DETERMINING THE FEASIBILITY OF MEASURING GENOTYPIC DIFFERENCES IN SKIN-SET

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Abstract

Replicated experiments with various potato cultivars having diverse tuber skin types demonstrated that the Halderson periderm shear tester was sufficiently sensitive to measure genotypic and phenotypic differences in skin-set (periderm maturity). All genotypes had lower skin-set readings at the bud end of the tuber compared to the equatorial region and stem end. The technique employed with the shear tester objectively resolved genotypic differences in the developmental time courses for skin-set and clearly showed that the periderm of Russet Burbank tubers matured more rapidly than the periderm of other cultivars with smooth skin types. However, a consistent relationship between periderm weight and skin-set could not be established. In postharvest experiments designed to describe the phenotypic effect of temperature and relative humidity on tuber periderm maturation, we found that high relative humidity retarded the development of skin-set. The ability to detect genotypic and phenotypic differences with this technique for measuring skin-set indicates that it is feasible to further develop a standardized procedure suitable for testing diverse skin types in breeding and certified seed programs as well as for use by growers of table stock, seed, and chipping potatoes.

Compendio

Experimentos con repeticiones, con varios cultivares de papa con tubérculos de diversos tipos de piel, demostraron que el probador Halderson de

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This research was supported in part by a grant from the Red River Valley Potato Grower's Association.

Accepted for publication February 22, 1993.

ADDITIONAL KEY WORDS: Periderm maturation, potato skinning, potato scuffing, potato feathering.

corte de periderma era suficientemente sensible para medir las diferencias genotípicas y fenotípicas en el establecimiento de la piel (madurez del periderma). Todos los genotipos tuvieron lecturas menores de establecimiento de la piel en el extremo apical del tubérculo en comparación con la región ecuatorial y el extremo basal. La técnica empleada con el probador de corte resolvió objetivamente las diferencias genotípicas en los periodos de desarrollo para el establecimiento de la piel y mostró claramente que el periderma de los tubérculos de Russet Burbank madura más rápidamente que el periderma de otros cultivares con tipos lisos de piel. Sin embargo, no se pudo establecer una relación consistente entre el peso del periderma y el establecimiento de la piel. En experimentos de poscosecha, diseñados para describir el efecto fenotípico de la temperatura y de la humedad relativa sobre la maduración del periderma del tubérculo, se encontró que una alta humedad relativa retarda el desarrollo del establecimiento de la piel. La capacidad para detectar las diferencias genotípicas y fenotípicas con esta técnica, para medir el establecimiento de la piel, indica que es factible desarrollar en el futuro un procedimiento estándar apropiado para probar diversos tipos de piel en los programas de mejoramiento y certificación de semilla al igual que para su utilización por los productores de papa para consumo directo, semilla y papas fritas a la inglesa.

Introduction

Skinning and bruising of potatoes during harvesting and handling operations result in costly market quality and disease problems affecting all sectors of the potato industry including fresh market, processing and seed. Potato tubers are often susceptible to skinning or scuffing because the biochemical process involved in bonding the native periderm (skin) to the underlying cell layers has not sufficiently advanced as part of the maturation process to provide protection against abrasion. This physiological bonding process, often referred to as "skin-set", occurs during the final stages of "periderm maturation" while the tubers are in the soil prior to harvest. The process of periderm maturation is initiated after growth ceases and the potato vines die in the field (8).

Genotypic differences in completion of skin-set, over a given period of time after vine death, have been subjectively observed by potato breeders and growers for many years. Genotypes which require prolonged times for adequate skin-set generally scuff badly at harvest and consequently sweat and sustain large moisture and weight losses during initial storage (1, 5, 6). Additionally, genotypes that scuff easily are prone to storage diseases and defects. Loss of moisture (shrinkage) from the tuber during storage, such as from skinning and bruising, exacerbates blackspot (4). Varietal differences in shrinkage from moisture loss were demonstrated during early storage, but not during the middle of the storage season (1); this is likely because some varieties were more prone to skinning and bruising than

others and consequently released more moisture vapor from the tubers until they were fully healed.

