1). The slices were paired on a 30 cm x 16 cm galvanized metal hardware cloth (12.5 x 12.5 mesh) set in a 31 x 17 x 8 cm clear plastic box. The bottom of the box was filled with tap water to a depth of approximately 2.5 cm. The slices were positioned 2.5 cm above the tap water on the hardware cloth.

Tuber slices were inoculated with isolates from *Erwinia carotovora* subsp. *atroseptica* (Eca), *E. carotovora* subsp. *carotovora* (Ecc) or *E. chrysanthemi* (Ech). Dr. Carol Ishimaru of Colorado State University provided the six *Erwinia* isolates used in this study. Isolates Eca CLR300 and Eca CLR310 were collected from potato stems. Isolates Ecc CLR30 and Ecc CLR240 were also collected from potato stems. Both isolates Ech CLR219 and Ech CLR229 were collected from irrigation ditch water in Colorado. All of these isolates were evaluated for pectolytic activity on crystal violet pectate medium (Cuppels and Kelman, 1974), florescence on King's medium B (King *et al.*, 1954), growth at 37 C, and sensitivity to erythromycin. They were characterized with the Biolog Microstation System and the Biolog GN Database (release 3.50; Biolog Inc., Hayward, CA) to determine carbohydrate utilization, and were further characterized via gas chromatographic analysis of fatty acid methyl esters (FAME) by Microbial ID, Inc. (Newark, DE).

Stock suspensions of the *Erwinia* isolates were maintained in sterile distilled water in capped test tubes at ± 20 C and in glycerin at -42 C. For inocu-

lum, transfers from the stock cultures were streaked on Difco nutrient agar. Single colonies were transferred from the nutrient agar to 50 ml Difco nutrient broth in a 125 Erhlenmeyer flask and agitated continuously at 100 rpm on an orbital shaker at 20-22 C for 24 hr. The inoculum concentration was adjusted to 10^8 cfu with nutrient broth. Suspensions of isolates of the respective subspecies were combined for the inoculation study. Nutrient broth was used as a control.

Immediately after weighing, one tuber slice was inoculated by positioning a 12.5 mm dia Schleicher and Schuell filter paper circle containing 200 µl of 10^8 cfu/ml of Eca, Ecc or Ech in the center of the slice. The other slice, which served as a control, was similarly inoculated with 200 µl of nutrient broth. Care was taken to inoculate the sides of the slices which were adjacent to each other. The boxes were fitted with their tight plastic top and placed in the dark in an incubator at either 20 C or 30 C for 48 hr.

There were eighteen treatment combinations in this experiment arranged as a 3 cultivar (Atlantic, Norchip, Superior) x 3 *Erwinia* subsp. (Eca, Ecc, Ech) x 2 temperature chamber (20 C, 30 C) factorial. Fifteen tuber slices were inoculated for each of the eighteen treatment combinations. The experiment was conducted 9-11 January, 1995 and repeated 24-26 January, 1995.

Following the 48 hr incubation period, the diameter of the macerated tissue was measured in mm at its widest point. Macerated tissue was removed from each slice with a spatula and the remaining tissue was weighed (WT2). Actual weight loss was calculated for each tuber slice as (WT1-WT2). In addition, four measures of proportional weight loss were calculated for each tuber slice: 1) PWT1 = WT1/WT2; 2) PWT2 = WT2/WT1; 3) PWT3 = [(WT1-WT2)/WT1]; and, 4) PWT4 = [(WT1-WT2)/WT2]. These four measures of proportional weight loss were each transformed using the square root, log and logit transformations.

Analyses of variance were conducted on all variables mentioned above using Proc Mixed in SAS (SAS Institute, 1993). For these analyses all treatment effects (cultivar, *Erwinia* subsp. and temperature) were considered fixed. Date and all interactions of date with cultivar, *Erwinia* subsp. and temperature were considered random. Residuals from the analysis of variance on each variable were plotted against their predicted values and visually examined for homogeneity of variance (Draper and Smith, 1981). The correlation between the "best" response variables was computed after date and treatment effects were removed from the analysis. A data set was created using residuals from Proc Mixed for each variable and the correlation between these residuals was calculated using Proc Corr in SAS (SAS Institute, 1985).

