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# Effect of Pyraclostrobin on Postharvest Storage and Quality of Sugarbeet Harvested Before and After a Frost

L.G. Campbell<sup>1</sup>, K. Klotz Fugate<sup>1</sup>, and L.J. Smith<sup>2</sup>

<sup>1</sup>USDA-ARS, Northern Crop Science Laboratory,  
Fargo ND 58102-2765; and

<sup>2</sup>Northwest Research and Outreach Center,  
University of Minnesota, Crookston, MN 56717

Corresponding author: Larry Campbell (larry.campbell@ars.usda.gov)

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## ABSTRACT

Pyraclostrobin and other strobilurin fungicides have been reported to have beneficial effects on productivity that cannot be attributed to disease control. Enhanced frost tolerance is one such effect that has been observed for sugarbeet (*Beta vulgaris* L.) after a late season foliar pyraclostrobin application. This phenomenon has been reported in some, but not all, sugarbeet trials, and may potentially affect root storage properties, especially when roots are harvested after a frost. Research was conducted to determine the effect of late season pyraclostrobin application on storage properties of roots harvested before and after a frost. The effects of pyraclostrobin on postharvest respiration rate, invert sugar and raffinose concentration were variable across environments and time in storage, and there were no apparent relationships between the storage properties measured. However, foliar applied pyraclostrobin resulted in a small (3.7 kg Mg<sup>-1</sup>) but significant increase in average extractable sucrose concentration compared to no pyraclostrobin control treatments. This increase was observed in roots harvested before and after a damaging frost after storage for 0 or 90 days.

**Additional key words:** *Beta vulgaris*, strobilurin, fungicide, frost tolerance

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*Cercospora beticola* Sacc., the causal organism of Cercospora leaf spot (Jacobsen and Franc, 2009) in sugarbeet (*Beta vulgaris* L.), is capable of rapidly producing strains that are resistant to frequently used fungicides (Hanson, 2010). Pyraclostrobin, methyl 2-[1-(4-chlorophenyl)pyrazol-3-yloxymethyl]-N-methoxycarbanilate, was introduced in 2003 as a fungicide which could, when used in conjunction with fungicides having other modes of action, control Cercospora leaf spot (CLS) while mitigating the development of fungicide resistant or tolerant *C. beticola* strains (Khan and Smith, 2005; Secor et al., 2010). As pyraclostrobin use as a fungicide increased, yield increases associated with pyraclostrobin application that were unrelated to CLS control were reported (Ag Notes, 2009a). These 'plant health benefits' were especially apparent after a frost (Ag Notes 2007; 2010). In many cases visual differences between the canopies of pyraclostrobin-treated areas and areas that were untreated or treated with an alternative fungicide were not apparent before a frost. However, after a frost, damage to the canopy was visibly reduced for plants that received a pyraclostrobin application as the last fungicide in a CLS management program. In 2008, 91% of the American Crystal Sugar Company (Moorhead, MN) growers applied pyraclostrobin 30-40 days before harvest (Ag Notes, 2009b); some for both CLS control and plant health benefits and others solely for the increased productivity potential attributable to plant health benefits. In a British trial, increased sugar yields associated with foliar-applied pyraclostrobin were attributed to "direct action of fungicides on plant function" and not to "disease intervention, even if the disease was not visually apparent" (Ober et al., 2004). In contrast, pyraclostrobin was not found to increase root yield or sugar yield in recent Michigan and North Dakota trials (Hubbell, et al., 2009; Khan and Carlson, 2009), or decrease postharvest respiration rate in the North Dakota trial (Khan and Carlson, 2009).

Yield increases not attributable to disease control have been reported in other crops in response to pyraclostrobin and other strobilurin fungicides (Bartlett et al., 2002; Kohle et al., 2002), but have not been observed in all crop-environment combinations (Swoboda and Pedersen, 2009). Bertelsen et al. (2001) postulated that strobilurins differed from triazoles in their ability to prevent germination of pathogenic, non-pathogenic, and saprophytic fungi spores which, in turn, halted energy-requiring host defense responses. Most research, however, has focused on the effect of strobilurins on physiological processes. Physiological processes affected by strobilurins include ethylene biosynthesis, antioxidant enzyme activities, endogenous hormone levels, nitrate reductase activity, photosynthetic activity, and the carbon dioxide compensation point (Grossmann and Retzlaf, 1997; Glaab and Kaiser, 1999; Wu and von Tiedemann, 2001, 2002; Nason et al., 2007). The ability of strobilurins to maintain a viable crop canopy and delay senescence was noted frequently in comparisons with other fungicides.

In many areas with a temperate climate, lengthening the growing

season by delaying sugarbeet harvest increases both yield and the risk of frost damage to the roots (Smith 2001; Milford et al., 2002; Yonts et al., 2009). Frost damage to roots can have a substantial detrimental effect on postharvest storage and processing. Frost-related tissue damage allows sucrose to leach out and provides an entry site for microorganisms that produce invert sugars and gums (Shore et al., 1983; Campbell and Klotz, 2006). As temperature decreases, raffinose concentrations also increase (Wyse and Dexter, 1971; Haagenson et al. 2008). Invert sugars, gums, and raffinose all increase sucrose loss to molasses and can make roots unprocessable if present in sufficient quantities.

The research summarized in this report investigates whether the beneficial effects attributed to pyraclostrobin provide protection from frost damage and reduce frost-associated storage and processing problems. In the first of two experiments, the effects of pyraclostrobin application on sugarbeet root storage properties in relation to fungicide application time and exposure to frost were determined. A second experiment determined the effect of pyraclostrobin application on sugarbeet storage properties in relation to multiple harvest dates following a damaging frost.

