Sugar Beet Cultivar Evaluation for Storability and Rhizomania Resistance

Carl A. Strausbaugh and **Imad Eujayl,** USDA-ARS NWISRL, 3793 North 3600 East, Kimberly, ID 83341; **Eugene Rearick,** Amalgamated Research, Inc., Twin Falls, ID 83301; and **Paul Foote** and **Dave Elison,** Amalgamated Sugar Co., 50 S. 500 W., Paul, ID 83347

ABSTRACT

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To reduce storage losses and improve resistance to rhizomania caused by *Beet necrotic yellow vein virus* (BNYVV), studies were initiated to establish a storage cultivar selection program. In 2006 and 2007, 30 or more commercial sugar beet (*Beta vulgaris*) cultivars were grown in soil naturally infested with BNYVV. At harvest, two root samples from each plot were collected and used to establish percent sugar. Additional samples were placed on top of an indoor pile (set point 1.7°C) and inside an outdoor pile in a randomized complete block design with four replications. After 142 and 159 days in indoor storage, sucrose reduction ranged from 13 to 90% in 2007 and 57 to 100% in 2008. Outdoor storage sucrose reduction ranged from 13 to 32% in 2007 and 28 to 60% in 2008. An average of 31 and 45% of the root surface was covered with fungal growth in 2007 and 2008, respectively. Cultivars that retained the most sucrose had resistance to BNYVV and the least fungal growth and weight loss. Indoor storage with BNYVV-infested roots allowed for the most consistent cultivar separation and will potentially lead to selection of cultivars for improved storability and rhizomania resistance.

Controlling the loss of sucrose during sugar beet storage has been an industry goal since the 1950s (11). In Idaho, sugar beet (*Beta vulgaris* L.) roots may be stored up to 160 days, allowing weather (primarily temperature and moisture) and microbes to negatively influence the sucrose stored in the roots, along with normal respiration and the buildup of impurities (3,5,6,9,34). Other factors can also influence sucrose loss such as scalping, impacts, and wounding during harvest and transport, mud and weeds in piles, and unusually high or low temperatures (2,6,10,13,16,19,33). Disease and drought stress during crop production may also predispose the roots to sucrose loss in storage (8,14,15,27,28). Rhizomania (8,28), curly top (27), Rhizoctonia root rot (15), Aphanomyces root rot (7), and Cercospora leaf spot (24) during crop production have all been shown to lead to increases in sucrose loss in storage.

Rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV), has been shown to be particularly damaging in storage; sucrose losses up to 94% have been documented (28). Since BNYVV was first discovered in the United States in 1984, the virus has spread through the major U.S. production areas (21,22). Currently, disease control for rhizomania largely relies on a single dominant gene, *Rz1* (21,22). However, resistance breaking isolates that can overcome *Rz1* have been documented in some commercial fields (1,17). Therefore, improving disease control for rhizomania and reducing storage losses will be important for maintaining factory efficiency and profitability. Selecting for resistance to BNYVV in cultivars is currently conducted through disease ratings and yield data collected from roots grown in field plots. This field approach is successful at identifying major gene resistance, but accurately selecting for the effects of minor genes is problematic. Cultivar selection for storability, although tried by some groups over recent decades, has also proven to be a challenge. In order to establish a cultivar selection approach for storability and improve on the selection for rhizomania resistance, investigations with rhizomania-infested sugar beet roots produced and stored under commercial conditions were conducted in indoor and outdoor piles.

Corresponding author: Carl A. Strausbaugh
E-mail: Carl.Strausbaugh@ars.usda.gov

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MATERIALS AND METHODS

Treatments. Thirty-two commercial sugar beet cultivars were evaluated in 2006 to establish a screen for storability. The study was repeated in 2007 with 30 commercial sugar beet cultivars. Twenty of the cultivars were the same in both studies; whereas the others varied because of availability. All the cultivar names were coded (B = Betaseed Inc., C = ACH Seed Inc., HH = Holly Hybrids, HM = Hilleshog, and SX = Seedex); respective companies can be contacted using the code to gain additional information on the cultivars. Cultivars contained at least the *Rz1* gene for resistance to BNYVV except for the susceptible check, HM070005, and five cultivars (B-16, HM070011, HM070021, SX001, and SX004) evaluated in 2006. Rhizomania was uniform and evident throughout the naturally infested commercial fields in both years based on foliar and root symptoms and enzyme-linked immunosorbent assay (ELISA) (data not presented). Other diseases were not evident in the fields, and the roots were free of visible root rot at harvest. Plots were arranged in a randomized complete block design with four replications as four-row plots 10.4 and 7.3 m long in 2006 and 2007, respectively, with rows 0.6 m apart. The plots were planted to a density of 352,123 seeds/ha and thinned to 117,374 plants/ha. The fields were managed using standard commercial cultural practices. At harvest, three 8-beet samples were collected in nylon mesh onion bags from each plot. For six cultivars, an additional 8-beet sample was pulled at the same time for testing under ambient conditions outdoors. All storage samples (including the outdoor samples) were held inside the commercial storage building set to hold 1.7°C (building cooled with ambient air) until the indoor and outdoor commercial piles were established. The indoor samples were then placed on top of a 9.1-m-high commercial indoor pile in the center of the building while maintaining the samples in the same block design used in the field. The outdoor samples were placed inside a commercial outdoor sugar beet pile inside a metal corrugated ventilation pipe (0.9 m diameter) on top of plywood in the same experimental block design used in the field. The samples inside the pipe were 10 to 14 m from the edge of the pile. The open end of the pipe was covered with straw bales. The pipe was located on top of a 30-cm layer of beet and was covered by roots to a height of 6.1 m. The pile was ventilated using the same perforated pipe placed 3.7 m on center. The storage pipe with the samples was placed in between ventilation pipes. The roots surrounding the pipe were from commercial cultivars and healthy in appearance (no visible rhizomania or rot symptoms). Temperature was recorded at 1-h intervals on a Hobo temperature sensor (Model H08-001-02; Onset Computer Corp., Bourne, MA) located in with the samples.