Using Mead's simple rule-of-thumb, the sample sizes necessary to detect differences between two means can be estimated (Mead, 1988). For the simple effect means in this experiment (3x3x2=18 treatment combinations) we have:

$$S.E._{diff} = (2s^2/r)^{1/2} = d/3$$

where r = number of replications

d = difference we desire to find significant

s^2 = mean square error from the analysis of variance

and, therefore,

$$r = 18s^2/d^2 \qquad (1)$$

To compare either cultivar or *Erwinia* main effect means:

$$S.E._{diff} = (2s^2/6r)^{1/2} = d/3$$

and therefore,

$$r = 3s^2/d^2 \qquad (2)$$

These equations may be rewritten in terms of the coefficient of variation (CV) when d is expressed as a proportion of the mean. This would then allow comparisons for r to be made for variables measured in different units. We can express s in terms of the coefficient of variation:

$$CV = s/\bar{x} \text{ or } s = CV * \bar{x}$$

Defining d as a proportion (p) of the mean ($\bar{x}$):

$$d = p * \bar{x}$$

We can rewrite equation (1) as:

$$r = 18\ (CV/p)^2$$

And, equation (2) as:

$$r = 3\ (CV/p)^2$$

Results and Discussion

An examination of the residuals plotted against their predicted values from the analysis of variance revealed that residuals from only six of the eighteen considered response variables were homogeneous (Table 1). Non-homogeneous distributions of the residuals were revealed by a general fanning pattern, indicating non-constant variance. Variables with such a distribution were eliminated from further consideration as a response variable for measuring resistance to soft rot. For residuals that were homogeneous, the residuals plotted against their predicted values were evenly scattered across the plot. Upon closer examination, three of these six variables were equivalent (logit (WT2/WT1), logit [(WT1-WT2)/WT1] and log [(WT1-WT2)/WT2]). Of these three, the variable logit (WT2/WT1) was retained for further consideration as a response variable for measuring resistance to soft rot.

All the observations were used only for the analysis involving diameter. Two observations could not be used in four analyses because the response variable was undefined for those particular observations. However, when logit [(WT1-WT2)/WT2] was evaluated as the response variable 63 observations

TABLE 1.—*Examination of different response variables for measuring resistance to soft rot* Erwinia *subsp. in potato tuber slices for which the residuals from the analysis of variance were homogeneous. The number of observations (N), the mean square error (se), the population mean ($\bar{x}$), and the coefficient of variation (cv) from the analysis of variance for each response variable are given.*

Variable	N	se	$\bar{x}$	cv
LOGIT (WT2/WT1)[A]	523	0.50	1.61	0.44
[(WT1-WT2)/WT1]$^{1/2}$	523	0.01	0.43	0.24
LOGIT[(WT1-WT2)/WT1][A]	523	0.50	1.61	0.44
LOG [(WT1-WT2)/WT2][A]	523	0.50	-1.61	0.44
LOGIT [(WT1-WT2)/WT2]	462	0.89	-1.56	0.61
DIAMETER	525	92.95	40.81	0.24

[A]LOGIT (WT2/WT1), LOGIT [(WT1-WT2)/WT1] and LOG [(WT1-WT2)/WT2 are mathematically equivalent.

were undefined. Ideally, transformations of the data should not result in a large number of the individual observations being unusable in the analysis. Therefore, logit [(WT1-WT2)/WT2] was dropped from further consideration as a response variable for measuring resistance to soft rot.

The coefficient of variation was largest for logit [(WT1-WT2)/WT2] and smallest for diameter of macerated tissue and [(WT1-WT2)/WT1]$^{1/2}$ (Table 1). These coefficients of variation for the different response variables were calculated from the analyses of variance combined for both dates. The coefficients of variation for the different response variables from the analyses of variance by date varied around these. Calculations using Mead's formula (Mead, 1988) used the former coefficients of variation for ease in presentation. To find a 20% difference between two treatment (cultivar x *Erwinia* subsp. x temperature) means significant, the number of tuber slices to be evaluated per treatment combination ranged from 26 when either diameter of macerated tissue or [(WT1-WT2)/WT1]$^{1/2}$ were used as the response variable to 168 when logit [(WT1-WT2)/WT2) was used as the response variable.

