Sugar Beet Root Storage Properties Are Unaffected by Cercospora Leaf Spot

Karen K. Fugate[1]*, Mohamad F. R. Khan[2,3]*, John D. Eide[1], Peter C. Hakk[2], Abbas M. Lafta[2], and Aiming Qi[4]

[1]USDA-ARS, Edward T. Schafer Agricultural Research Center, Fargo, ND, 58102, USA
[2]Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA
[3]University of Minnesota Extension Service, St. Paul, MN 55108, USA
[4]Centre for Agriculture, Food and Environmental Management Research, School of Life and Medical Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK

*Corresponding authors: Karen Fugate; E-mail: karen.fugate@usda.gov
Mohamed Khan; E-mail: mohamed.khan@ndsu.edu

Co-authors' E-mails: J. Eide: john.eide@usda.gov
P. Hakk: peter.hakk@ndsu.edu
A. Lafta: abbas.lafta@usda.gov
A. Qi: a.qi@herts.ac.uk

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Abstract

Cercospora leaf spot (CLS; causal agent *Cercospora beticola* Sacc.) is endemic in many sugar beet production regions due to the widespread distribution of *C. beticola* and the inability of current management practices to provide complete control of the disease. Roots harvested from plants with CLS, therefore, are inevitably incorporated into sugar beet root storage piles, even though the effects of CLS on root storage properties are largely unknown. Research was conducted to determine the effects of CLS on storage properties including root respiration rate, sucrose loss, invert sugar accumulation, loss in recoverable sucrose yield, and changes in sucrose loss to molasses with respect to CLS disease severity and storage duration. Roots were obtained from plants with four levels of CLS severity in each of three production years, stored at 5°C and 95% relative humidity for up to 120 days, and evaluated for storage characteristics after 30, 90 and 120 days storage. No significant or repeatable effects of CLS on root respiration rate, sucrose loss, invert sugar accumulation, loss in recoverable sucrose yield, or change in sucrose loss to molasses were detected after 30, 90 or 120 days storage regardless of the severity of CLS disease symptoms. Therefore, no evidence was found that CLS accelerates sugar beet storage losses and it is concluded that roots harvested from plants with CLS can be stored without additional or specialized precaution, regardless of CLS symptom severity.

Cercospora leaf spot (CLS), caused by *Cercospora beticola* Sacc., is a fungal disease of sugar beet (*Beta vulgaris* L.) that develops under wet, warm and humid environmental conditions and causes necrotic lesions on leaves and petioles (Asher and Hanson 2006; Jacobsen and Franc 2009). As the disease becomes increasingly severe, leaf lesions merge, causing large areas of necrosis on leaves, leaf death, and occasionally complete defoliation of plants (Windels et al. 1998). The disease is found in sugar beet production areas worldwide and is the most damaging foliar disease of sugar beet in production regions where warm and humid conditions occur (Rangel et al. 2020). Although lesions occur only on above-ground portions of the plant, CLS reduces root mass and sucrose content by decreasing shoot photosynthetic capacity and altering root/shoot carbon partitioning (Khan et al. 2008; Shane and Teng 1992; Windels et al. 1998). CLS also reduces sugar yield after processing by elevating concentrations of sodium ions and amino-nitrogen-containing compounds in the root (Shane and Teng 1992). These root components hinder processing and increase sugar loss to molasses. When severe, CLS reduces sugar yield by as much as 42%, although infections affecting as little as 3% of a plant's leaf surface area cause economic loss (Shane and Teng 1992; Windels et al. 1998).

CLS is managed by a diversified approach that involves the planting of varieties with genetic resistance to *C. beticola*, cultural practices that reduce pathogen populations (i.e., cultivation and crop rotations), and timely, repeated applications of fungicides (Bosemark 2006; Windels et al. 1998). However, disease management rarely, if ever, provides complete disease control since varieties have only partial resistance to the pathogen and *C. beticola* strains have emerged with resistance to many commonly used fungicides (Rangel et al. 2020; Smith and Gaskill 1970; Taguchi et al. 2011). As a result, where CLS is endemic, nearly all plants display

some symptoms of CLS at time of harvest, and roots that are stored prior to processing inevitably are obtained from *C. beticola*-infected plants.