## MATERIALS AND METHODS

### Plant materials and treatments.

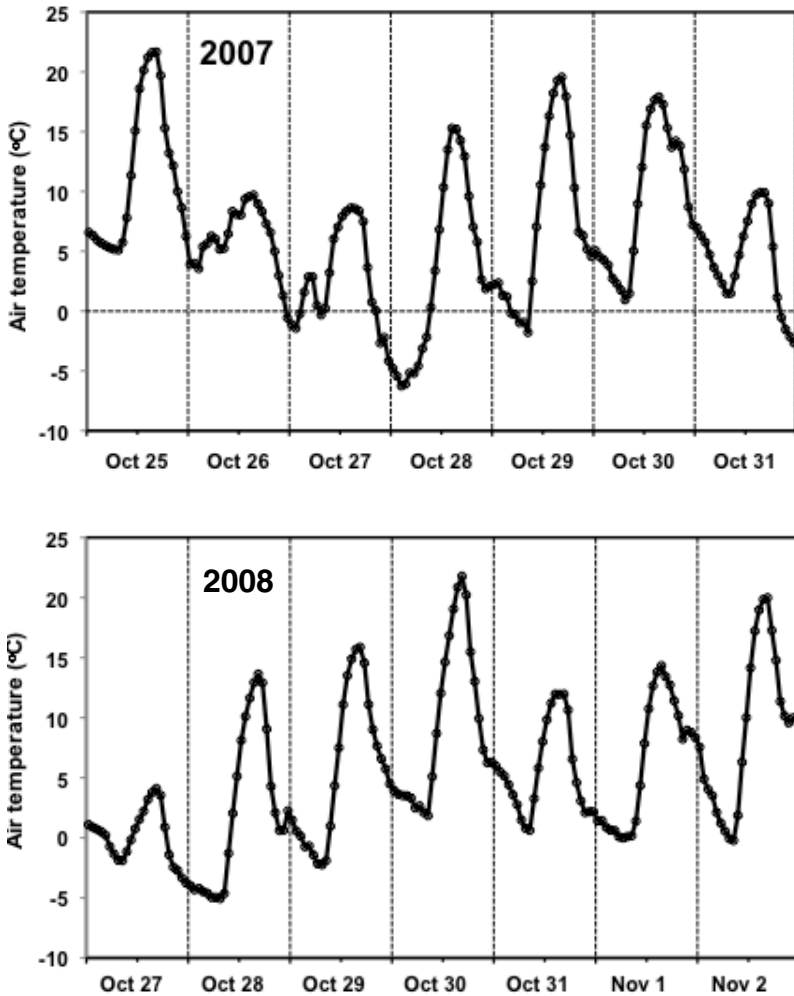
#### Experiment 1:

#### Effect of pyraclostrobin application timing and frost.

Field plots were established near Crookston, MN and Prosper, ND in 2007 and 2008. Experimental units were six-row plots, 10.6 m long, with rows spaced 56 cm apart. A randomized complete block design with three replicates and a 3 X 2 factorial treatment arrangement consisting of two pyraclostrobin application dates and an untreated check and two harvest dates was used. Pyraclostrobin (Headline<sup>®</sup>, 2.09 EC; BASF Corp., Raleigh, NC) treatments consisted of an early and a late foliar application, and were applied as broadcast treatments to the center four rows of each treated plot at a rate of 657 mL ha<sup>-1</sup>. Half of the plots were harvested prior to an anticipated frost; the other half were harvested immediately after a frost that caused visible damage to crown tissue (Milford et al., 2002; Yonts et al., 2009) of the untreated check.

In 2007, the early pyraclostrobin treatment was applied on 25 August; the late treatment was applied on 19 or 20 September. In 2008, early and late pyraclostrobin treatments were applied 27 August and 10 September, respectively. No additional fungicide treatments were applied to any plots in either year, and no symptoms of CLS or any other foliar disease were observed. At Prosper, pre-frost samples were harvested on 25 October 2007 and 27 October 2008. Post-frost samples were harvested 28 October in both years. Hourly temperature readings for Prosper are presented in Figure 1 (<http://ndawn.ndsu.nodak.edu>).

**Figure 1.** Hourly air temperatures from 25 to 30 October 2007 and 27 October to 2 November 2008, Prosper, North Dakota (<http://ndawn.ndsu.nodak.edu>)



At Crookston in 2007 and 2008, pre-frost samples were harvested 26 October. Post-frost samples were harvested the following day. At both locations, the two center rows of each plot were harvested. Plots at Prosper were hand harvested. At Crookston, plots were mechanically defoliated and immediately harvested with a commercial two-row lifter modified to harvest experimental plots.

**Experiment 2:****Effect of pyraclostrobin and harvest date following a frost**

Field trials were conducted near Prosper, ND in 2007 and 2008 using a split-plot design with three replicates. Main plots were 10.6 m long and 20 rows wide, with 56 cm between rows. The two main plot treatments were an untreated check and a broadcast pyraclostrobin treatment which was applied two times at a rate of 657 mL ha<sup>-1</sup> (2.09 EC) per application. Pyraclostrobin was applied on 25 August and 19 September in 2007 and 27 August and 10 September in 2008. Sub-plot treatments were date of harvest. Roots were hand harvested from a single row on each of 5 days in 2007 and 7 days in 2008. The first harvest date was prior to an anticipated frost; the remaining harvest dates occurred after a frost that resulted in visual damage to the crowns of the untreated plants (Milford et al., 2002; Yonts et al., 2009). The pre-frost harvest dates were 25 and 27 October in 2007 and 2008, respectively. The initial post-frost harvest date was 28 October in both years.

**Postharvest handling of roots**

For all trials, harvested roots were promptly transported to Fargo, ND. Roots were washed, randomized and 10 to 12 roots were combined to form an experimental unit. Each 10- to 12-root sample was stored in a perforated plastic produce bag at 4.5°C and 90-95% relative humidity.

**Respiration rate determination**

Respiration rate was determined by placing a 10- to 12-root sample in a 23-L sealed bucket equipped with inlet and outlet tubes through which ambient air was continuously circulated at a flow rate of 350 to 450 mL min<sup>-1</sup>. Buckets were equilibrated for 24 h, and the CO<sub>2</sub> concentration of the air from the exit tube was determined with an infrared CO<sub>2</sub> analyzer (Licor LI-6252, Lincoln, NE). The CO<sub>2</sub> concentration of ambient air from the exit tube of an empty bucket was subtracted from this measurement and the respiration rate was expressed as mg CO<sub>2</sub> produced per kg of roots per hour.