Although we know that the process of skin-set takes place during the final stages of periderm maturation, nothing is published or known about the biology or biochemistry of skin-set. Only recently have fluorescent histochemical probes been described for use in separately detecting deposition of the two components of ligno-suberin during periderm development (7).

Despite the financial losses and marketing problems created by inadequate skin-set and ensuing scuffing of tubers, only subjective tests to evaluate the progress of skin-set development had been available. However, recently J. L. Halderson developed a device to objectively measure tuber skin shear strength of Russet Burbank potatoes (2, 3). The Halderson skin shear strength testing device consists of a rubber tip mounted on the end of a spring loaded shaft which is connected to a torquometer. Using the torquometer, the rubber tip is pressed against the tuber skin with a prescribed amount of force which is controlled via the spring loaded mechanism. The rubber tipped shaft of the device is then turned, while simultaneously maintaining the pressure against the tuber skin, until the skin (periderm) is sheared from the adjoining cells. The reading obtained from the torquometer is then recorded. The torsional force required to shear the skin from adjoining cells is a mechanical means of quantitatively assessing the relative extent of biological bonding of skin to adjoining cells (i.e. skin-set) as maturation of tuber periderm advances. The device was designed to determine the effectiveness of various vine killing treatments and to determine when the periderm of Russet Burbank tubers was sufficiently mature, or "set", to withstand harvest without excessive skinning.

Our main objective in this research was to determine if the Halderson skin shear strength testing device was sufficiently sensitive to detect genotypic and phenotypic differences in skin-set. Subsequent research could then be conducted to develop a standardized procedure for use in breeding and certified seed programs having diverse genotypes and skin types.

Materials and Methods

Plant Materials and Treatments

The cultivars used in this research (Russet Burbank, Norchip, Kennebec and Redsen) were planted and tested in 1990 and 1991 in non-irrigated test plots at the Potato Research Farm in Grand Forks County, ND. The four cultivars were grown as references and tested along with 33 breeding selections in 1990 and 28 selections in 1991, data not included. Norland potatoes were used in place of Russet Burbank in postharvest experiments requiring earlier tuber development to determine phenotypic effects of temperature and relative humidity on skin-set progress of very immature periderm. Breeding selections were provided by Dr. Robert Johansen, North

Dakota State University, and Dr. Florian Lauer, University of Minnesota. For experiments requiring vine killing to initiate skin-set, Diquat was applied per manufacturers recommendation (473 ml diquat and 946 ml crop oil in 151 l water sprayed/acre).

The phenotypic effect of temperature and relative humidity on tuber skin-set development was determined using immature tubers which were removed from live vines four to five weeks before normal harvest. The immature tubers were placed in three separate, darkened controlled environment chambers equipped with temperature and humidity controls (18C and 50% RH, 18C and 95% RH, and 10C and 95% RH) where they were allowed to mature. This treatment of the immature tubers eliminated the environmental variations experienced in the field and provided for strict control of defined environmental conditions during periderm maturation. Tuber samples were periodically removed from the chambers and their skin-set measured.

Sampling

Tubers were obtained from the field 7, 14, 21 and 28 days after vine killing to construct a time course for skin-set development. Immediately after hand digging, the tubers were placed in paper bags to avoid skin desiccation prior to skin-set measurements, which quickly followed digging. In 1990, the field sampling plan consisted of hand digging tubers from two or more plants and randomly selecting three tubers. Skin-set measurements were obtained in triplicate from the equatorial region of each of the three tubers. A similar sampling plan was followed in 1991, except 6 tubers were selected and two measurements were taken from the equatorial region of each tuber.

Separate samples were collected to determine the variation in skin-set for different locations on the tuber surface. Three tubers from each of the three plants were collected for each cultivar seven days after vine killing the 1990 crop. Three skin-set measurements were obtained at each location (stem end, equatorial region, and bud end) on the surface of each tuber.

The phenotypic effect of temperature and relative humidity on the time course for skin-set development was determined by sampling tubers maturing in each of the three controlled environment chambers (see Plant Materials and Treatment section). Three tubers of each cultivar were removed from each controlled environment chamber for each point of the time course and triplicate skin-set measurements were obtained from the equatorial region of each tuber.