2006 Samples. The trial was located in a commercial field 11.3 km north of Rupert, ID. The field had been in spring barley in 2005 and was planted to sugar beet on 10 April 2006. The plants were mechanically topped (leaving a 3-cm-diameter scar), and the center two rows were harvested on 6 October 2006 with a commercial harvester. The samples were placed inside the Twin Falls outdoor ventilated pile on 19 October. The indoor samples were placed on top of the pile on 20 October. On 26 February 2007, roots were retrieved after 142 days in storage.

2007 Samples. The trial was located in a commercial sprinkler-irrigated sugar beet field 4.8 km southeast of Rupert, ID where winter wheat was grown in 2006. The roots were mechanically topped (leaving a 3-cm-diameter scar), and the center two rows were collected with a commercial harvester on 27 September 2007. The samples were placed inside the Twin Falls outdoor ventilated pile on 17 October. The indoor samples were placed on top of the pile on 26 October. On 4 March 2008, roots were retrieved after 159 days in storage.

Rhizomania, fungal growth, and rot ratings. On 1 February each year, the indoor roots were visually evaluated while still lying on the storage pile for the percentage of surface area covered by primarily basidiomycete fungal growth (29). The outdoor samples were not rated for fungal growth because the fungal growth could not be evaluated without disturbing the samples inside the pipe. After samples were retrieved from the storage piles at the end of the storage period, the roots were evaluated for rhizomania symptoms using a 0 to 9 disease index where: 0 = no symptoms; 1 = root growth normal, minor bearding, and no discoloration; 2 = taproot slightly constricted and bearded; 3 = taproot moderately constricted, bearded, and discolored with very little adhering soil; 4 = similar to 3 except more adhering soil; 5 = taproot wine-glass shaped, discolored, and brittle and feeder roots bearded with soil adhering; 6 = damage to taproot severe with heavy bearding just below the crown; 7 = taproot destroyed and severe bearding below the crown with root area a ball of soil; 8 = similar to 7 except root necrotic into the crown area; and 9 = root dead. The index was similar to one published previously (32) and was utilized in a continuous manner (all numbers between 0 and 9 possible) rather than categorically. At the same time, surface rot was also visually evaluated as the percentage of root area associated with discolored tissue such as dry black rot and/or wet bacterial rot.

Weight analysis. Prior to placing the storage samples in the pile, each sample was weighed. The samples were reweighed when retrieved from the storage pile. These weights were used to determine reduction in root weight.

Sugar analysis. Two samples collected from each plot at harvest were submitted to the Amalgamated Tare Lab in Paul, ID. Percent sugar was determined using an Autopol 880 polarimeter (Rudolph Research Analytical, Hackettstown, NJ) and a half-normal weight sample dilution and aluminum sulfate clarification method (ICUMSA Method GS6-3 1994) (4). Conductivity was measured using a Foxboro conductivity meter Model 871EC (Foxboro, Foxboro, MA), and nitrate was measured using a multimeter Model 250 (Denver Instruments, Denver, CO) with Orion probes 900200 and 9300 BNWP (Krackler Scientific, Inc., Albany, NY).

Percent sugar for samples coming out of storage was determined by Amalgamated Research Inc., Twin Falls, ID using gas chromatography, because polarimeter readings can be affected by impurities that accumulate during storage. The gas chromatographic method was similar to ICUMSA Method GS4/7/8/5-2 (2002) with the following modifications: the internal standard used was D(-)-salicin[2-(hydroxymethyl)phenyl-β-D-glucopyranoside, and equal volumes (to ±0.01 ml) of a solution of internal standard in dimethylformamide were dispensed into weighed samples and standards using a volumetric dispenser (4). The gas chromatography analysis averaged 1.395% higher than the polarimeter reading on samples evaluated in previous work (28). To establish percent reduction in sugar at harvest versus storage, only samples from within the same plot were compared. Percent sugar reduction was established using the following equation: % reduction in pounds of sucrose = (1 − {[(% $\text{sucrose}_{\text{storage sample}}$ − 1.395) ×

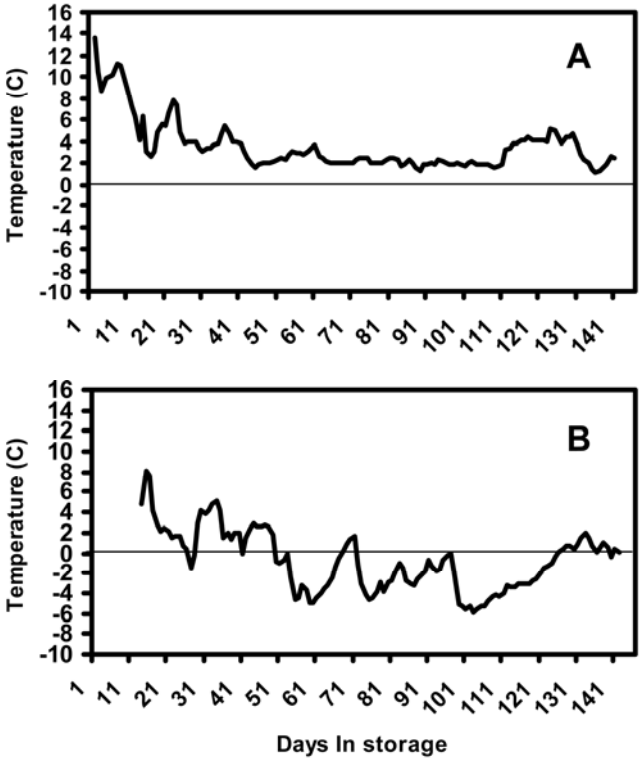

Fig. 1. Average daily temperature (°C) during storage in commercial sugar beet piles from 6 October 2006 to 26 February 2007 in **A,** an indoor storage facility in Paul, ID, and **B,** an outdoor pile in Twin Falls, ID.