**Extractable sucrose and carbohydrate impurity determinations****Sample preparation**

In experiment 1, the 10- to 12-root samples were converted to brei using a tarehouse beet saw. The brei was rapidly mixed and a portion frozen for later sucrose analysis. A second portion of the brei was used to collect expressed juice by the method of Dexter et al. (1967), and the expressed juice was used to determine clear juice purity. In experiment 2, each of the 10 to 12 roots in a sample was quartered longitudinally, and one quarter section from each taproot was separated into crown and root portions by bisection at the lowest leaf scar. All crowns from a sample were combined and ground into brei using an electric

meat grinder (model #32, LEM Products, Harrison, OH). The brei was mixed for homogeneity and a portion frozen for later analysis of carbohydrate impurities. Root sections were similarly collected, prepared and used. The remaining three quarters of all roots from each experimental unit were combined, converted to brei using a beet saw and used for sucrose analysis and clear juice purity as described above.

### **Analytical methods**

Sucrose concentration was determined polarimetrically using aluminum sulfate-clarified brei samples (McGinnis, 1982). Clear juice purity was determined by the method of Dexter et al. (1967). Extractable sucrose concentration was calculated using sucrose concentration and purity measurements as previously described (Dexter et al., 1967) and expressed as kg sucrose per Mg of roots. Extractable sucrose concentration at harvest was expressed on a fresh weight basis. Extractable sucrose concentrations for the samples 90 days after harvest (DAH) were adjusted to account for slight changes in water content between sampling dates and corrected such that dry matter concentration 90 DAH was equal to that of the corresponding sample at harvest (0 DAH). Dry matter concentration was determined by weighing a portion (~20 g) of each brei sample before and after oven drying at 80°C.

Glucose, fructose and raffinose concentrations were determined colorimetrically using end point, enzyme-coupled assays and aluminum sulfate-clarified brei samples (Spackman and Cobb, 2001; Klotz and Martins, 2007). Invert sugar concentration was calculated by addition of glucose and fructose concentrations. Invert sugar and raffinose concentrations are reported as grams per 100 grams of sucrose (100 g S), which was measured colorimetrically on the same sample.

### **Statistical analysis**

The SAS GLM procedure (ver. 9.1, SAS Institute, Inc, Cary, NC) was used for the analyses of variance with  $\alpha = 0.10$ . The 10% significance level was chosen over the frequently used 5% level to reduce the probability of a type II error. As is the case with many agricultural experiments, treatments were selected because of frequent anecdotal reports of their beneficial effects. In situations in which it seems likely that the treatments are, in reality, unequal, the consequences of a type II error (declaring two treatments equal when, in fact, they are different) frequently are at least as important as the consequences of a type I error (declaring two treatments different when, in fact, they are equal) (Carmer, 1976; Chew, 1976). Increasing the protection against a type I error ( $\alpha$ ) increases the probability of committing a type II error. In practice, the economic consequence of either type of error depends upon the relative cost and benefits of a treatment.

Linear contrasts provided a comparison between the average of the early and late pyraclostrobin applications with the untreated check in the application-timing trial (Experiment 1). Three linear contrasts were used in the date-of-harvest trial (Experiment 2) to compare data

for the before-frost harvest (average of pyraclostrobin and untreated) with the average of all after-frost harvest dates and to compare data for the pyraclostrobin treatment with the untreated check harvested before a frost and for the average of all after-frost harvest dates. The “estimate” function of the SAS GLM procedure was used to estimate actual differences when the corresponding linear contrast was significant ( $P = 0.10$ ). A significant F-test for treatments or harvest date is not a prerequisite for determining the significance of the linear contrast (Chew, 1976).

A paired t-test was used to obtain a combined estimate of the difference between a pyraclostrobin treatment and untreated controls. The 56 paired comparisons (means of three reps) in both trials, including all application dates and harvest dates were included in the analysis (SAS PROC TTEST). These paired observations also were the basis for the regression of the extractable sucrose concentration when pyraclostrobin was applied relative to extractable sucrose concentration without pyraclostrobin (SAS PROC REG).

## RESULTS

### Experiment 1:

#### Effect of pyraclostrobin application timing and frost.

At Crookston, the air temperature prior to the post-frost harvest in 2007 was  $-2^{\circ}\text{C}$  or lower for 4 h with a low of  $-3.4^{\circ}\text{C}$  (<http://www.nwroc.umn.edu/weather>); in 2008, the low temperature prior to the after-frost harvest was  $-6.1^{\circ}\text{C}$  and was  $-2^{\circ}\text{C}$  or lower for 5 h at Crookston. The minimum air temperature prior to the post-frost harvest at Prosper was  $-7^{\circ}\text{C}$  and  $-5^{\circ}\text{C}$  in 2007 and 2008, respectively. In both years, the temperature was  $-2^{\circ}\text{C}$  or lower for at least 12 h prior to harvest (Fig. 1). Visual differences in the canopies at the time of harvest due to pyraclostrobin treatment were not apparent either before or after a frost in any environment.

Environment, harvest date, and the environment X harvest date interaction effects were significant for respiration rate 30 DAH (Table 1). Environment means ranged from  $3.80 \text{ mg kg}^{-1} \text{ h}^{-1}$  at Crookston in 2007 to  $6.33 \text{ mg kg}^{-1} \text{ h}^{-1}$  at Crookston in 2008. Respiration rates of roots from Prosper were  $4.70$  and  $4.98 \text{ mg kg}^{-1} \text{ h}^{-1}$  in 2007 and 2008, respectively ( $\text{LSD}_{0.10} = 0.41$ ). Roots harvested before a frost had an average respiration rate of  $4.20 \text{ mg kg}^{-1} \text{ h}^{-1}$ , compared to an average respiration rate of  $5.71$  ( $\text{LSD}_{0.10} = 0.21$ ) for roots harvested after a frost (Table 2). The respiration rate of roots harvested after a frost was approximately twice that of roots harvested before a frost at Prosper in 2007. The other three environments had respiration rate increases of 13% to 34% after the frost. Although the pyraclostrobin treatment effect was not significant, the significant contrast ( $P = 0.07$ ) between the average of the early and late applications and the untreated check (Table 1) indicated that a foliar application of pyraclostrobin decreased the respiration rate 30 DAH by  $0.25 \text{ mg kg}^{-1} \text{ h}^{-1}$  ( $\text{SE} = 0.13$ ).