Skin-Set Measuring Procedure

The skin-set testing device and operational procedure were initially developed by J.L. Halderson and R.C. Henning (2, 3). We attached the Halderson skin-set testing device to a Snap-on "Torqometer" (registered

trade mark name), model TQSO50FUA (0-96 oz in/0-678 mNm range). The torqometer was equipped with a "main pointer", which provided real-time readings, and a "follow-up pointer", which indicated the maximum torque reading reached in shearing the skin during a skin-set measurement (Figure 1). The maximum torque reading was recorded as the measured skin-set value.

A small steel flywheel (0.9 cm thick X 2.0 cm diameter) was fabricated and fitted to the shaft of the skin-set tester to provide sufficient inertia to prevent the torquometer's main pointer from recoiling past zero as the skin abruptly sheared; this was necessary to maintain calibration of the main pointer at zero at the start and end of each test. For the 1990 crop, the skin-set testing device was fitted with a 1.3 cm^2 rubber tip (size 0 test tube stopper) to grip the native periderm while it was being torsionally delaminated from the cortical tissue beneath it. The rubber tip of the device was applied to the tuber with a spring setting of 31 N as a standard contact force perpendicular to the tuber surface. For the 1991 crop, a 1.5 cm^2 rubber tip (size 1 test tube stopper) and 53 N spring setting was used. Results from the 1990 and 1991 crop were analyzed separately because of the above differences in testing parameters.

Periderm Weights

Skin disks from the various genotypes were obtained by excising the native periderm from the equatorial region of the tubers with a number 13 cork borer (2.1 cm diameter) and then using forceps to carefully pull the periderm away from the cortical tissue beneath it. The skin disks were then dried overnight at 90C, cooled in a desiccator and the dry weight determined. All periderm weights were the average of triplicate determinations from each of three or more tubers.

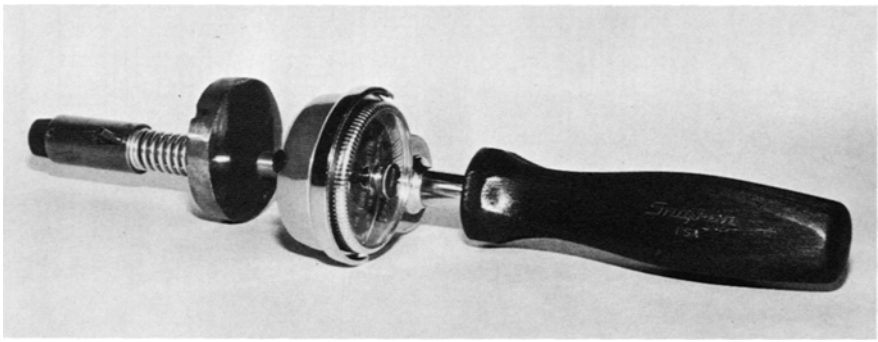


FIG. 1. The Halderson periderm shear tester, fitted with a flywheel to maintain calibration, and attached to a torquometer equipped with a follow-up pointer.

Results and Discussion

Skin-set measurements obtained from immature tubers (1990 crop, 1.3 cm^2 periderm sampled per measurement) seven days after vine killing revealed that the bud end of the tuber had lower skin-set readings than either the stem end or equatorial region (Fig. 2). As shown in Figure 2, these differences can be relatively large. We found that this differential in skin-set across the tuber surface existed for all varieties and breeding selections (data not shown) tested. Variations in scuffing, sometimes observed during harvest, might be partially explained by these differentials in skin-set across the tuber surface. The existence of such differentials must be considered when developing a final standardized technique to measure skin-set among genotypes.