In northern production regions of the U.S., sugar beet roots are stored in large outdoor piles or ventilated sheds for up to 120 days before processing or freezing for long-term storage (Bernhardson 2009; Campbell and Klotz 2006b; Tungland et al. 1998). Storage piles are cooled with ambient winter air to aid in the preservation of root sucrose content and processing quality during storage (Campbell and Klotz 2006b). Nevertheless, root sucrose content declines, primarily due to root respiration; carbohydrate impurities that hamper processing such as invert sugars (i.e., glucose and fructose) accumulate; and root quality deteriorates, causing increases in sucrose loss to molasses during processing (Campbell and Klotz 2006b). Past research established that production diseases such as Aphanomyces root rot (causal agent, *Aphanomyces cochlioides* Drechsl.), Fusarium yellows (causal agent, *Fusarium oxysporum* f. sp. *betae*), Rhizoctonia root and crown rot (causal agent, *Rhizoctonia solani* Kühn), and rhizomania (causal agent, *Beet necrotic yellow vein virus*) accelerate sucrose loss and quality deterioration during storage, with the deterioration of storage properties proportional to disease symptom severity (Campbell and Klotz 2006a; Campbell et al. 2008, 2011, 2014; Klotz and Campbell 2009; Strausbaugh et al. 2008). The effect of CLS on the ability of roots to maintain sucrose content and processing quality during postharvest storage, however, is unclear. CLS is claimed to increase incidence of fungal storage diseases based on an observed, inverse relationship between genetic resistance to CLS and incidence of storage rots after 141 days in storage (Smith and Ruppel 1971). However, the authors of this study found no significant relationship between CLS severity and storage rot incidence. In another study, CLS was reported to have

63 little effect on root sucrose or amino-nitrogen concentrations during storage (Kenter et al.
64 2006). This study, however, was limited in scope and duration in that it examined changes in
65 only two storage properties and only after 25 days of storage (Kenter et al. 2006).
66 To clarify the effect of CLS on sugar beet root storage properties, research was
67 conducted to evaluate changes in multiple storage properties of roots obtained from plants
68 with varying levels of CLS disease symptoms. Storage properties that were evaluated included
69 root respiration rate, sucrose content, invert sugar accumulation, sucrose loss to molasses, and
70 recoverable sugar content. These storage properties were evaluated after 30, 90 and 120 days
71 of storage to allow interactions of CLS disease severity and storage duration on root storage
72 properties to be evaluated. Ultimately, the goal of this study was to generate information to
73 assist sugar beet agronomists and storage pile managers tasked with preserving the sucrose
74 and quality of roots harvested from *C. beticola*-infected plants by determining whether a level
75 of CLS severity exists that impacts storage to an extent that would preclude roots from being
76 incorporated into storage piles or require their segregation for early processing.
77

78 **Materials and Methods**

79 **Plant material and treatments.** Sugar beet roots were obtained in 2018, 2019, and 2020
80 from field plots planted to commercial, CLS susceptible hybrids that were inoculated with *C.*
81 *beticola* and treated with different fungicide regimes to produce plants with four levels of CLS
82 disease symptom severity. Plants were grown in fields near Foxhome, MN using a randomized
83 block design with four replications. Plots (3.35 x 9.14 m, width x length) were six-rows wide
84 with 0.56 m between rows and 0.12 m spacing within rows. CLS-susceptible varieties Hilleshög

9528 (Hilleshög Seed LLC, Longmont, CO), Seedex Cruze (Seedex, Inc., Fargo, ND), and Hilleshög HM4448RR were planted in 2018, 2019, and 2020, respectively. Plots were planted in the first two weeks of May in all years and maintained using recommended agronomic practices (Khan 2018). All plots were inoculated with 5.60 kg ha^{-1} dried *C. beticola*-infected leaves on 28 June, 12 July, and 6 July in 2018, 2019, and 2020, respectively. *C. beticola*-infected leaves were obtained from CLS-infected fields near Foxhome, MN with the identity of the pathogen confirmed by genetic testing and morphological characterization after culture. To obtain plants with four levels of CLS disease symptoms, four programs of fungicide treatments, as described in Table 1, were applied each year to the center four rows of plots using a CO_2 pressurized, four-nozzle boom sprayer with 11002 TT TwinJet (TeeJet Technologies, Wheaton, IL) nozzles calibrated to 414 KPa pressure and delivering 159 L ha^{-1}. Before harvest, entire plants in the middle two rows of each plot were visually rated for CLS disease severity using the 1 to 10 scoring scale of Jones and Windels (1991), summarized in Supplemental Table S1, to obtain an average CLS rating for each plot. Roots from the middle two rows of plots were manually harvested in September, on the 27th, 10th, and 11th in 2018, 2019, and 2020, respectively, and weighed to determine root yield at harvest. Forty arbitrarily selected roots from each plot were washed and randomly divided into four 10-root samples. One 10-root sample from each plot was ground to brei on the day of harvest using a pilot-scale brei saw. The ground root material (brei) was rapidly mixed for uniformity and a sample of brei that was sufficient to fill a standard 9 cm petri dish was collected, frozen, and stored at -80 °C for later analysis. The remaining 10-root samples per plot were stored under favorable storage conditions in a 5 °C, 95% relative humidity cold room and used for respiration rate determinations after 30, 90, and 120 d in

storage. Following respiration rate determinations, each 10-root sample was ground to brei as described above.