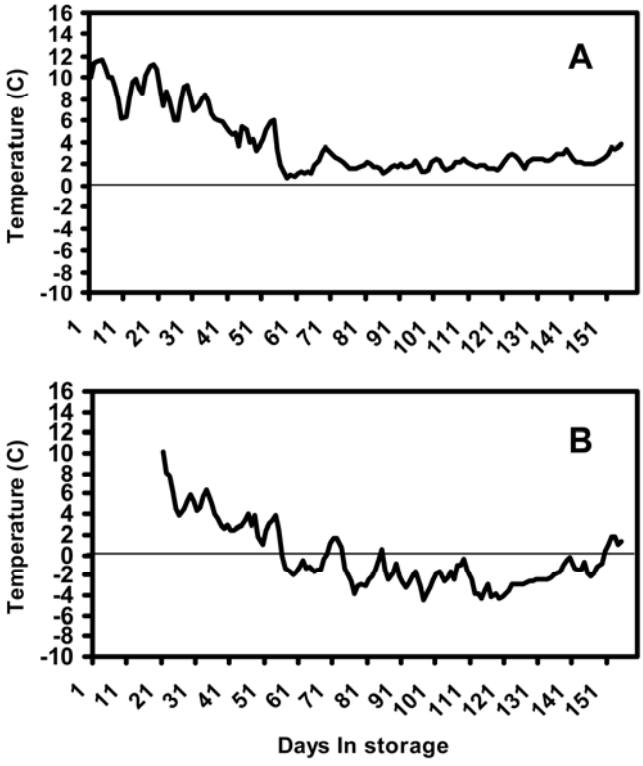

Fig. 2. Average daily temperature (°C) during storage in commercial sugar beet piles from 27 September 2007 to 4 March 2008 in **A,** an indoor storage facility in Paul, ID, and **B,** an outdoor pile in Twin Falls, ID.

$weight_{storage\ sample}$]/(% $sucrose_{harvest\ sample}$ × $weight_{harvest\ sample}$)}) × 100. Estimated recoverable sucrose = extraction × 0.01 × gross sucrose and extraction = 250 + [1255.2 × (conductivity – 15000) × (% sucrose – 6185)]/{% sucrose × [98.66 – (7.845 × conductivity)]}.

Basidiomycete pathogenicity test. The pathogenicity test used roots of the cultivar B-16 that were produced on the USDA South Research Farm in Kimberly, ID using the Poncho Beta seed treatment (60 g a.i. clothianidin + 8 g a.i. beta-cyfluthrin/100,000 seed) and standard cultural practices (26). The plants had a very low curly top rating of 1 at the end of the growing season using a disease index (26) of 0 to 9, where 0 = healthy and 9 = dead. No other disease or pest problems were evident on these plants. The plants were mechanically topped and harvested on 9 October 2007, and the roots were placed in a cold storage room at 3°C and 90% relative humidity. All roots used in the pathogenicity test came from the same plot. The pathogenicity test consisted of 7 treatments: 6 basidiomycete isolates (F566, F568, F570, F574, F580, and F583) and an uninoculated check. Each sugar beet root served as an experimental unit. The roots were arranged in a randomized complete block design with five replications. Hyphal tipped cultures grown on Difco potato dextrose agar (PDA; Becton Dickinson & Co., Sparks, MD) for 2 weeks at 21°C served as the source of inoculum. A 5-mm-diameter plug 12 mm in length was pulled from the shoulder of the root with a cork borer. Then, a 4-mm plug from the inoculum plate was placed into the hole and the plug reinserted. The root was placed on top of the indoor commercial sugar beet pile in Paul, ID for 53 days. The root was then split in half through the plugged area and the distance that discoloration extended from the plug was measured. A 10-mm cubed piece of root from the transition zone (discolored to healthy appearing tissue) was cut from near the plug. The cube was disinfested in 0.6% sodium hypochlorite (NaOCl) for 60 s and then rinsed in sterilized reverse osmosis water for 60 s. The surface area of each cubed piece was then removed, and a 2 × 2 mm piece was placed on PDA amended with streptomycin sulfate at 200 mg/liter and incubated at 21°C. Fungal isolates were identified using a light microscope. The experiment was repeated once.

Data analysis. Data were analyzed in SAS (23) using the general linear models procedure (Proc GLM), and Fisher's protected least significant difference was used for mean comparisons. Correlations based on Spearman's coefficient of rank correlation and linear regression analyses (Proc Reg) were conducted in SAS (23).

Table 1. Disease and weight reduction data for 32 commercial sugar beet cultivars harvested in 2006 from a commercial field naturally infested with rhizomania in Paul, ID

Cultivar[u]	Rhizomania rating[v]	Fungal growth (%)[w]	Root rot (%)[x]	Weight reduction (%)[y]
HH001	1.0 k	38 c-h	36 a-f	5.2 f-k
B-23	1.2 jk	14 h-k	8 g	4.9 h-k
B-27	1.2 jk	29 e-k	30 a-g	6.1 e-k
HH019	1.4 i-k	12 jk	13 d-g	5.2 f-k
B-26	1.5 i-k	22 g-k	19 c-g	4.2 jk
HH003	1.5 i-k	12 i-k	15 c-g	3.1 k
HH004	1.5 i-k	35 c-j	19 c-g	5.1 g-k
B-31	1.6 i-k	9 k	18 c-g	4.8 h-k
B-4	1.6 i-k	24 f-k	27 b-g	6.3 d-k
HH005	1.6 i-k	36 c-i	20 c-g	11.9 ab
HM070007	1.7 i-k	8 k	14 c-g	4.5 i-k
HM070012	1.9 h-k	28 e-k	12 d-g	5.7 e-k
HM070015	2.0 h-k	30 d-k	38 a-e	6.8 c-k
B-28	2.1 h-k	16 h-k	10 e-g	5.0 g-k
HM070004	2.1 h-k	19 h-k	38 a-e	4.7 i-k
C-21	2.2 h-k	29 e-k	30 a-g	4.5 i-k
HM070001	2.2 h-j	28 e-k	41 a-c	7.8 c-j
C-2	2.4 g-j	17 h-k	9 fg	4.6 i-k
HM070018	2.4 g-j	23 g-k	18 c-g	5.8 e-k
C-17	2.5 g-i	22 g-k	27 b-g	5.8 e-k
SX002	2.5 g-i	24 f-k	30 a-g	5.8 e-k
SX005	2.6 f-i	18 h-k	6 g	13.8 a
HH002	3.0 e-h	18 h-k	11 d-g	5.9 e-k
HM070014	3.5 d-g	36 c-i	36 a-f	5.8 e-k
B-30	3.8 c-f	54 b-d	58 a	8.9 b-f
SX006	3.9 c-e	58 a-c	22 b-g	9.3 b-e
SX004	4.4 b-d	48 b-f	29 b-g	10.4 a-c
HM070011	4.8 a-c	49 b-e	29 b-g	9.3 b-e
HM070005	4.8 a-c	46 b-g	39 a-d	8.7 b-g
B-16	5.0 a-c	81 a	50 ab	10.0 a-d
SX001	5.2 ab	49 b-e	28 b-g	8.0 c-i
HM070021	5.8 a	62 ab	28 b-g	8.5 b-h
Overall mean	2.7	31	25	7
$P > F$[z]	<0.0001	<0.0001	0.0504	<0.0001
LSD ($P \leq 0.05$)	1.2	24	28	3.8