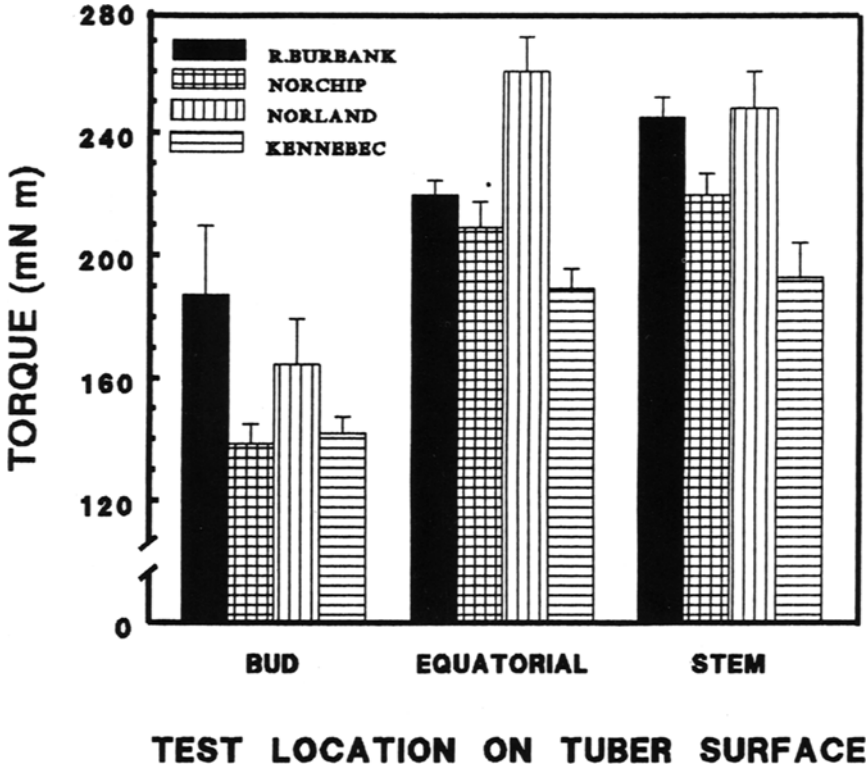


FIG. 2. Variations in skin-set/periderm maturity readings (torque in mN m) for different locations on the tuber surface. Note that the reading for the bud area of the tuber is lowest for all cultivars. These data were obtained from the 1990 crop, seven days after vine killing. Bars indicate ± SE of the mean.

Results from the 1990 crop and 1991 crop clearly demonstrated that the Halderson device, using the technique employed here, was sufficiently sensitive to detect genotypic differences in skin-set as periderm matured after vine killing. Skin-set measurements on the cultivars used in these experiments reflected the well-organized rapid development of skin-set of Russet Burbank tubers and the undesirably slow development of skin-set of Redsen and Kennebec tubers after vine killing (Fig. 3). The technique is clearly capable of detecting differences in skin-set among various genotypes at two, three and four weeks after vine killing. These time intervals are generally regarded as the most important for obtaining proper skin-set for harvesting.

Although tubers having russet type periderm are recognized for their "thicker" and more durable skin in harvesting/handling operations, the rela-