The best response variables for measuring soft rot resistance were diameter of macerated tissue and [(WT1-WT2)/WT1]$^{1/2}$. The residuals from the analysis of variance for these two variables were homogeneous and the coefficients of variation were the smallest of all response variables examined. Even so, to find a 20% difference in any two treatment means (18 means in this study) significant at the 5% level would require a sample size of 26 tuber slices for each treatment combination. This may explain why some researchers believe that the tuber slice method is too variable for reliable results (Lojkowska and Kelman, 1994). In evaluating potato germplasm, a researcher would rarely be interested in comparing all 18 treatment combinations (cultivar x *Erwinia* subsp. x temperature effect means). To find a 20% difference between

two cultivar means (main effect means) significant at the 5% level with 0.83 power, five tuber slices would need to be evaluated for each treatment combination using either the diameter of macerated tissue or $[(WT1-WT2)/WT1]^{1/2}$. The number of slices needed per treatment will depend on the experimental design and the comparisons of interest. The present experiment is simply an example of the issues that a researcher needs to address. In the early stages of a breeding program when the number of tubers available for testing is small, a researcher could still expect to distinguish between more resistant and more susceptible cultivars when either the diameter of macerated tissue or $[(WT1-WT2)/WT1]^{1/2}$ was used as the response variable.

The partial correlation between diameter of macerated tissue and $[(WT1-WT2)/WT1]^{1/2}$ after date and treatment effects were removed was highly significant (r=0.58**). However, one variable could only explain about 36% of the variation in the other. Possible sources of variation in both types of measurements can be envisioned. Tissue in the center of a potato slice will only occasionally rot in a true circular pattern. Oftentimes, edges of the macerated tissue will be uneven. However, choosing the maximum diameter of macerated tissue is fairly straightforward even if the pattern is oddly shaped. The experimenter has only to make measurements at all sites that are potentially the maximum diameter and then, in fact, choose the maximum diameter. There are considerably more sources contributing to the variation of $[(WT1-WT2)/WT1]^{1/2}$. First, there are slight variations in initial weight and final weight due to water loss depending on how long the tuber slices are exposed to ambient temperature conditions. Second, there is the potential for the introduction of great variation from the physical removal of macerated tissue by the experimenter. However, $[(WT1-WT2)/WT1]^{1/2}$ gives a relative estimate of the volume of affected tissue for comparisons between treatments. These measurements will have value as long as the depth of the macerated tissue does not extend through to the other side of the tuber slice. Finally, there is usually more variation associated with a response variable composed of several measured variables, such as $[(WT1-WT2)/WT1]^{1/2}$, than in either of the original measured variables, in this case WT1 and WT2. Nevertheless, both the diameter of macerated tissue and $[(WT1-WT2)/WT1]^{1/2}$ measure important components of resistance to soft rot *Erwinias*. The diameter of macerated tissue is a measurement on a two-dimensional plane (the surface of the slice) and $[(WT1-WT2)/WT1]^{1/2}$ is a measurement in three-dimensions (volume).

The tuber slice method used in this experiment measured the reaction of the cultivar to bacterial challenge at the wound site and its capacity for and rate of wound healing. All nutrient broth inoculated slices in each plastic box suberized, indicating the presence of enough healthy tissue to initiate a healing response. In this respect, diameter was a measurement of the response of the injured tuber slice to bacterial challenge, and $[(WT1-WT2)/WT1]^{1/2}$ was a measurement of the response of the injured plus non-injured tissue in the tuber slice to bacterial challenge.

There were no significant differences among the responses of the cultivars to these *Erwinia* subspecies when either diameter of macerated tissue or [(WT1-WT2)/WT1]$^{1/2}$ was used as the response variable (Table 2). These three cultivars cover a broad range in specific gravity. Atlantic is a very high specific gravity cultivar, Norchip is intermediate, and Superior is low. Thus, there was no apparent relationship between resistance to soft rot and specific gravity in these cultivars. This agrees with the findings of Hidalgo and Echandi (1983) who observed no correlation between dry matter content and susceptibility to tuber rot in *Tuberosum*, although they did find a significant negative correlation between dry matter content and susceptibility to tuber rot in Andigena. Also, there were no significant interactions between cultivar and *Erwinia* subsp. These results are in agreement with those of other researchers who have reported that cultivars or selections are ranked similarly for the different *Erwinia* subspecies (Austin *et al.*, 1988; Lapwood *et al.*, 1984; Lyon, 1989; Wolters and Collins, 1994).