[u] All cultivar names were coded (B = Betaseed Inc., C = ACH Seed Inc., HH = Holly Hybrids, HM = Hilleshog, and SX = Seedex), but the respective companies can be contacted using the code to gain additional information on the cultivars.

[v] Rhizomania ratings were conducted using a disease index of 0 to 9 (0 = no symptoms, 9 = root dead). Cultivars contained at least the *Rz1* gene for resistance to *Beet necrotic yellow vein virus* except for the susceptible check, HM070005, and cultivars B-16, HM070011, HM070021, SX001, and SX004.

[w] Fungal growth = percentage of root surface area covered by fungal growth on 1 February 2007. Fungal growth was primarily an undescribed basidiomycete.

[x] Root rot = percentage of root surface area covered by discolored tissue on 26 February 2007.

[y] Weight reduction = percent reduction in root weight after storage when compared to that determined at harvest.

[z] $P > F$ was the probability associated with the F value. Means followed by the same letter did not differ significantly based on Fisher's protected least significant difference (LSD) value with $P \leq 0.05$.

RESULTS

Temperature. During the 2006–2007 indoor storage season, temperatures in the building hit set point of 1.7°C on 19 November 2006 (43 days in storage) and maintained temperature until 26 January 2007 (112 days in storage) when temperatures began to rise (Fig. 1A). During the 2006–2007 outdoor storage season, temperatures in the pipe dropped below 0°C on 26 November 2006 (50 days in storage) and stayed below zero for 71 of the next 76 days (Fig. 1B). The lowest average daily temperature during this period was –5.9°C. During the 2007–2008 indoor storage season, temperatures in the building hit set point on 22 November 2007 (56 days in storage) and maintained temperature until almost the beginning of March 2008 (Fig. 2A). During the 2007–2008 outdoor storage season, temperatures in the pipe dropped below 0°C on 21 November 2007 (55 days in storage) and stayed below zero until 23 February 2008 (149 days in storage) except for 5 days (Fig. 2B). The lowest average daily temperature during this period was –4.4°C.

Rhizomania ratings. Rhizomania was uniform and significant ($P < 0.0001$) both years (Tables 1 and 2). The mean rating for the susceptible check cultivar, HM070005, was 4.8 in 2006 and 4.5 in 2007. In 2006, the five cultivars (B-16, HM070011, HM070021, SX001, and SX004) lacking the *Rz1* gene all had a rhizomania rating similar to the susceptible check.

Fungal growth. The white cottony fungal growth of a basidiomycete (29) was evident in mid-December both years. By the beginning of February, fungal growth was considerable in both years (Tables 1 and 2). The majority (>95%) of the fungal growth was the basidiomycete (29), but

Penicillium spp. and *Botrytis* spp. were also present on some roots (data not shown). An average of 31 and 45% of the root surface was covered with fungal growth in 2006 and 2007, respectively. When using Spearman's rank correlation coefficient, fungal growth (r = 0.5608, P = 0.0101) correlated when comparing the same 20 cultivars included in both studies. Based on regression analysis, a significant positive relationship existed between the rhizomania rating and fungal growth in 2006 (r^2 = 0.42), but there was no relationship in 2007 (r^2 = 0.00) (Table 3).

Root rot. Root surface rot (discolored tissue) was considerable by the end of the storage season in both years (Tables 1 and 2). In 2006 the rot averaged 25%, but in 2007 it more than doubled to 57%. In 2006 and 2007, surface rot was related (r^2 = 0.26 and r^2 = 0.18, respectively) to fungal growth on the surface (Table 3). In 2006, there was no difference (P = 0.8591) when comparing the six check cultivars indoors (31%) versus outdoors (30%) for root rot. The 2006 outdoor percent rot means for HH004, HH001, HM070005, B-31, B-26, and HM070014 were 40, 34, 30, 27, 26, and 25, respectively; these means were not significantly different (P = 0.5616). Indoors means for the same cultivars were significantly different (Table 1).

In 2007, there was a significant difference (P = 0.0012) when comparing the six check cultivars indoors (64%) versus outdoors (50%) for root rot. The 2007 outdoors percent rot means for HH001, HM070005, HH004, B-26, HM070014, and B-31 were 69, 66, 62, 44, 36, and 24, respectively; some means were significantly different (P = 0.0074, LSD = 25). Indoors, some means were also significantly different (Table 2). When using Spearman's rank correlation coefficient, root surface rot was not correlated (r = 0.3918, P = 0.0876) when comparing the same 20 cultivars included in both studies.

Weight reduction. In 2006, root weight was reduced by an average of 7%; whereas in 2007, there was a 19% reduction (Tables 1 and 2). Indoors, there were significant differences in weight reduction between cultivars as well. In 2006, when comparing the six check cultivars indoors (6%) versus outdoors (17%) for weight reduction, there was a significant difference (P = 0.0002). The 2006 outdoors percent weight reduction means for HM070005, HH001, HH004, HM070014, B-31, and B-26 were 22, 18, 17, 16, 15, and 15, respectively; some means were significantly different (P = 0.0219, LSD = 4). One mean for these cultivars was significantly different indoors (Table 1). In 2007, when comparing the six check cultivars indoors (20%) versus outdoors (13%) for weight reduction, there was a significant difference (P = 0.0134). The 2007 outdoors percent weight reduction means for B-31, HH001, HM070005, HM070014, HH004, and B-26 were 16, 15, 15, 13, 11, and 10, respectively; these means were not significantly different (P = 0.1960). Indoors, one mean for these same cultivars was significantly different (Table 2).