Respiration rate. Respiration rate of 10-root samples was measured at 5 °C by infrared gas analysis using an open system (Campbell et al. 2011). Root samples were weighed and contained in 23 liter, tightly sealed buckets that were modified with an inlet and outlet to permit air circulation. Inlet and outlet openings were created by drilling one small hole in the bottom and lid of the bucket, respectively, and Tygon tubes (16 mm OD; United States Plastic Corp., Lima, OH, USA) were inserted through these holes. Air was pumped continuously into the bucket's inlet tube, at a rate of 475 mL $minute^{-1}$ and carbon dioxide concentration of air exiting the bucket's outlet tube was quantified with a LI-COR LI-7000 gas analyzer (Lincoln, NE, USA).

Chemical Analyses. Brei samples were clarified with a 0.3% (w:v) aluminum sulfate solution using the protocol of McGinnis (1982) and filtrates of the resulting suspensions were used for all chemical analyses. Sucrose concentration was determined polarimetrically using a Rudolf Research Analytical Autopol 880 saccharimeter (Hackettstown, NJ, USA). Sodium (Na) and potassium (K) concentrations were determined by flame photometry (Corning model 410C, Corning, NY, USA). Amino-nitrogen (amino-N) concentration was determined spectroscopically (Shimadzu, model UV-1601, Kyoto, Japan) using the ICUMAS copper method (International Commission for Uniform Methods of Sugar Analysis 2007). Sucrose loss to molasses (LTM) was calculated using the equations of Carruthers et al. (1962) as modified by American Crystal Sugar Company (Moorhead, MN, USA) where LTM = {[(Na x 3.5) + (K x 2.5) + (amino-N x 9.5)]/1100} x 1.5 with Na, K and amino-N expressed in ppm and LTM as g kg^{-1}. Recoverable sugar concentration was calculated by subtracting LTM from the sucrose concentration. Invert sugar

concentration was the sum of glucose and fructose concentrations which were individually determined using spectroscopic, end point, enzyme-coupled assays as previously described (Klotz and Martins 2007; Spackmann and Cobb 2001). All data are expressed as a function of root fresh weight.

Statistical analysis. Data from each year were analyzed separately due to the use of different varieties and different fungicide regimes that yielded plants with different CLS ratings in each year of the study. All measurements were made using composite random samples comprised of ten roots per field plot with unique samples analyzed at each time point. Significant differences between treatments were determined by analysis of variance (ANOVA) using Minitab software (ver. 19, State College, PA, USA), with CLS disease rating assumed to be a fixed effect. Fisher's least significant difference (LSD) was used to identify treatment means that differed when significant treatment differences were detected by ANOVA ($p \leq 0.05$). P values for all comparisons are available in Supplementary Tables S2 and S3. For all analyses, $\alpha = 0.05$ and $n = 4$.

Results

The inoculation of field plots with *Cercospora beticola* and the use of different fungicide programs to control the pathogen (described in Table 1) successfully generated plants with four levels of CLS disease symptoms in each of the three years of the study. No fungicide program, however, was able to produce plots free of disease or with disease ratings below 3.0 in any year (Table 2). In 2018, mean CLS ratings of plots ranged from 3.0 to 9.8. In 2019, CLS plot ratings were similar to those of 2018 and ranged from 3.0 to 8.8. In 2020, weather conditions hindered

CLS control causing disease ratings to be greater than the previous two years. In this final year of the study, plot CLS ratings ranged from 5.5 to 10.0 with symptoms ranging from moderate levels of leaf spotting to loss of entire leaves.

Plants with intermediate to high CLS ratings had reduced root yield and recoverable sugar per hectare relative to plants with mild CLS symptoms in all years (Table 2). In 2018, root yield and recoverable sugar were reduced by 24 and 35%, respectively, in plots with the highest CLS ratings relative to plots with the lowest ratings (CLS ratings 9.8 vs. 3.0). In 2019, root yield and recoverable sugar were reduced by 32 and 37%, respectively, in plots with the most severe symptoms relative to plots with the mildest symptoms (CLS ratings 8.8 vs. 3.0). Plots with more moderate symptoms, i.e., those with ratings of 5.8-6.0, had reduced root yield and recoverable sugar per hectare in both 2018 and 2019, although these reductions were statistically significant in 2019 only. In 2020, all plots had intermediate to high disease ratings (CLS ratings 5.5 to 10.0) and had values for root yield and recoverable sugar per hectare that were greatly reduced from those recorded in 2018 and 2019.