**Table 1.** Sources of variation, value of F, and significance level of F-test, P(F), for respiration rate 30 and 90 days after harvest (DAH) and extractable sucrose concentration 0 and 90 DAH of sugarbeet with and without foliar applied pyraclostrobin harvested before and after a damaging frost, Crookston, MN and Prosper, ND, 2007-2008.

Source of variation	Respiration Rate				Extractable Sucrose			
	30 DAH		90 DAH		0 DAH		90 DAH	
	F	P(F)	F	P(F)	F	P(F)	F	P(F)
Environment (Env)	44.59	<0.01	48.80	<0.01	23.30	<0.01	4.62	0.04
Pyraclostrobin (Pyr)	1.72	0.19	3.57	0.04	2.33	0.11	1.19	0.32
Harvest date (Har)	144.60	<0.01	87.12	<0.01	2.85	0.09	0.30	0.59
Env X Pyr	0.69	0.66	3.40	0.01	0.23	0.96	0.42	0.86
Pyr X Har	0.27	0.76	2.58	0.09	1.45	0.25	1.06	0.36
Env X Har	24.74	<0.01	14.71	<0.01	0.38	0.77	1.62	0.20
Env X Pyr X Har	2.23	0.60	2.97	0.02	1.35	0.26	0.98	0.45
Pyr Contrast†	3.44	0.07	3.77	0.06	4.61	0.04	2.37	0.13

† Pyr contrast = difference between the average of the early plus late pyraclostrobin treatments and the untreated check; [(early + late) / 2 - untreated check].



All main and interaction effects were significant ( $P < 0.10$ ) for respiration rate 90 DAH (Table 1). Trends observed in the 90-DAH respiration rates were similar to those recorded 30 DAH. Environment mean respiration rates ranged from  $3.55 \text{ mg kg}^{-1} \text{ h}^{-1}$  for Crookston in 2007 to  $6.66 \text{ mg kg}^{-1} \text{ h}^{-1}$  for Crookston in 2008. Respiration rates of roots from Prosper were  $3.60$  and  $4.42 \text{ mg kg}^{-1} \text{ h}^{-1}$  in 2007 and 2008, respectively ( $\text{LSD}_{0.10} = 0.55$ ). Roots harvested before a frost had an average respiration rate of  $4.04 \text{ mg kg}^{-1} \text{ h}^{-1}$ , compared to an average respiration rate of  $5.08 \text{ mg kg}^{-1} \text{ h}^{-1}$  ( $\text{LSD}_{0.10} = 0.19$ ) for roots harvested after a frost (Table 2). Roots harvested from the early pyraclostrobin application had a lower respiration rate ( $4.36 \text{ mg kg}^{-1} \text{ h}^{-1}$ ) than roots from either the late pyraclostrobin application ( $4.61 \text{ mg kg}^{-1} \text{ h}^{-1}$ ) or the untreated check ( $4.71 \text{ mg kg}^{-1} \text{ h}^{-1}$ ;  $\text{LSD}_{0.10} = 0.23$ ). Furthermore, the  $0.23 \text{ mg kg}^{-1} \text{ h}^{-1}$  ( $\text{SE} = 0.12$ ) difference between the average of the early and late application dates and the untreated check indicated that a foliar application of pyraclostrobin decreased the respiration rate 90 DAH ( $P = 0.06$ ). The unique response of roots from Crookston in 2008 contributed to the significance of the pyraclostrobin treatment X environment X harvest date interaction. In all environments, roots harvested after a frost had higher respiration rates 90 DAH than roots harvested before a frost (Table 2) and, with the exception of Crookston in 2008, the increases in respiration rate were similar for all three treatments within an environment. Pyraclostrobin treatment had little or no apparent effect on the respiration rate of roots harvested before a frost at Crookston in 2008. However, the respiration rate increase due to frost was less for the early pyraclostrobin application ( $0.65 \text{ mg kg}^{-1} \text{ h}^{-1}$ ) than for the late pyraclostrobin application ( $3.03 \text{ mg kg}^{-1} \text{ h}^{-1}$ ) or the untreated check ( $2.44 \text{ mg kg}^{-1} \text{ h}^{-1}$ ).

Environment, harvest date, and the difference between the average of the two pyraclostrobin treatments and the untreated check were the only significant sources of variation for extractable sucrose concentration at harvest (Table 1). Extractable sucrose concentrations ranged from  $150 \text{ kg Mg}^{-1}$  at Prosper in 2007 to  $185 \text{ kg Mg}^{-1}$  at Crookston in 2008 (Table 2). The extractable sucrose concentration of roots from Crookston in 2007 and Prosper in 2008 was  $173$  and  $171 \text{ kg Mg}^{-1}$ , respectively ( $\text{LSD}_{0.10} = 8$ ). The difference in extractable sucrose concentration between the harvest dates was significant ( $\text{LSD}_{0.10} = 1.5$ ) but small;  $171 \text{ kg Mg}^{-1}$  before a frost versus  $169 \text{ kg Mg}^{-1}$  after a frost. The significant difference between the average of the pyraclostrobin treatments and the untreated check indicated that a pyraclostrobin application increased extractable sucrose concentration at harvest by an average of  $3.4 \text{ kg Mg}^{-1}$  ( $\text{SE} = 1.6$ ).