Root and sucrose yield. The root yields and estimated recoverable sucrose at harvest were typical for the rhizomania resistant cultivars both years (Tables 4 and 5). Yields for the susceptible check cultivar, HM070005, were considerably reduced as expected. Cultivars were significantly different for these yield data both study years.

Sucrose content and reduction. Sucrose content was significantly different both years among cultivars (Tables 4 and 5). In 2006, when comparing the means for reduction in sucrose with the six check cultivars indoors (38%) versus outdoors (21%), there was a significant difference (P = 0.0085). The 2006 outdoors percent reduction in sucrose means for HM070005, HH004, HH001, B-31, B-26, and HM070014 were 32, 26, 20, 19, 14, and 13, respectively; these means were not significantly different (P = 0.0906). Indoors, some means for these cultivars were significantly different (Table 4). In 2007, when comparing the means for reduction in sucrose with the check cultivars indoors (88%) versus outdoors (45%), there was a significant difference (P = 0.0016). The 2007 outdoor percent reduction in sucrose means for HM070005, HH004, HH001, HM070014, B-31, and B-26 were 60, 58, 54, 40, 31, and 28, respectively; these means were not significantly different (P = 0.0885). Indoors, means for some of the same cultivars were significantly different (Table 4). When comparing the same 20 cultivars included in both studies using Spearman's rank correlation coefficient, sugar reduction results (r = 0.5535, P = 0.0114) correlated.

Table 2. Disease and weight reduction data for 30 commercial sugar beet cultivars harvested in 2007 from a commercial field naturally infested with rhizomania in Rupert, ID

Cultivar[u]	Rhizomania rating[v]	Fungal growth (%)[w]	Root rot (%)[x]	Weight reduction (%)[y]
HH003	1.0 e	64 a-d	36 j-m	16.4 f-i
HH006	1.1 de	19 f-i	21 m	16.6 f-i
HM070007	1.2 de	50 a-g	56 c-j	15.9 hi
HH008	1.2 de	75 ab	49 g-l	15.8 hi
C-23	1.3 de	25 e-i	56 c-j	17.2 e-i
SX005	1.4 de	19 f-i	48 g-l	19.2 b-h
HH011	1.4 de	69 a-c	68 b-g	19.2 b-i
B-30	1.5 de	60 a-d	52 e-k	21.2 a-e
B-26	1.5 de	38 c-i	31 k-m	18.1 c-i
HH004	1.5 de	68 a-d	79 a-c	21.4 a-d
C-21	1.5 de	50 a-g	71 a-g	20.5 a-f
HH001	1.5 de	82 a	78 a-d	17.6 c-i
B-18	1.5 de	35 d-i	63 b-i	18.2 b-i
C-3	1.6 c-e	39 c-i	59 c-j	15.0 i
C-2	1.6 c-e	18 g-i	50 f-l	19.7 b-h
HM070004	1.6 c-e	52 a-f	52 e-k	18.8 b-i
HM070015	1.7 b-e	40 c-i	66 b-h	22.2 ab
SX008	1.8 b-e	56 a-e	52 e-k	19.2 b-h
HM070001	1.8 b-e	14 hi	42 h-m	17.4 d-i
B-31	1.9 b-e	19 f-i	26 lm	19.2 b-i
HH014	1.9 b-e	46 b-h	54 d-k	17.1 f-i
HM070003	1.9 b-e	42 b-i	75 a-e	19.3 b-h
B-28	2.0 b-d	10 i	38 j-m	16.1 g-i
B-32	2.0 b-d	36 c-i	40 i-m	20.1 a-g
HH005	2.0 b-d	58 a-e	87 ab	17.5 c-i
HM070014	2.0 b-d	54 a-e	74 a-f	21.7 a-c
B-4	2.0 b-d	50 a-g	71 a-g	18.0 c-i
HH002	2.4 bc	42 b-i	64 b-i	19.7 b-h
C-17	2.6 b	64 a-d	71 a-g	16.4 f-i
HM070005	4.5 a	53 a-f	94 a	24.2 a
Overall mean	1.8	45	57	19
$P > F$[z]	<0.0001	0.0004	<0.0001	0.0037
LSD ($P \leq 0.05$)	0.9	33	25	4.2

[u] All cultivar names were coded (B = Betaseed Inc., C = ACH Seed Inc., HH = Holly Hybrids, HM = Hilleshog, and SX = Seedex), but the respective companies can be contacted using the code to gain additional information on the cultivars.

[v] Rhizomania ratings were conducted using a disease index of 0 to 9 (0 = no symptoms, 9 = root dead). All cultivars contained at least the *Rz1* gene for resistance to *Beet necrotic yellow vein virus* except for the susceptible check, HM070005.

[w] Fungal growth = percentage of root surface area covered by fungal growth on 1 February 2008. Fungal growth was primarily an undescribed basidiomycete.

[x] Root rot = percentage of root surface area covered by discolored tissue on 4 March 2008.

[y] Weight reduction = percent reduction in root weight after storage when compared to that determined at harvest.

[z] $P > F$ was the probability associated with the F value. Means followed by the same letter did not differ significantly based on Fisher's protected least significant difference (LSD) value with $P \leq 0.05$.