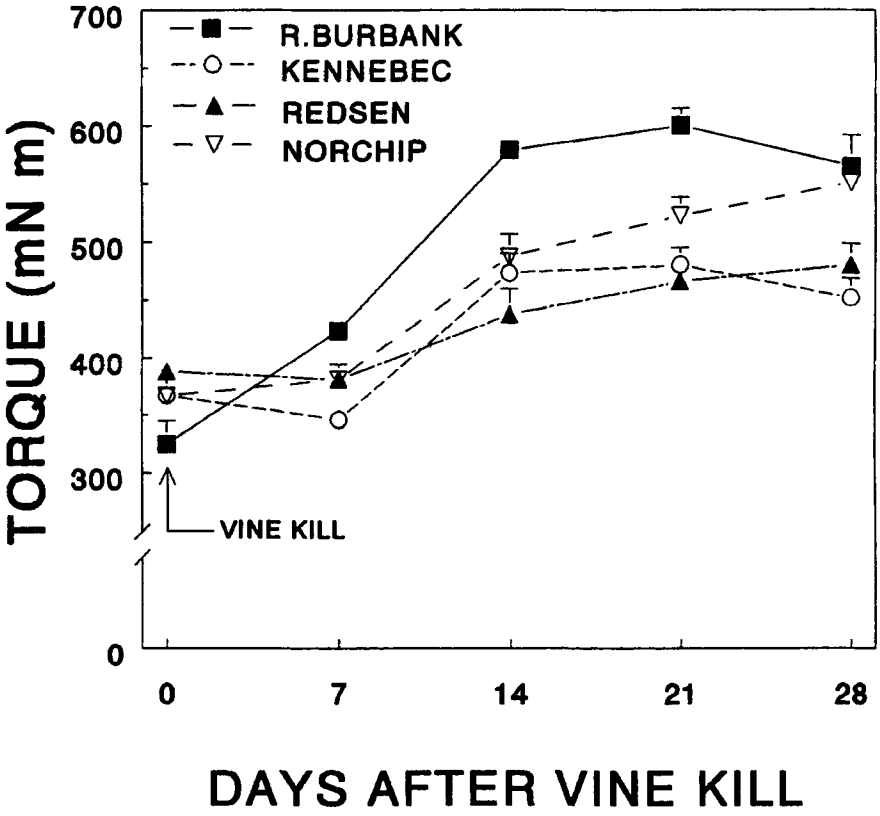


FIG. 3. Changes in skin-set after vine killing. Note that genotypic differences in the time courses for skin-set development are easily detected. These data were obtained from the 1991 crop. Bars indicate ± SE of the mean.

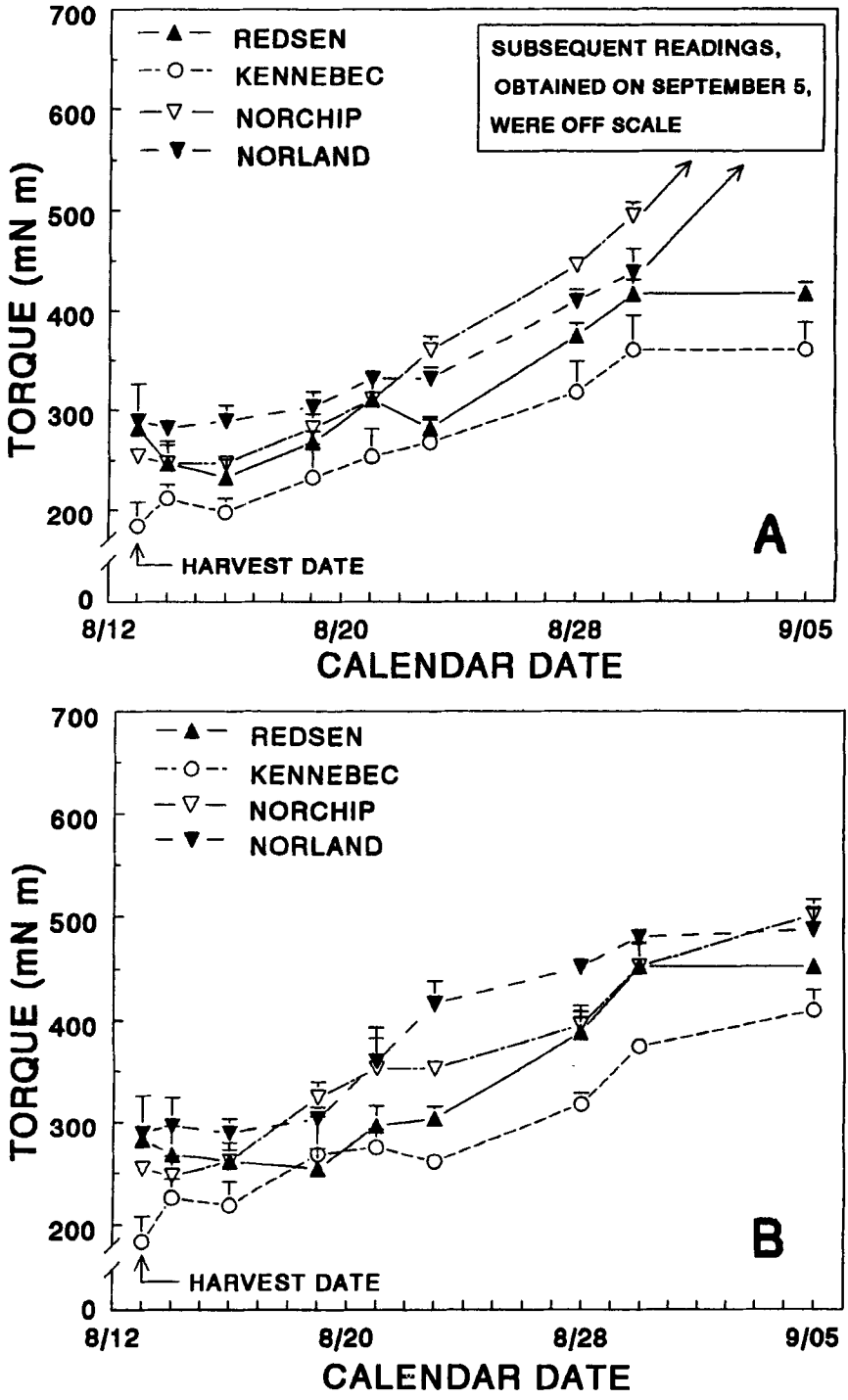
tionship between periderm weight and skin-set was not totally consistent (table 1). Although Redsen tubers had a periderm weight similar to Norchip and higher than Kennebec tubers, Redsen tubers had the lowest skin-set reading (table 1). Similar variations in skin-set readings *vs* periderm weight also existed for the breeding selections (data not shown). These results indicate that, although periderm weight may be important to tuber durability against bruise damage in mature potatoes which have fully set skin, periderm weight is not a totally reliable diagnostic tool to predict skin-set.