The only significant source of variation for extractable sucrose concentration 90 DAH was that due to environment (Table 1). Environment means ranged from  $136 \text{ kg Mg}^{-1}$  for Prosper in 2007 to  $162 \text{ kg Mg}^{-1}$  for Crookston in 2007. The extractable sucrose concentration of roots from Prosper in 2008 and Crookston in 2007 was  $144$  and  $156 \text{ kg Mg}^{-1}$ , respectively ( $\text{LSD}_{0.10} = 14$ ). The grand-mean extractable sucrose

**Table 2.** Effects of foliar applied pyraclostrobin on sugarbeet harvested before and after a damaging frost on respiration rate 30 and 90 days after harvest (DAH) and extractable sucrose concentration 0 and 90 DAH, Crookston, MN and Prosper, ND, 2007-2008.

Pyraclostrobin treatment	Crookston, MN				Prosper, ND				Mean		Mean
	2007		2008		2007		2008		Before	After	
	Before	After	Before	After	Before	After	Before	After			
<b>Respiration rate 30 DAH, mg kg<sup>-1</sup> h<sup>-1</sup></b>											
Pyraclostrobin-early	3.85†	3.73	5.39	7.48	2.62	6.27	4.57	5.01	4.11	5.62	4.87
Pyraclostrobin-late	3.31	4.35	5.12	7.11	3.50	5.70	4.75	5.11	4.17	5.57	4.87
No pyraclostrobin	3.53	4.00	5.76	7.12	3.24	6.85	4.69	5.74	4.30	5.93	5.12
Mean	3.57	4.03	5.42	7.24	3.12	6.27	4.67	5.29	4.20	5.71	4.95
<b>Respiration rate 90 DAH, mg kg<sup>-1</sup> h<sup>-1</sup></b>											
Pyraclostrobin-early	3.36	3.57	5.57	6.22	2.88	4.57	4.24	4.46	4.02	4.70	4.36
Pyraclostrobin-late	3.30	4.03	5.46	8.49	2.91	4.17	4.21	4.29	3.97	5.25	4.61
No pyraclostrobin	3.31	3.77	5.87	8.31	2.88	4.21	4.51	4.84	4.14	5.28	4.71
Mean	3.33	3.79	5.64	7.67	2.89	4.32	4.32	4.53	4.04	5.08	4.59
<b>Extractable sucrose 0 DAH, kg Mg<sup>-1</sup></b>											
Pyraclostrobin-early	179	171	183	190	155	149	173	169	173	170	171
Pyraclostrobin-late	175	172	187	187	147	156	173	171	170	171	171
No pyraclostrobin	173	171	185	176	151	146	172	167	170	165	168
Mean	176	171	185	184	151	150	173	169	171	169	170

	<b>Extractable sucrose 90 DAH, kg Mg<sup>-1</sup></b>										
Pyraclostrobin-early	163	163	161	156	142	132	148	145	153	149	151
Pyraclostrobin-late	165	165	163	158	133	144	138	146	149	153	151
No pyraclostrobin	167	167	156	142	143	123	142	145	149	144	147
Mean	165	165	160	152	139	133	143	145	150	149	149

†Mean of three replicates; Relevant F-tests and their corresponding probabilities are presented in Table 1.

**Table 3.** Sources of variation, significance level of F-tests P(F), and LSDs (P = 0.10) for comparing main and interaction effects, and standard errors (SE) of contrasts for date-of-harvest trial (Experiment 2), Prosper, ND 2007-2008.

Year	Variable	Source of variation							Contrasts					
		Treatment		Harvest date		Treatment X harvest date			Before frost vs after frost <sup>‡</sup>		Pyraclostrobin vs none			
		DAH	LSD	P(F)	LSD	P (F)	LSD <sup>†</sup>	P(F)	SE	P (F)	SE	P(F)	SE	P(F)
<b>2007</b>														
	Respiration	30	ns	0.16	0.52	0.02	ns	0.11	ns	0.71	ns	0.39	0.21	0.02
		60	0.16	0.10	0.28	0.01	0.48	0.03	0.12	<0.01	0.22	0.09	ns	0.36
		90	ns	0.49	ns	0.43	ns	0.97	ns	0.31	ns	0.65	ns	0.74
	Ext. sucrose	0	ns	0.86	ns	0.29	ns	0.90	ns	0.55	ns	0.44	ns	0.99
		90	ns	0.89	8.90	0.04	ns	0.65	4.02	0.01	ns	0.41	ns	0.81
	Invert sugar													
	Crown	0	ns	0.44	0.88	0.04	ns	0.81	0.40	0.05	ns	0.92	ns	0.11
		90	ns	0.36	0.46	0.02	ns	0.65	ns	0.21	0.38	0.09	ns	0.52
	Roots	0	0.06	0.01	ns	0.59	ns	0.41	ns	0.60	ns	0.75	ns	0.48
		90	ns	0.37	ns	0.48	ns	0.46	ns	0.90	ns	0.20	ns	0.40
	Raffinose													
	Crown	0	ns	0.70	ns	0.22	ns	0.24	ns	0.17	ns	0.26	ns	0.98
		90	ns	0.35	ns	0.56	1.31	0.03	ns	0.42	ns	0.58	ns	0.22
	Root	0	ns	0.68	ns	0.47	ns	0.90	ns	0.30	ns	0.48	ns	0.75
		90	ns	0.55	ns	0.63	0.63	0.02	ns	0.17	ns	0.47	ns	0.61