Table 3. Regression analysis for disease and yield variables in sugar beet storage studies

Independent variable[z]	Dependent variable	Slope	Intercept	r^2	Probability
2006					
Fungal growth	ERS	–85	8,883	0.47	<0.0001
Rz rating	ERS	–1,187	9,416	0.43	<0.0001
Rz rating	Fungal growth	0	1	0.42	<0.0001
Weight reduction	ERS	–479	9,503	0.34	<0.0001
Fungal growth	Root rot	0	10	0.26	<0.0001
Root rot	ERS	–56	7,655	0.18	<0.0001
Sucrose at harvest	ERS	1,407	–16,266	0.14	<0.0001
Nitrates at harvest	ERS	–6	5,356	0.01	0.0676
Conductivity at harvest	ERS	–3,780	9,145	0.00	0.1790
2007					
Fungal growth	ERS	–18	2,444	0.11	0.0002
Rz rating	ERS	–635	2,748	0.13	<0.0001
Rz rating	Fungal growth	0	41	0.00	0.4789
Weight reduction	ERS	–151	4438	0.11	0.0002
Fungal growth	Root rot	0	42	0.18	<0.0001
Root rot	ERS	–36	3,688	0.28	<0.0001
Sucrose at harvest	ERS	532	–6,522	0.06	0.0061
Nitrates at harvest	ERS	–1	1,936	0.01	0.4346
Conductivity at harvest	ERS	–262	1,802	0.00	0.8942

[z] Fungal growth = percentage of root surface area covered by fungal growth. Rz rating = rhizomania rating. Weight reduction = percent reduction in root weight after storage when compared to that determined at harvest. Root rot = percentage of root surface area covered by discolored tissue. ERS = estimated recoverable sucrose at the end of storage.

When comparing the 2006 sucrose reduction with other variables using regression analysis, correlations were as follows: fungal growth (r^2 = 0.47), rhizomania rating (r^2 = 0.43), weight reduction (r^2 = 0.34), surface rot (r^2 = 0.18), percent sucrose at harvest (r^2 = 0.14), nitrates at harvest (r^2 = 0.01), and conductivity at harvest (r^2 = 0.00) (Table 3). When comparing sucrose reduction with the same variables in 2007, correlations were as follows: surface rot (r^2 = 0.28), rhizomania rating (r^2 = 0.13), fungal growth (r^2 = 0.11), weight reduction (r^2 = 0.11), percent sucrose at harvest (r^2 = 0.06), nitrates at harvest (r^2 = 0.01), and conductivity at harvest (r^2 = 0.00) (Table 3).

Basidiomycete pathogenicity test. Because the pathogenicity tests did not differ (P = 0.6700), variances were homogeneous (P = 0.0859), and no interactions were evident (P > 0.28), these data were analyzed together. All basidiomycete isolates lead to significant (P = 0.0001) rot (5.6 to 2.6 mm); whereas no rot was evident in the uninoculated check. Isolate F570 produced more rot (5.6 mm) than the other isolates (3.6 to 2.6 mm). Koch's postulates could

Table 4. Yield data for 32 commercial sugar beet cultivars harvested in 2006 from a commercial field naturally infested with rhizomania in Paul, ID

Cultivar[v]	Nitrate (ppm)	Cond. (mmhos)[w]	Sucrose content (%)	Root yield (t/ha)	ERS at harvest (kg/ha)[x]	Sucrose reduction (%)	ERS in Feb (kg/ha)[y]
B-23	152 c-i	0.74 d-i	16.07 b-f	96.6 a-c	13,168 a-c	19 gh	10,629 a
HH002	118 e-l	0.70 g-i	16.07 b-f	88.8 d-j	12,193 b-g	13 h	10,586 a
B-26	129 d-l	0.72 e-i	17.01 a	94.8 a-e	13,784 a	26 e-h	10,247 ab
B-31	115 e-l	0.71 f-i	16.95 a	92.8 b-h	13,447 ab	24 f-h	10,236 ab
HM070007	137 c-l	0.65 i	16.97 a	84.3 i-m	12,348 b-g	19 gh	10,013 a-c
B-28	202 a-d	0.74 d-i	16.33 a-d	93.3 b-h	12,939 a-d	29 e-h	9,312 a-d
HH003	150 c-j	0.73 d-i	15.99 b-f	98.9 ab	13,430 ab	31 e-h	9,240 a-e
C-21	107 f-l	0.76 d-h	16.14 b-e	90.8 c-i	12,409 b-g	28 e-h	8,944 a-f
B-4	129 d-l	0.80 b-f	17.02 a	89.2 c-j	12,791 a-e	31 e-h	8,722 a-f
C-17	188 a-f	0.82 b-e	15.70 d-g	92.8 b-h	12,208 b-g	31 e-h	8,366 a-g
HH019	244 ab	0.90 ab	15.60 d-g	86.3 h-l	11,138 g-i	27 e-h	8,164 a-g
B-27	148 c-j	0.76 d-h	16.32 a-d	94.4 a-f	13,070 a-c	37 d-h	8,110 a-g
HM070012	148 c-j	0.67 hi	16.67 ab	80.0 l-n	11,472 f-i	32 e-h	7,710 b-g
C-2	151 c-j	0.78 c-g	16.05 b-f	93.9 a-g	12,736 a-f	40 d-g	7,700 b-g
HH005	263 a	0.86 a-c	14.74 h	100.9 a	12,353 b-g	39 d-g	7,663 b-g
HM070018	187 a-f	0.72 d-i	16.56 a-c	80.9 k-n	11,418 g-i	33 e-h	7,593 b-g
HH001	138 c-l	0.82 a-d	16.28 a-d	95.9 a-d	13,146 a-c	42 d-g	7,591 b-g
HM070004	142 c-k	0.73 d-i	16.57 a-c	86.1 h-l	12,174 b-g	38 d-g	7,577 b-g
HH004	218 a-c	0.88 ab	15.41 e-h	89.2 c-j	11,393 g-i	36 d-h	7,259 c-h
SX002	117 e-l	0.69 g-i	16.27 a-d	82.7 j-m	11,536 e-i	37 d-h	7,241 c-h
SX005	181 b-g	0.92 a	15.84 c-f	86.5 g-l	11,304 g-i	42 d-g	6,601 d-h
HM070014	105 g-l	0.76 d-h	15.03 gh	81.1 k-n	10,298 ij	38 d-g	6,427 e-h
HM070015	147 c-k	0.73 d-i	16.16 b-e	88.1 e-k	12,105 c-g	49 b-e	6,215 f-i
HM070001	195 a-e	0.78 c-g	16.06 b-f	86.5 g-l	11,757 d-g	48 b-f	6,103 f-i
SX006	102 g-l	0.73 d-i	15.66 d-g	78.5 mn	10,453 h-j	45 c-f	5,776 g-j
HM070011	69 j-l	0.75 d-i	15.06 gh	64.1 pq	8,210 k	45 c-f	4,515 h-k
B-30	157 c-h	0.77 c-g	15.90 b-f	87.2 f-l	11,726 d-h	70 ab	3,528 i-l
SX001	95 h-l	0.76 d-h	16.11 b-f	57.8 rq	7,882 k	59 b-d	3,170 j-l
HM070005	70 i-l	0.76 c-h	15.36 f-h	59.9 rq	7,765 k	60 b-d	3,076 j-l
SX004	145 c-k	0.76 c-h	15.57 d-g	74.4 no	9,813 j	68 a-c	3,008 j-l
HM070021	64 kl	0.70 f-i	15.95 b-f	55.8 r	7,599 k	69 a-c	2,390 kl
B-16	58 l	0.80 b-f	14.96 gh	68.1 op	8,514 k	90 a	831 l
Overall mean	143	0.76	16.01	84.3	11,456	40	7,016
$P > F$[z]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD	83	0.10	0.78	7.6	1,281	24	2,853