The ability to detect the effect of factors phenotypically influencing skin-set is of key importance for research directed at reducing skinning at harvest. The sensitivity of the technique for measuring skin-set easily provided for detection and establishment of environmental effects that may influence the development of skin-set of various genotypes in the field. We found that environmental conditions surrounding the tuber played a significant role in the development of skin-set (Figs. 4A, B and C). The tubers tested, to obtain the data in Figures 4A, B and C (1991 crop), were very immature when dug and consequently required significant maturation to achieve skin-set; this immaturity allowed us to obtain a time course for skin-set development with a wide range of measurements. When immature tubers of the four cultivars stored at 18C and 50% RH (Fig. 4A) were tested, all genotypes increased in skin-set, but with detectable differences. Norland and Norchip tubers, which are regarded as having good resistance to skinning and damage during harvesting operations, increased their skin-set, under these environmental conditions, to the extent that the skin-set readings were off-scale 24 days after digging. However, the skin-set readings of Redsen and Kennebec tubers plateaued at a lower level during the latter period of the time course. This lower plateauing of skin-set readings is consistent with the characteristic scuffing problems typical for these two cultivars.

High humidity retarded the later stages of skin-set development (Fig. 4B) for Norchip and Norland tubers. At 18C and 95% RH, the skin-set measurements of all four cultivars plateaued near the end of the time course. By holding the temperature constant at 18C and elevating the relative

TABLE 1.—*Periderm weights of cultivars and the corresponding skin-set readings three weeks after vine killing.*

Cultivar	Periderm Dry Weight (mg/sq cm periderm)		Skin-Set Readings (torque mN m)	
	$\bar{x}$	se	$\bar{x}$	se
Russet Burbank	3.93	(0.35)	600.2	(2.1)
Redsen	2.54	(0.23)	466.1	(1.2)
Norchip	2.43	(0.49)	522.6	(2.2)
Kennebec	1.91	(0.09)	480.2	(2.1)