Root storage respiration rate was unaffected by CLS disease severity (Table 3). In all years of the study, root respiration measured after 30, 90 or 120 d in storage was unaffected by the CLS disease rating prior to harvest. Although root respiration rate generally increased with time in storage, as measured by the difference in respiration between 120 and 30 d ($\Delta_{(120\ d\ -\ 30\ d)}$, Table 3), this time-dependent increase in respiration was nonsignificant in 2018 and 2020 and for three of four treatments in 2019. Moreover, any changes in respiration rate due to increased time in storage were unrelated to CLS disease ratings.

Foliar CLS symptoms prior to harvest affected root sucrose concentration at harvest and after 30, 90, and 120 d storage (Table 4). In 2018, sucrose concentration was reduced in roots from plants with CLS ratings of 6.0 and 9.8 at all sampling times, although reductions that occurred after 120 d storage were not statistically significant. Similarly, sucrose concentration was reduced in roots from plots with the highest CLS rating in 2019 and 2020 at all time points. In all years, reductions in sucrose content due to higher CLS severity were evident at harvest (0 d) indicating that reductions in sucrose content occurred during preharvest production of the root. Reductions in sucrose concentration after 30, 90 or 120 d storage in roots with higher CLS ratings reflected the lower sucrose concentrations these roots had at harvest and were not due to an acceleration in sucrose loss during storage. This was evidenced by the absence of any relationship between CLS rating and sucrose loss in storage, as measured by the difference in sucrose concentration at 120 and 0 d ($\Delta_{(120\ d\ -\ 0\ d)}$, Table 4). Therefore, CLS reduced root sucrose concentrations at harvest, but the severity of CLS had no influence on sucrose loss during storage.

No relationship between CLS symptom severity and invert sugar concentration at harvest or after storage was evident in any year of the experiment (Table 4). All differences in invert sugar concentrations on the day of harvest were nonsignificant, regardless of CLS rating, with invert concentrations low and less than 0.2% of the root weight. Differences in invert sugar concentration in stored roots with differing CLS ratings were also nonsignificant, except for a single time point in 2018 (30 d) and in 2020 (90 d). The effect of storage on invert sugar concentration ($\Delta_{(120\ d\ -\ 0\ d)}$) was variable between years, with invert concentrations after 120 d storage increasing in 2018, remaining largely unchanged in 2019, and declining in 2020. More

importantly, any changes in invert sugar concentration during storage bore no relationship to CLS ratings of the plots from which roots were harvested. Therefore, CLS severity had no influence on invert sugar concentration at harvest or invert sugar formation during storage.

CLS disease severity also had no consistent effect on sucrose loss to molasses at harvest or after storage (Table 5). LTM is a calculated estimate of sucrose present in the root that cannot be recovered during processing due to melassingenic root impurities that limit sucrose crystallization from concentrated root extracts (Junghans et al. 1998). At time of harvest, roots with the highest two CLS ratings had LTM values that were similar to, greater than, or less than roots with the lowest ratings in 2018, 2019, and 2020, respectively. Although statistically higher values for LTM were recorded for roots with the most severe CLS symptoms after 30 d storage in 2020, no changes in LTM in roots with higher CLS ratings were recorded at any time during 2018, 2019 or after 90 or 120 d in 2020. Moreover, the severity of disease symptoms did not affect the change in LTM during storage ($\Delta_{(120\ d\ -\ 0\ d)}$) in 2018 or 2019, although LTM increased modestly in roots with the highest two CLS ratings in 2020. Therefore, CLS disease severity had no influence on LTM at harvest and no consistent effect on changes in LTM during storage.

Recoverable sugar is an estimate of the quantity of sugar that will be produced from roots after processing and is calculated using root sucrose concentration and the concentrations of non-sucrose impurities that cause sucrose losses in the factory. Severity of CLS symptoms prior to harvest significantly affected recoverable sugar at harvest, after 30 and 90 d storage in all years, and after 120 d storage in all years except 2018 (Table 5). In 2018 and 2019, a progressive reduction in recoverable sugar with incremental increases in CLS rating was generally observed at all sampling times. Similarly, recoverable sucrose was reduced in roots

from plots with the highest CLS rating in 2020 at all time points. In all years, reductions in recoverable sugar due to greater disease severity were evident at harvest (0 d) indicating that losses in recoverable sugar content occurred prior to harvest. Reduced quantities of recoverable sugar after 30, 90 or 120 d storage in roots with higher CLS ratings generally reflected the reduced recoverable sugar values of these roots at harvest. No acceleration in the loss of recoverable sugar during storage occurred during 2018 and 2019, as evidenced by the absence of any relationship between CLS rating and the change in recoverable sugar between 120 and 0 d ($\Delta_{(120\ d\ -\ 0\ d)}$). However, in 2020, roots from plots with the highest CLS rating lost an additional 6 kg Mg^{-1} more in recoverable sugar relative to roots with the lowest CLS ratings. Therefore, CLS reduced root recoverable sugar content at harvest, but the severity of CLS generally had little to no influence on recoverable sugar losses during storage.