<b>2008</b>														
Respiration	30	ns	0.40	ns	0.21	0.81	0.09	ns	0.88	ns	0.40	0.12	0.09	
	60	ns	0.62	0.57	0.01	ns	0.62	ns	0.28	ns	0.86	ns	0.18	
	90	ns	0.46	ns	0.28	ns	0.78	ns	0.39	ns	0.57	ns	0.26	
Ext. sucrose	0	ns	0.48	4.35	0.01	ns	0.88	2.02	<0.01	ns	0.79	1.53	0.04	
	90	ns	0.36	ns	0.85	ns	0.78	ns	0.37	ns	0.76	3.04	0.02	
Invert sugar														
Crown	0	ns	0.19	ns	0.49	ns	0.21	ns	0.45	ns	0.28	0.32	0.08	
	90	ns	0.41	ns	0.20	ns	0.38	ns	0.88	ns	0.19	ns	0.62	
Roots	0	ns	0.33	0.84	0.02	ns	0.57	0.37	0.04	ns	0.81	0.28	0.02	
	90	ns	0.52	1.36	0.09	ns	0.70	ns	0.45	ns	0.60	ns	0.68	
Raffinose														
Crown	0	ns	0.24	0.08	0.06	0.14	0.01	0.04	<0.01	0.07	0.09	ns	0.94	
	90	ns	0.12	ns	0.38	ns	0.85	ns	0.58	ns	0.74	ns	0.50	
Root	0	ns	0.68	0.09	0.02	ns	0.48	0.04	0.01	0.08	0.06	ns	0.89	
	90	ns	0.98	ns	0.99	ns	0.63	ns	0.91	ns	0.29	ns	0.66	

† LSD<sub>0.10</sub> for comparing difference between the pyraclostrobin treatment and the untreated check for same, or different, harvest dates.

‡ Difference between mean of roots harvested from the pyraclostrobin treatment and the untreated check before a damaging frost and the mean of roots harvested on all the after-frost dates from both the pyraclostrobin treatment and untreated check.

§ Difference between the pyraclostrobin treatment and untreated check for roots harvested before a damaging frost.

¶ Difference between the pyraclostrobin treatment and untreated check for roots harvested after a damaging frost, all harvest dates.

concentration 90 DAH was 149 kg Mg<sup>-1</sup>; 21 kg Mg<sup>-1</sup> less than the 170 kg Mg<sup>-1</sup> at harvest (Table 2).

### **Experiment 2:**

#### **Effect of pyraclostrobin and harvest date following a frost.**

The first post-frost roots were harvested 28 October in 2007 and 2008 after air temperatures dropped to -7°C and -5°C, respectively. In both years the temperature was below -2°C for approximately 12 hours (Fig. 1). In 2007, daily average soil temperature (10-cm depth, bare soil) near the Prosper site on 25 October was 7°C, decreased to 5°C on 28 October, and fluctuated between 7°C and 8°C for the next three days (<http://ndawn.ndsu.nodak.edu>). The average soil temperature on 25 October 2008 was 6°C, decreased to 3°C on 27-28 October and then increased to 7°C by 2 November.

Harvest date and the contrast between pyraclostrobin and no-pyraclostrobin treatments for roots harvested after a frost were the only significant sources of variation for respiration rate 30 DAH in 2007 (Table 3). Roots harvested before a frost had a respiration rate of 3.76 mg kg<sup>-1</sup> h<sup>-1</sup> (Table 4); roots harvested four days later had a respiration rate of 4.38 mg kg<sup>-1</sup> h<sup>-1</sup> (LSD<sub>0.10</sub> = 0.52). Roots harvested after a frost from plots that were treated with pyraclostrobin had a 0.57 mg kg<sup>-1</sup> h<sup>-1</sup> (SE = 0.21) lower respiration rate than untreated roots harvested after a frost. In 2007, the main effects, treatment and harvest date, and the interaction between the two were significant for respiration rate 60 DAH (Table 3). The average respiration rate over all harvest dates for the pyraclostrobin treatment was 3.47 mg kg<sup>-1</sup> h<sup>-1</sup>, compared to 3.31 mg kg<sup>-1</sup> h<sup>-1</sup> (LSD<sub>0.10</sub> = 0.16) for roots from untreated areas (Table 4). The average respiration rate of roots harvested after a frost was 0.66 mg kg<sup>-1</sup> h<sup>-1</sup> (SE = 0.13) greater than the respiration rate of roots harvested before a frost. The respiration rate of roots harvested before a frost from untreated plots was 0.39 mg kg<sup>-1</sup> h<sup>-1</sup> (SE = 0.22) lower than the respiration rate of roots from plots treated with pyraclostrobin, 60 DAH. Differences among treatments, harvest dates, and the interactions between treatments and harvest dates were not significant for respiration rate 90 DAH in 2007 (Tables 3 & 4).

Only the treatment X harvest date interaction and the contrast between respiration rates of roots from the pyraclostrobin treatment and the no-pyraclostrobin treatment harvested after a frost were significant 30 DAH in 2008 (Table 3). The average respiration rate of roots from the pyraclostrobin treatment harvested after a frost was 0.22 mg kg<sup>-1</sup> h<sup>-1</sup> (SE = 0.12) less than the average respiration rate of roots from the no-pyraclostrobin treatment harvested after a frost (Table 4). Harvest date was the only significant source of variation for respiration rate 60 DAH. Roots harvested 30 October 2008 had an average respiration rate of 5.85 mg kg<sup>-1</sup> h<sup>-1</sup> 60 DAH; the other harvest dates ranged from 4.36 mg kg<sup>-1</sup> h<sup>-1</sup> to 4.68 mg kg<sup>-1</sup> h<sup>-1</sup> (LSD<sub>0.10</sub> = 0.57). Respiration rates for both the pyraclostrobin (5.76 mg kg<sup>-1</sup> h<sup>-1</sup>) and the no-pyraclostrobin (5.93 mg kg<sup>-1</sup> h<sup>-1</sup>) treatments 60 DAH were highest for roots

harvested 30 October (Table 4). Air temperatures 25 hours before the 30 October harvest ranged from 1°C to 16°C and remained above 0°C for the remaining harvest dates (Fig. 1). Minimum temperatures of -5°C and -2°C were recorded on 28 and 29 October, respectively. None of the differences in respiration rate among treatments or harvest dates or interactions between treatment and harvest date were significant 90 DAH (Table 3). Harvest date and the contrast between roots harvested before a frost and the average of the four post-frost harvest dates 90 DAH were the only significant sources of variation for extractable sugar concentration in 2007 (Table 3). Roots harvested before a frost (25 October) had an average extractable sucrose concentration of 150 kg Mg<sup>-1</sup> 90 DAH. The average extractable sucrose concentration of roots harvested after a frost ranged from 134 kg Mg<sup>-1</sup> for 31 October to 144 kg Mg<sup>-1</sup> for roots harvested 29 October (LSD<sub>0.10</sub> = 9) with an average of 138 kg Mg<sup>-1</sup>, 11 kg Mg<sup>-1</sup> (SE = 4.0) less than that for roots harvested before a frost.