TABLE 2.—*Analysis of variance of diameter of macerated tissue and [(WT1-WT2)/WT1]$^{1/2}$. Cultivar,* Erwinia *subsp. and temperature were considered fixed effects; date and all two-, three-, and four-way interactions involving date were considered random effects. F-tests on Type III means squares were computed for fixed effects. Estimates of the variance components for the respective random effects were not significantly different from zero.*

	Tests of Fixed Effects			
			Diameter	[(WT1-WT2)/WT1]$^{1/2}$
Source	Ndf[1]	Ddf[2]	Type III F	Type III F
Cultivar (C)	2	2	1.14	0.27
Erwinia (E)	2	2	0.07	3.53
Temp (T)	1	1	36.62†	162.55*
C x E	4	3	2.37	0.89
C x T	2	2	0.22	1.59
E x T	4	3	16.7†	31.77*
C x E x T	4	3	1.51	0.78

	Tests of Random Effects			
	Diameter		[(WT1-WT2)/WT1]$^{1/2}$	
Source	Variance	Z	Variance	Z
Date (D)	0	.	0	.
D x C	49.97	1.10	0.0061	1.04
D x E	15.19	0.94	0.0017	0.80
D x T	0	.	0.0003	0.36
D x C x E	0	.	0.0003	0.32
D x C x T	1.39	0.37	0.0001	0.11
D x E x T	0	.	0	.
D x C x E x T	0.74	0.22	0.0009	0.88

†,*Significant at the 10% and 5% levels, respectively.

[1,2]Numerator and denominator degrees of freedom for F-tests of significance, respectively.

The *Erwinia* subsp. x temperature interaction was significant for diameter of macerated tissue and $[(WT1\text{-}WT2)/WT1]^{1/2}$. Significance was tested at the 10% level because of few degrees of freedom for the tests. At 20 C, the largest diameter of macerated tissue and greatest losses for $[(WT1\text{-}WT2)/WT1]^{1/2}$ were observed with Eca (Table 3). At 30 C, the largest diameter of macerated tissue and greatest losses for $[(WT1\text{-}WT2)/WT1]^{1/2}$ were observed with Ech. There was relatively little change in diameter between 20 and 30 C for Eca. There was a large change in diameter and $[(WT1\text{-}WT2)/WT1]^{1/2}$ between 20 and 30 C for Ech.

TABLE 3.—*Mean* $[(WT1\text{-}WT2)/WT1]^{1/2}$ *and mean diameter in mm (Dia) of macerated tissue of tuber slices from three potato cultivars for three* Erwinia *subspecies at 20 or 30 C.*

	Temperature			
	20 C		30 C	
Erwinia	$[(WT1\text{-}WT2)/WT1]^{1/2}$	Dia	$[(WT1\text{-}WT2)/WT1]^{1/2}$	Dia
Eca	0.31	40	0.47	41
Ecc	0.26	36	0.53	44
Ech	0.29	35	0.71	48

This paper has examined some of the statistical assumptions underlying the analysis of variance for different response variables for measuring resistance to soft rot. In addition to determining the best response variable for measuring resistance to soft rot, it is hoped that this paper will re-acquaint readers with the importance of examining the assumptions underlying the analysis of variance before analyzing their data. In any experiment, residuals from the analysis of variance should always be plotted against their predicted value and examined for homogeneity before the analysis proceeds further. This may be of particular importance in an experiment of this type, where considerable variation can be introduced through the choice of potato cultivars, isolates of *Erwinia* subsp. and environmental conditions of the test. Often, some transformation of the data is needed before performing statistical analyses to avoid violating the statistical assumptions that underlie the analysis of variance. We also hope that Mead's simple rule of thumb (Mead, 1988) will provide researchers with a quick and easy method of determining an appropriate sample size for comparing treatment means with some precision.

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