Discussion

Moderate to severe symptoms of Cercospora leaf spot were associated with reductions in root yield, sucrose content, and weights of recoverable sugar per tonne (RST) and recoverable sugar per hectare (RSH) at time of harvest. These reductions were expected since reductions in root yield, sucrose content, RST and RSH have been reported in a multitude of studies (Khan et al. 2007; Shane and Teng 1992; Smith and Martin 1978; Smith and Ruppel 1973; Vogel et al., 2018). Moderate or severe CLS symptoms, however, had no apparent effect on root invert sugar content or sucrose loss to molasses at harvest in the present study. This contrasts with Smith and Martin (1978) who found a decline in root purity in roots harvested from plants with CLS which would cause more sucrose to be lost to molasses during processing

but agrees with Shane and Teng (1992) who found no clear effect of CLS on LTM at harvest. To our knowledge, no earlier studies examined the effect of CLS on invert sugar concentration at harvest.

Despite affecting root and sucrose yield at harvest, moderate to severe CLS infections had no effect on root storage respiration rate, sucrose loss in storage, or loss in recoverable sugar per tonne after 30, 90 or 120 d storage. Although sucrose content and recoverable sugar per tonne were lower in roots with greater CLS symptomatology relative to roots with milder symptoms after 30, 90 and 120 d storage, the reduced sugar content and RST in these roots after storage reflected the lower sugar content and RST of these roots at harvest. In fact, all roots, regardless of CLS rating, lost sucrose or RST at similar rates during storage. Similarly, root storage respiration rates were unaltered by CLS symptom severity of the plants from which they were harvested. Root respiration is the primary cause of sucrose loss in storage, with estimates that this metabolic process is responsible for 60 to 80% of the total sucrose lost during storage (Wyse and Dexter 1971).

In addition to having no effect on respiration rate, sucrose loss or loss in recoverable sugar per tonne, CLS had no effect on invert sugar accumulation or sucrose loss to molasses after 30, 90 or 120 days storage. Invert sugars often accumulate during storage and degrade during processing to colored and acidic compounds that increase sucrose loss to molasses and impede white sugar production (Dutton and Huijbregts 2006). The accumulation of invert sugars in stored roots is often attributable to the development of storage rots (Liebe and Varrelmann 2016; Schnepel and Hoffmann 2016). That severe CLS did not elevate invert sugar concentrations during storage, therefore suggests that CLS did not promote the postharvest

development of storage rots. This contrasts with Smith and Ruppel (1971) who concluded that CLS predisposed roots to storage rots based on their observation of greater storage rot incidence in roots with lower genetic resistance to CLS. Storage-related changes in sucrose loss to molasses were generally nonsignificant and unrelated to CLS symptom severity in all years of the study. LTM is a calculation of the sucrose that is not recovered during processing due to the melassingenic effects of sodium, potassium and amino nitrogen-containing compounds present in roots (Dutton and Huijbregts 2006). That neither invert sugar accumulation nor LTM were affected by CLS severity indicates that CLS did not escalate losses in root processing quality during storage.

From the results of this study, it is concluded that roots obtained from plants with CLS suffer no additional storage losses due to this production disease and can be stored without specialized precautions, regardless of the severity of disease symptoms. This contrasts with other production diseases such as Aphanomyces root rot and Rhizoctonia root and crown rot for which a threshold level of disease severity exists that precludes roots from storage or Fusarium yellows and rhizomania that impact root quality to such an extent during prolonged storage to warrant segregation of diseased roots for early processing (Campbell and Klotz 2006a; Campbell et al. 2008, 2011, 2014; Klotz and Campbell 2009; Strausbaugh et al. 2008). That CLS had no effect on sugar beet root storage properties is perhaps not surprising since CLS is principally a foliar disease. Although lesions on occasion have been noted on root crowns and infection via the root system has been reported, CLS symptoms appear almost exclusively on leaf and petioles of infected plants (Khan et al. 2008; Vereijssen et al. 2004). This contrasts with sugar beet diseases that were previously found to impact storage (i.e., Aphanomyces root rot,

Rhizoctonia root and crown rot, Fusarium yellows, and rhizomania) which are caused by pathogens that infect, multiply in, and damage root tissues and are generally considered to be root diseases (Asher and Hanson 2006; Stevens et al 2006).