In 2008, harvest date, the contrast between roots harvested before a frost and the average of the six post-frost harvest dates, and the contrast between roots from the pyraclostrobin treatment and the untreated check harvested after a frost were significant sources of variation for extractable sucrose concentration at harvest (0 DAH). The only significant source of variation for extractable sucrose concentration 90 DAH was the contrast between roots from the pyraclostrobin treatment and the untreated check harvested after a frost (Table 3). The initial (0 DAH) extractable sucrose concentration of roots harvested before a frost was 158 kg Mg<sup>-1</sup> and roots harvested after a frost had extractable sucrose concentrations ranging from 160 kg Mg<sup>-1</sup> to 176 kg Mg<sup>-1</sup> (LSD<sub>0.10</sub> = 5). After 90 days in storage (90 DAH), differences among harvest dates were not significant and ranged from 140 kg Mg<sup>-1</sup> to 146 kg Mg<sup>-1</sup> for roots harvested after a frost, compared to 147 kg Mg<sup>-1</sup> for roots harvested before a frost. The average extractable sucrose concentration of roots from the pyraclostrobin treatment that were harvested after a frost was 3.4 kg Mg<sup>-1</sup> (SE = 1.5) greater than that of roots harvested from untreated plots after a frost, 0 DAH. By 90 DAH the extractable sucrose concentration of roots harvested after a frost from the pyraclostrobin treatment in 2008 was 7.7 kg Mg<sup>-1</sup> (SE = 3.0) greater than that of roots from the untreated check harvested after a frost.

In 2007, harvest date and the contrast between the before frost harvest and the average of the four after frost harvest dates were the only significant sources of variation for invert sugar concentration of crowns at harvest (0 DAH). Ninety days after harvest, harvest date and the differences between the invert sugar concentrations in the crowns of roots harvested before a frost from the pyraclostrobin treatment and roots harvested before a frost from the no-pyraclostrobin treatment were significant. The invert sugar concentration 0 DAH of crowns of roots harvested before a frost was 2.54 g (100 g S)<sup>-1</sup>, compared to a range of 0.87 g (100 g S)<sup>-1</sup> (28 October) to 2.15 g (100 g S)<sup>-1</sup>,

**Table 4.** Effects of foliar applied pyraclostrobin (Pycsn) and harvest date on respiration rate of sugarbeet 30, 60, and 90 days after harvest (DAH) and extractable sucrose concentration 0 and 90 DAH, Prosper, ND, 2007-2008.

Year / Harvest date	Respiration rate, mg kg <sup>-1</sup> h <sup>-1</sup>						Extractable sucrose, kg Mg <sup>-1</sup>			
	30 DAH		60 DAH		90 DAH		0 DAH		90 DAH	
	Pycsn	None	Pycsn	None	Pycsn	None	Pycsn	None	Pycsn	None
<b>2007</b>										
Oct 25	3.58 <sup>†</sup>	3.95	3.06	2.67	3.17	3.03	154	150	153	146
28	3.50	4.02	2.90	3.01	3.24	3.08	156	157	139	132
29	3.24	4.79	3.71	4.13	3.45	3.47	155	154	145	144
30	3.06	3.43	4.07	3.74	3.31	3.39	157	154	137	144
31	4.46	4.30	3.62	3.01	3.22	3.09	147	150	132	136
Mean	3.57	4.10	3.47	3.31	3.28	3.21	154	153	141	140
<b>2008</b>										
Oct 27	4.49	4.75	4.50	4.41	4.32	4.08	158	157	148	146
28	4.73	4.75	4.53	4.83	4.12	4.07	163	157	143	137
29	4.21	4.17	4.70	4.40	4.01	4.58	172	168	149	138
30	4.35	5.16	5.76	5.93	4.59	4.92	177	172	148	136
31	4.53	4.66	4.39	4.34	4.28	4.26	177	171	146	145
Nov 1	4.84	4.47	4.13	4.94	4.71	4.64	169	168	153	139
2	4.23	5.01	4.11	4.75	4.09	4.56	176	176	143	140
Mean	4.49	4.71	4.56	4.80	4.30	4.44	170	167	147	140

<sup>†</sup> Mean of three replicates; LSDs for comparing means of interest are presented in Table 3 when the probability of a significant F-test was less than or equal to 10%.



(29 October) for roots harvested after a frost ( $\text{LSD}_{0.10} = 0.88$ ). Crowns of roots harvested before a frost had  $0.85 \text{ g (100 g S)}^{-1}$  ( $\text{SE} = 0.40$ ) more invert sugar than the average of those harvested after a frost, 0 DAH. In contrast to the relatively high invert sugar concentrations of the before frost samples 0 DAH, the invert sugar concentration 90 DAH in the crowns of roots harvested prior to a frost was relatively low,  $1.13 \text{ g (100 g S)}^{-1}$ , with a range of  $0.94 \text{ g (100 g S)}^{-1}$  (28 October) to  $1.90 \text{ g (100 g S)}^{-1}$  (31 October) for harvests after a frost ( $\text{LSD}_{0.10} = 0.46$ ). The invert sugar concentration 90 DAH of crowns from the pyraclostrobin treatment harvested before a frost was  $0.68 \text{ g (100 g S)}^{-1}$  ( $\text{SE} = 0.38$ ) greater than the concentration in crowns of roots from the untreated plots harvested before a frost. Treatment 0 DAH was the only significant factor for invert sugar concentration in the true root in 2007. The average invert sugar concentration 0 DAH of roots from the pyraclostrobin treatment was  $1.25 \text{ g (100 g S)}^{-1}$ , compared to  $1.44 \text{ g (100 g S)}^{-1}$  for roots from untreated plots ( $\text{LSD}_{0.10} = 0.06$ ). The average invert sugar concentration of true root tissue 90 DAH was  $1.03 \text{ g (100 g S)}^{-1}$ .