[v] All cultivar names were coded (B = Betaseed Inc., C = ACH Seed Inc., HH = Holly Hybrids, HM = Hilleshog, and SX = Seedex), but the respective companies can be contacted using the code to gain additional information on the cultivars.
[w] Cond. = conductivity during sucrose analysis at harvest.
[x] ERS at harvest = estimated recoverable sucrose at harvest.
[y] ERS in Feb = estimated recoverable sucrose at the end of storage on 26 February 2007.
[z] $P > F$ was the probability associated with the F value. Means followed by the same letter did not differ significantly based on Fisher's protected least significant difference (LSD) value with $P \leq 0.05$.

not be proven with the basidiomycete because only *Penicillium* spp. and not the basidiomycete were reisolated from the rotted areas.

DISCUSSION

Cultivar selection for storability using an indoor storage facility gave more consistent significant differences than outdoor storage. By combining the indoor storage approach with roots from an infested rhizomania field, both storability and rhizomania resistance could be addressed at the same time. To perform well in the storage assay, cultivars had to possess both good rhizomania resistance and storability.

Rhizomania has become widespread in the major production areas of the United States and other areas of the world (21,22,31), so developing and maintaining cultivars resistant to BNYVV will be very important to the sugar beet industry. Commercial cultivars currently possess the *Rz1* gene for resistance to BNYVV, but resistance breaking strains have been discovered in the United States (1,17). Rhizomania can severely impact yield variables in susceptible cultivars but recently has been shown to impact storability as well (8,28). Even by early December in outdoor piles under ambient conditions, infested roots can suffer significant sucrose reductions (28). For roots to perform well in storage, they need resistance to BNYVV as well as storability. Selecting cultivars with improved performance for both traits will be important to the profitability of the sugar beet industry when BNYVV is present in the field.

A basidiomycete (29) was the primary fungus present on the root surface in the storage studies. By the beginning of February, some roots were approaching 100% coverage by the basidiomycete. The basidiomycete fungal growth was evident on both roots stored outdoors and indoors. The white cottony growth is not very hardy since it desiccates immediately if exposed to dry ambient conditions outside the pile or storage building. Thus, finding this fungus growing as white cottony masses on roots on the surface of an outdoor pile would not be likely. Indoors, this fungus has been frequently found growing on commercial sugar beet roots on the surface of the pile. The basidiomycete has been shown to grow over a range of at least 3 to 22°C (29). The optimum temperature for growth has been shown to be between 12 and 16°C with no growth occurring at 30°C (29). Prior studies have noted the importance of other fungi such as *Botrytis, Penicillium* spp., *Phoma*, *Fusarium*, *Rhizopus*, and *Aspergillus* in storage (5,12,18). More research should be conducted to determine if the basidiomycete is pathogenic and what if any interaction it may have with these other fungi.

Based on regression, the basidiomycete was correlated with surface rot and sucrose reduction both years. The basidiomycete fungal growth was correlated with the rhizomania rating in 2006, but in 2007 there was no relationship. The lack of relationship with the rhizomania rating in 2007 may have been affected by the lack of highly susceptible cultivars, other than the susceptible check, and fungal growth and surface rot in storage that bordered on being overwhelming.

If sugar beet roots lose more than 25 to 30% of their weight, then vital root functions are disrupted and the root cannot resist microbial development (6,30).

Table 5. Yield data for 30 commercial sugar beet cultivars harvested in 2007 from a commercial field naturally infested with rhizomania in Paul, ID