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Table 1. Fungicides and application dates that were used to obtain varying severities of Cercospora leaf spot symptoms on sugar beets produced in a field near Foxhome, MN in 2018, 2019, and 2020

Disease Severity	2018		2019		2020	
	Fungicide[a]	Date[b]	Fungicide	Date	Fungicide	Date
Group 1 *lowest*	Minerva Duo	07/05	Super Tin/Proline/NIS	07/22	Super Tin/Proline/NIS	07/20
	Super Tin/Topsin	07/18	Super Tin/Proline/NIS	08/01	Super Tin/Proline/NIS	07/31
	Proline/Badge SC/NIS	07/31	Super Tin/Proline/NIS	08/14	Super Tin/Proline/NIS	08/12
	Mankocide	08/16	Super Tin/Proline/NIS	08/28	Super Tin/Proline/NIS	08/24
Group 2	Super Tin/Manzate Max/Topsin	07/18	Super Tin/Manzate Max/Topsin	07/22	Proline/NIS/Badge SC	07/20
	Super Tin/Manzate Max/Topsin	07/31	Super Tin/Manzate Max/Topsin	08/01	Proline/NIS/Badge SC	07/31
	Super Tin/Manzate Max/Topsin	08/16	Super Tin/Manzate Max/Topsin	08/14	Proline/NIS/Badge SC	08/12
	Super Tin/Manzate Max/Topsin	08/31	Super Tin/Manzate Max/Topsin	08/28	Proline/NIS/Badge SC	08/24
Group 3	Minerva Duo	07/05	Gem	07/22	Inspire XT/Badge SC	07/20
	Super Tin/Topsin	07/18	Gem	08/01	Inspire XT/Badge SC	07/31
	Proline/Badge SC/NIS	07/31	Gem	08/14	Inspire XT/Badge SC	08/12
			Gem	08/28	Inspire XT/Badge SC	08/24
Group 4 *highest*	none		none		Topsin	07/20
					Topsin	07/31
					Topsin	08/12
					Topsin	08/24

[a]Minerva Duo (Advan, LLC, Durham, NC, USA); 1.17 L ha^{-1}
Super Tin (United Phosphorus, Inc., King of Prussia, PA, USA); 0.585 L ha^{-1}
Topsin (Nippon Soda Co., Edison, NJ, USA); 1.46 L ha^{-1}
Proline (Bayer CropScience, Research Triangle Park, NC, USA); 0.365 L ha^{-1} in 2018; 0.417 L ha^{-1} in 2019 and 2020
Badge SC (ISAGRO S.p.A., Morrisville, NC, USA); 4.68 L ha^{-1} in 2018, 2.34 L ha^{-1} in 2020
NIS (nonionic surfactant; Prefer 90, CHS, Inc., Inver Grove Heights, MN, USA); 0.125% v/v
Mankocide (Certis USA, LLC, Columbia, MD, USA); 4.82 kg ha^{-1}
Manzate Max (United Phosphorus, Inc.); 3.74 L ha^{-1}
Gem (Bayer CropScience); 0.263 L ha^{-1}
Inspire XT (Syngenta Crop Protection, LLC, Greensboro, NC, USA) 0.512 L ha^{-1}
[b]date of fungicide application

Table 2. Mean sugar beet root yield and recoverable sugar yield of roots harvested from field plots with varying severity of Cercospora leaf spot (CLS) symptoms in 2018, 2019, and 2020

Year	CLS rating[v] (1 to 10)	Yield (Mg ha^{-1})	Recoverable sugar (kg ha^{-1})
2018	3.0	68.8 ab[x,y]	9794 ab
	3.3	75.5 a	10530 a
	6.0	60.5 bc	7622 bc
	9.8	52.2 c	6372 c
2019	3.0	71.1 a	9761 a
	3.5	67.9 a	9158 a
	5.8	58.1 b	7569 b
	8.8	48.2 c	6128 b
2020	5.5	33.4 ns[z]	4798 ns
	6.5	23.5	3210
	8.0	28.5	3957
	10.0	32.1	4259

[v]plants rated on a 1 to 10 scale based on foliar CLS symptoms prior to harvest

[x]means within a column for a given production year that are followed by different letters are significantly different based on Fisher's LSD, with $\alpha = 0.05$ and $n = 4$

[y]p values for all comparisons are available in Supplementary Table S2

[z]ns, not significant ($\alpha = 0.05$)

Table 3. Mean respiration rate of stored sugar beet roots obtained from plants with varying levels of Cercospora leaf spot (CLS) disease symptoms in 2018, 2019, and 2020

Production year	CLS rating[v] (1 to 10)	Respiration rate (mg kg^{-1} h^{-1})			
		30 d	90 d	120 d	$\Delta_{(120\ d - 30\ d)}$[w,x]
2018	3.0	2.48 ns[x,y]	nd[z]	2.42 ns	-0.06
	3.3	2.71	nd	2.89	0.18
	6.0	2.41	nd	2.73	0.32
	9.8	2.76	nd	3.10	0.35
2019	3.0	2.18 ns	3.66 ns	4.16 ns	1.98
	3.5	2.55	3.55	3.70	1.16
	5.8	2.72	3.39	3.64	0.92
	8.8	2.94	3.48	4.22	1.28 *
2020	5.5	2.85 ns	2.38 ns	4.75 ns	1.90
	6.5	2.94	3.14	3.94	1.00
	8.0	2.79	2.41	4.70	1.91
	10.0	3.07	2.87	3.63	0.56