REDSEN
KENNEBEC
NORCHIP
NORLAND
SUBSEQUENT READINGS, OBTAINED ON SEPTEMBER 5, WERE OFF SCALE
TORQUE (mN m)
700
600
500
400
300
200
0
HARVEST DATE
A
8/12
8/20
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CALENDAR DATE
REDSEN
KENNEBEC
NORCHIP
NORLAND
TORQUE (mN m)
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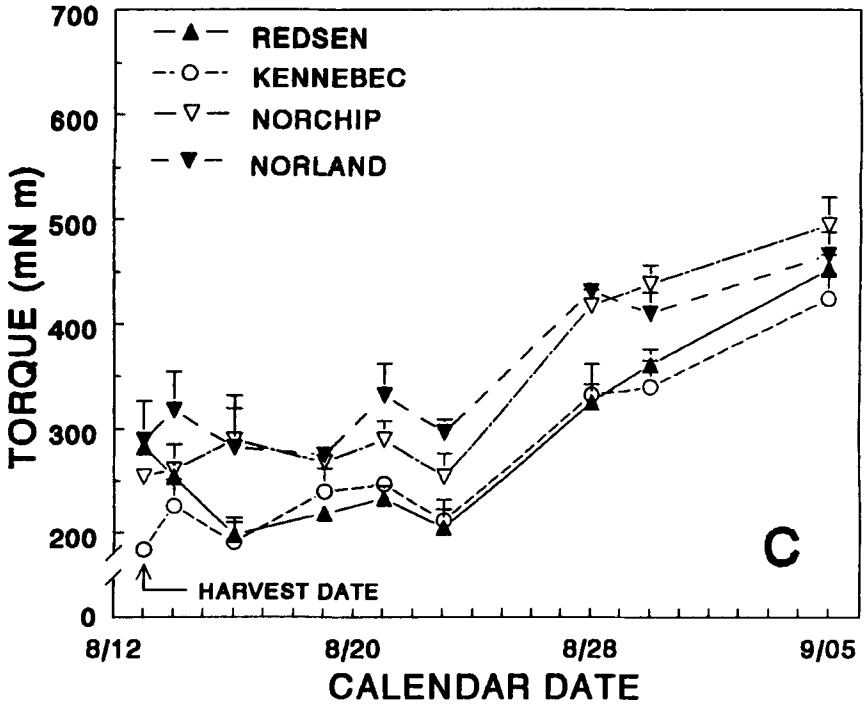

FIG. 4. The effect of temperature and relative humidity on the advancement of skin-set (Fig. 4A, 18C and 50% RH; Fig. 4B, 18C and 95% RH; and Fig. 4C, 10C and 95% RH). Note that lower relative humidity allows skin-set to progress (Fig. 4A) while higher relative humidity retards skin-set. These data were obtained from the 1991 crop. Bars indicate ± SE of the mean.

humidity from 50 to 95%, the desired phenotypic increase in skin-set, demonstrated earlier by Norchip and Norland cultivars, was lost. A similar time course was obtained when the temperature was reduced to 10C while the relative humidity was, again, held at 95% (Fig. 4C). Redsen and Kennebec, cultivars with known scuffing problems, had skin-set readings below those of Norchip and Norland during the last 21 days of the time course. Norchip and Norland tubers demonstrated good progress in developing skin-set at 18C and 50% RH, but remained repressed in their development of skin-set when 95% RH was maintained at both 10C and 18C. These results suggest that moisture conditions can be an important factor affecting development of skin-set. For these cultivars and environmental conditions, it took approximately one week after the tubers were removed from the live plants in the field and were placed in controlled environment chambers before appreciable progress in the development of skin-set was detected. The trends observed in these postharvest experiments (Fig. 4A, B and C)

may be similar to those occuring with very immature tubers in the soil after vine killing as they respond to prevailing temperature and moisture conditions and undergo skin-set. However, the many variations in cultural practices and growing conditions that exist across the U.S. likely elicit variations in the effect of temperature and moisture on skin-set development in the field.

The results of our experiments indicate that it is feasible to develop a refined, standardized procedure for the Halderson periderm shear tester to measure genotypic and phenotypic differences in skin-set development. A standardized procedure would be useful to researchers in variety development and certified seed programs, as well as to growers of commercial varieties. The procedure may also be useful in objectively identifying genotypes as resources for biological research employing molecular techniques to hasten skin-set in all varieties. The original testing guidelines were developed for the thicker, russeted skin of the Russet Burbank cultivar. Therefore, the appropriateness of several test parameters that may affect the ability to obtain skin-set measurements on other skin types must be considered when developing a standardized procedure for use on all genotypes. Such test parameters might include the type and size of rubber tip used to grip and shear the periderm, amount of pressure applied to the test surface, number and location of tests on the tuber surface and the number of tubers and plants sampled.

Acknowledgments

We thank Roderick Wiles and Scott Goecke for their technical assistance and Dr. J.L. Halderson for his cooperation and advice in using the skin-set testing device.

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