Cultivar[v]	Nitrate (ppm)	Cond. (mmhos)[w]	Sucrose content (%)	Root yield (t/ha)	ERS at harvest (kg/ha)[x]	Sucrose reduction (%)	ERS in March (kg/ha)[y]
HH006	223 a-h	0.716 a-e	15.04 h-k	98.0 a-e	12,654 b-g	57 h	5,605 a
B-31	152 f-i	0.678 b-f	15.81 b-e	92.4 d-h	12,605 b-g	70 gh	3,731 ab
C-3	193 c-i	0.681 b-f	15.67 c-h	102.2 a-d	13,700 a-c	77 e-g	3,118 bc
HH014	127 i	0.636 d-f	16.63 a	86.1 gh	12,369 c-h	76 fg	2,924 b-d
HH003	290 ab	0.750 a-c	14.87 i-k	98.0 b-e	12,288 e-h	79 c-g	2,664 b-e
B-30	195 c-i	0.700 b-e	15.20 e-j	103.1 a-c	13,374 a-f	81 c-g	2,596 b-e
C-23	294 ab	0.742 a-c	15.84 b-e	98.6 a-e	13,260 a-g	81 c-g	2,559 b-e
HM070007	181 d-i	0.594 f	16.33 ab	84.5 h	11,983 gh	78 d-g	2,526 b-e
B-28	192 c-i	0.676 b-f	15.73 b-g	105.8 ab	14,234 a	82 b-g	2,521 b-e
HH008	168 e-i	0.674 b-f	16.02 a-d	92.1 e-h	12,648 b-g	82 b-g	2,214 b-f
C-2	223 a-h	0.714 a-e	15.02 i-k	104.2 a-c	13,303 a-g	85 a-g	2,049 b-g
HM070003	138 hi	0.675 b-f	15.35 e-i	97.5 b-e	12,827 b-g	84 b-g	2,048 b-g
HH002	215 b-i	0.657 c-f	15.77 b-f	93.3 d-h	12,645 b-g	84 a-g	1,949 b-g
B-26	142 g-i	0.695 b-e	16.13 a-c	92.6 d-h	12,987 a-g	86 a-g	1,765 b-g
HH004	228 a-h	0.724 a-e	15.38 d-i	106.5 ab	13,919 ab	87 a-f	1,744 b-g
SX008	214 b-i	0.684 b-f	15.09 g-k	100.9 a-e	13,019 a-g	88 a-f	1,717 b-g
C-21	221 b-h	0.696 b-e	15.35 e-i	101.3 a-e	13,260 a-g	89 a-f	1,509 c-g
B-32	272 a-d	0.764 ab	15.07 h-k	107.6 a	13,666 a-d	89 a-f	1,492 c-g
HM070004	229 a-h	0.721 a-e	15.16 f-k	94.8 c-g	12,217 e-h	90 a-f	1,348 c-g
SX005	242 a-f	0.730 a-d	14.92 i-k	104.2 a-c	13,192 a-g	90 a-f	1,300 c-g
HH005	315 a	0.752 a-c	14.68 jk	101.8 a-d	12,625 b-g	89 a-f	1,265 c-g
HM070001	254 a-e	0.719 a-e	14.66 jk	102.2 a-d	12,712 b-g	92 a-f	1,094 c-g
C-17	232 a-g	0.722 a-e	15.32 e-i	99.8 a-e	12,986 a-g	92 a-e	1,029 d-g
HH011	169 e-i	0.678 b-f	15.67 c-h	100.9 a-e	13,529 a-e	93 a-d	937 d-g
HM070014	186 c-i	0.658 c-f	14.65 jk	87.6 f-h	11,082 h	92 a-f	927 d-g
B-4	158 f-i	0.718 a-e	15.24 e-j	101.8 a-d	13,211 a-g	94 a-d	817 e-g
HH001	274 a-c	0.805 a	14.54 k	100.9 a-e	12,309 d-h	94 a-c	653 e-g
B-18	207 b-i	0.629 ef	15.42 d-i	100.9 a-e	13,446 a-e	97 ab	309 fg
HM070005	242 a-f	0.734 a-d	13.47 l	64.1 i	7,395 i	100 a	0 g
HM070015	264 a-d	0.753 a-c	14.84 i-k	96.2 c-f	12,044 f-h	100 a	0 g
Overall mean	215	0.702	15.30	97.5	12,709	86	1,814
$P > F$[z]	0.0028	0.0468	<0.0001	<0.0001	<0.0001	0.0004	0.0007
LSD	92	0.098	0.64	9.6	1,378	16	2,085

[v] All cultivar names were coded (B = Betaseed Inc., C = ACH Seed Inc., HH = Holly Hybrids, HM = Hilleshog, and SX = Seedex), but the respective companies can be contacted using the code to gain additional information on the cultivars.

[w] Cond. = conductivity during sucrose analysis at harvest.

[x] ERS at harvest = estimated recoverable sucrose at harvest.

[y] ERS in March = estimated recoverable sucrose at the end of storage on 4 March 2008.

[z] $P > F$ was the probability associated with the F value. Means followed by the same letter did not differ significantly based on Fisher's protected least significant difference (LSD) value with $P \leq 0.05$.

Weight reduction was significant in both years, but mean values were less than 25%, so roots should have retained the ability to resist microbial development.

During both storage seasons, the outdoor piles afforded excellent storage conditions. The weather turned cold in early December and the average daily temperature stayed below 0°C most of the winter, thus creating ideal outdoor storage conditions. Root sucrose reduction within the outdoor piles was less than that on the surface of the indoor piles both years. Similar sucrose reduction data were collected in Idaho in 1978 when samples in controlled storage lost more sucrose than those in outdoor piles (20). However, during storage seasons when prolonged periods of warm wet weather follow freezing weather, storing sugar beet roots in outdoor piles can be much more challenging than the conditions during the 2006–2007 and 2007–2008 storage seasons.

In previous studies, sucrose was lost at the rate of 0.2 to 0.5 lb per ton of sugar beet roots per day (9,20). Based on these data, sugar companies could expect to lose from 8 to 17% of their sucrose in 100 days with healthy roots under good storage conditions in an outdoor pile. Because our studies were conducted for 142 and 159 days, we expected to lose at least 11 to 27% of the sucrose. Our sucrose losses ranged from 13 to 90% in 2006 and 57 to 100% in 2007 indoors, and from 13 to 32% in 2006 and 28 to 60% in 2007 outdoors. These data should not be considered surprising given that the roots were compromised by BNYVV, fungal growth, and surface rot. Regression data indicated that fungal growth, rhizomania rating, weight reduction, and surface rot were all related to sucrose reduction both years. The percentage of variation explained by these regression data changed between years; thus additional research will be required to identify which variable may be most important over time.

Previous studies have shown that if 20% or more of the root surface is affected by fungal growth, root respiration increases 100% (18). Indoors, the root surface was covered with an average of 31 and 45% in 2006 and 2007, respectively. The rank correlations for fungal growth (r = 0.56) and sugar reduction (r = 0.55) between years were significant. These data show that even though fungal growth and sucrose reduction varied between years, the ranking of the cultivars between years for these traits was similar. In previous storage work outdoors (28) and indoors (25) without the influence of disease, establishing significant differences between cultivars for reduction in sucrose was not possible. When utilizing rhizomania-infested sugar beet roots, significant differences in sucrose reduction and estimated recoverable sugar were possible both years. Cultivars that performed well possessed both storability and rhizomania resistance. Thus, the indoor storage approach with rhizomania-infested roots should allow for reliable separation and ranking of sugar beet cultivars for storability and rhizomania resistance.

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