[v]plants rated on a 1 to 10 scale based on foliar CLS symptoms prior to harvest

[w]difference after 120 d in storage relative to value at 30 d. Significant changes denoted with asterisks ($\alpha = 0.05$)

[x]p values for all comparisons are available in Supplementary Tables S2 and S3

[y]ns, not significant ($\alpha = 0.05$)

[z]nd, not determined

Table 4. Mean sucrose and invert sugar concentration of sugar beet roots obtained from plants with varying levels of Cercospora leaf spot (CLS) disease symptoms in 2018, 2019, and 2020 as a function of time in storage

Year	CLS rating[u] (1 to 10)	Sucrose concentration (g kg^{-1})					Invert sugar concentration (g kg^{-1})				
		0 d	30 d	90 d	120 d	$\Delta_{(120\ d - 0\ d)}$[v,w]	0 d	30 d	90 d	120 d	$\Delta_{(120\ d - 0\ d)}$
2018	3.0	158 a[w,x]	158 a	nd[y]	157 ns[z]	-1	1.97 ns	0.90 a	nd	4.22 ns	2.25
	3.3	157 a	157 a	nd	152	-5	1.21	0.99 a	nd	2.88	1.66
	6.0	141 b	136 b	nd	137	-4	1.40	1.14 ab	nd	4.38	2.98
	9.8	137 b	140 b	nd	135	-2	1.33	1.42 b	nd	3.70	2.37
2019	3.0	145 a	144 a	142 a	145 a	0	0.11 ns	0.11 ns	0.15 ns	0.32 ns	0.21
	3.5	136 ab	140 ab	139 a	141 a	6	0.12	0.12	0.17	0.18	0.06
	5.8	135 ab	136 b	137 a	138 ab	3	0.12	0.11	0.13	0.21	0.09
	8.8	128 b	131 c	127 b	132 c	4	0.10	0.11	0.18	0.19	0.09
2020	5.5	152 a	152 a	156 a	147 a	-5	1.09 ns	0.88 ns	0.44 c	0.62 ns	-0.47 *
	6.5	145 bc	143 b	138 c	137 b	-8 *	0.90	0.74	1.16 a	0.61	-0.29
	8.0	148 ab	148 a	145 b	145 a	-3	0.84	0.61	0.82 b	0.69	-0.14
	10.0	140 c	141 b	135 c	132 c	-8	0.75	0.72	0.71 b	0.65	-0.11

[u]plants rated on a 1 to 10 scale based on foliar CLS symptoms prior to harvest

[v]difference after 120 d in storage relative to value at harvest (0 d). Significant changes denoted with asterisks ($\alpha = 0.05$)

[w]p values for all comparisons are available in Supplementary Tables S2 and S3

[x]means within a column for a given year that are followed by different letters are significantly different based upon Fisher's LSD ($\alpha = 0.05$; n = 4)

[y]nd, not determined

[z]ns, not significant ($\alpha = 0.05$)

Table 5. Mean sucrose loss to molasses (LTM) and recoverable sugar (RS) of sugar beet roots obtained from plants with varying levels of Cercospora leaf spot (CLS) disease symptoms in 2018, 2019, and 2020 as a function of time in storage

Year	CLS rating[u] (1 to 10)	Sucrose loss to molasses (g kg^{-1})					Recoverable sugar (kg Mg^{-1})				
		0 d	30 d	90 d	120 d	$\Delta_{(120\ d - 0\ d)}$[v,w]	0 d	30 d	90 d	120 d	$\Delta_{(120\ d - 0\ d)}$
2018	3.0	17.2 ns[x]	15.0 ns	nd[y]	17.0 ns	-0.2	143 a[w,z]	143 a	nd	140 ns	-3
	3.3	17.9	16.5	nd	15.2	-2.7	139 ab	140 a	nd	137	-2
	6.0	15.8	15.7	nd	14.7	-1.1	126 bc	120 b	nd	123	-3
	9.8	15.9	16.2	nd	14.7	-1.2	122 c	124 b	nd	121	-1
2019	3.0	9.0 b	8.0 ns	7.7 ns	11.8 ns	2.8	136 a	136 a	135 a	133 a	-2
	3.5	8.7 b	9.2	8.8	12.0	3.3 *	127 ab	131 ab	130 ab	129 a	2
	5.8	11.7 a	8.6	10.1	13.3	1.6	124 b	127 b	127 b	125 ab	1
	8.8	11.6 a	10.4	10.3	13.7	2.1	116 b	121 c	116 c	118 b	2
2020	5.5	9.1 a	7.2 b	8.9 ns	9.5 ns	0.4	143 a	145 a	148 a	137 a	-5
	6.5	9.5 a	7.5 b	9.5	9.5	-0.1	135 bc	135 b	129 c	128 b	-8 *
	8.0	6.8 b	7.6 b	8.8	8.8	2.0 *	141 ab	141 a	136 b	137 a	-5
	10.0	6.7 b	9.1 a	8.9	9.3	2.7 *	133 c	132 b	127 c	122 c	-11

[u]plants rated on a 1 to 10 scale based on foliar CLS symptoms prior to harvest

[v]difference after 120 d in storage relative to value at harvest (0 d). Significant changes denoted with asterisks ($\alpha = 0.05$)

[w]p values for all comparisons are available in Supplementary Tables S2 and S3

[x]ns, not significant ($\alpha = 0.05$)

[y]nd, not determined

[z]means within a column for a given year that are followed by different letters are significantly different based upon Fisher's LSD ($\alpha = 0.05$; n = 4)

Table S1. Cercospora leaf spot symptom severity rating scale of Jones and Windels (1991)[a]

Rating	Spots per leaf	Percent leaf area affected
1	1-5	0.1
2	6-12	0.35
3	13-25	0.75
4	26-50	1.5
5	51-75	2.5
6	nd[b]	3
7	nd	6
8	nd	12
9	nd	25
10	nd	50

[a]Jones, R. K., and Windels, C. E. 1991. A management model for Cercospora leaf spot of sugarbeets. Univ. Minn. Ext. Serv. Publication AG-FO-5643-E.

[b]nd, not determined

Table S2. Significance levels for differences in sugar beet root traits that were related to CLS symptom severity in 2018, 2019, and 2020

		p value[a]			
Year	Trait	harvest / 0 d	30 d	90 d	120 d
2018	root yield	0.015	--	--	--
	recoverable sugar per hectacre	0.005	--	--	--
	respiration rate	nd[b]	0.467	nd	0.710
	sucrose concentration	0.010	0.005	nd	0.174
	invert sugar concentration	0.169	0.040	nd	0.827
	sucrose loss to molasses	0.866	0.897	nd	0.861
	recoverable sugar per tonne	0.026	0.008	nd	0.252
2019	root yield	0.001	--	--	--
	recoverable sugar per hectacre	0.001	--	--	--
	respiration rate	nd	0.250	0.538	0.845
	sucrose concentration	0.034	0.000	0.000	0.016
	invert sugar concentration	0.129	0.920	0.768	0.324
	sucrose loss to molasses	0.035	0.151	0.125	0.604
	recoverable sugar per tonne	0.022	0.001	0.001	0.033
2020	root yield	0.511	--	--	--
	recoverable sugar per hectacre	0.466	--	--	--
	respiration rate	nd	0.910	0.603	0.651
	sucrose concentration	0.017	0.000	0.000	0.000
	invert sugar concentration	0.197	0.074	0.001	0.850
	sucrose loss to molasses	0.000	0.044	0.616	0.689
	recoverable sugar per tonne	0.031	0.000	0.000	0.000

[a]p value from ANOVA

[b]nd, not determined

Table S3. Significance levels for changes in sugar beet storage traits that were related to time in storage in 2018, 2019 and 2020

		p value[a]			
Year	Trait	Group 1[b]	Group 2	Group 3	Group 4
2018	respiration rate	0.901	0.596	0.627	0.452
	sucrose concentration	0.554	0.382	0.624	0.521
	invert sugar concentration	0.305	0.056	0.073	0.082
	sucrose loss to molasses	0.965	0.220	0.486	0.551
	recoverable sugar per tonne	0.723	0.582	0.733	0.749
2019	respiration rate	0.141	0.052	0.093	0.046
	sucrose concentration	0.931	0.332	0.401	0.359
	invert sugar concentration	0.149	0.123	0.059	0.059
	sucrose loss to molasses	0.129	0.045	0.443	0.203
	recoverable sugar per tonne	0.714	0.675	0.733	0.680
2020	respiration rate	0.130	0.228	0.095	0.504
	sucrose concentration	0.168	0.006	0.299	0.096
	invert sugar concentration	0.023	0.144	0.345	0.221
	sucrose loss to molasses	0.507	0.889	0.014	0.002
	recoverable sugar per tonne	0.154	0.004	0.127	0.053

[a]p values associated with t-tests used to determine whether a trait was affected by storage duration. For respiration rate, t-tests were used to identify if data collected after 30 days differed from that collected after 120 days. For all other traits, comparisons were made between data collected after 0 and 120 days in storage

[b]Groups refer to roots obtained from plants with four different levels of CLS disease symptoms that were utilized in each year of the study. Group 1 refers to roots from plants with the lowest CLS rating; Group 4 refers to roots from plants with the highest CLS rating