The only significant source of variation for invert sugar concentration of the crowns 0 DAH in 2008 was the contrast between the pyraclostrobin treatment and the no-pyraclostrobin treatment harvested after a frost. The average invert sugar concentration of the crown tissue 0 DAH from the pyraclostrobin treatment harvested after a frost was  $0.59 \text{ g (100 g S)}^{-1}$  ( $\text{SE} = 0.32$ ) lower than the concentration in crowns from untreated plots harvested after a frost. In 2008, differences among invert sugar concentrations of true root tissue attributable to harvest date were significant at harvest (0 DAH) and 90 DAH. The contrast between the before frost harvest and the average of the six after frost harvests and the difference between the pyraclostrobin treatment and the no-pyraclostrobin treatment for roots harvested after a frost were significant 0 DAH. Invert sugar concentrations of root tissue 0 DAH ranged from  $0.64 \text{ g (100 g S)}^{-1}$  for roots harvested before a frost to  $2.50 \text{ g (100 g S)}^{-1}$  for roots harvested 29 October ( $\text{LSD}_{0.10} = 0.84$ ). Ninety days after harvest, the invert sugar concentration in root tissue ranged from  $1.81 \text{ g (100 g S)}^{-1}$  for the 2 November harvest to  $3.92 \text{ g (100 g S)}^{-1}$  for the 1 November harvest; the concentration in roots harvested before a frost (27 October) was  $3.28 \text{ g (100 g S)}^{-1}$  ( $\text{LSD}_{0.10} = 1.37$ ). Root tissue of roots harvested before a frost had  $0.82 \text{ g (100 g S)}^{-1}$  ( $\text{SE} = 0.37$ ) less invert sugar than the average of those harvested after a frost, 0 DAH. The invert sugar concentration 0 DAH of root tissue from the pyraclostrobin treatment harvested after a frost was  $0.74 \text{ g (100 g S)}^{-1}$  ( $\text{SE} = 0.28$ ) greater than the concentration in root tissue of roots from the untreated plots harvested after a frost.

No consistent pattern of relative raffinose concentration was observed in 2007 and 2008. In 2007, the treatment X harvest date interaction was significant for both the crowns and true roots 90 DAH (Table 3). This was largely due to a relatively high raffinose concentration in the crowns of roots from the pyraclostrobin treatment harvested on 29 October and a relatively low concentration in the true

**Table 5.** Effects of foliar applied pyraclostrobin (Pycsn) and harvest date on raffinose and invert sugar concentration in crown and true-root tissue 0 and 90 days after harvest (DAH), Prosper, ND 2007-2008.

Year / Harv. date	Crown				Root			
	0 DAH		90 DAH		0 DAH		90 DAH	
	Pycsn	None	Pycsn	None	Pycsn	None	Pycsn	None
<b>2007</b>	<b>Invert sugar, g (100 g sucrose)<sup>-1</sup></b>							
Oct. 25	2.51 <sup>†</sup>	2.58	1.47	0.79	1.67	1.41	0.68	1.32
28	0.90	0.83	1.12	0.77	0.87	0.59	0.98	0.88
29	2.60	1.70	1.31	1.34	0.99	2.09	0.78	1.70
30	2.11	1.23	1.44	1.47	0.94	1.89	0.61	0.79
31	2.40	1.80	2.00	1.79	1.80	1.08	1.35	1.17
Mean	2.10	1.63	1.46	1.23	1.25	1.44	0.88	1.17
	<b>Raffinose, g (100 g sucrose)<sup>-1</sup></b>							
Oct. 25	0.21	0.66	1.57	1.91	0.20	0.47	0.97	1.20
28	0.60	0.55	1.64	0.78	0.38	0.31	1.15	0.52
29	0.88	0.38	2.77	0.90	0.80	0.75	1.11	0.56
30	1.16	1.02	1.34	1.40	0.54	0.56	0.92	0.91
31	0.33	1.01	0.86	1.98	0.40	0.74	0.33	1.18
Mean	0.64	0.72	1.64	1.40	0.46	0.57	0.87	0.88
<b>2008</b>	<b>Invert sugar, g (100 g sucrose)<sup>-1</sup></b>							
Oct. 27	1.65	0.79	1.71	3.30	0.72	0.55	3.59	2.98
28	0.73	1.63	2.51	1.00	0.94	0.90	2.62	1.58
29	1.71	1.69	3.07	3.56	2.92	2.09	2.36	2.17
30	0.97	2.70	1.95	2.14	1.24	1.35	3.71	3.57
31	1.38	2.82	1.84	3.93	2.49	0.97	2.51	3.77
Nov. 1	1.30	0.98	2.68	3.62	1.82	0.78	4.63	3.20
2	1.38	1.19	1.70	0.96	1.54	0.44	1.62	2.01
Mean	1.30	1.69	2.21	2.64	1.67	1.01	3.01	2.75
	<b>Raffinose, g (100 g sucrose)<sup>-1</sup></b>							
Oct. 27	0.17	0.05	0.07	0.12	0.34	0.19	0.04	0.14
28	0.14	0.34	0.06	0.05	0.10	0.18	0.07	0.08
29	0.36	0.17	0.14	0.02	0.05	0.06	0.14	0.03
30	0.20	0.18	0.06	0.14	0.25	0.18	0.11	0.11
31	0.26	0.22	0.10	0.09	0.21	0.20	0.06	0.14
Nov. 1	0.21	0.26	0.17	0.29	0.21	0.21	0.11	0.13
2	0.21	0.19	0.17	0.34	0.13	0.15	0.16	0.05
Mean	0.22	0.20	0.11	0.15	0.19	0.17	0.10	0.10

<sup>†</sup>Mean of three replicates; LSD's for comparing means of interest are presented in Table 3 when the probability of a significant F-test was less than or equal to 10%.

roots from the pyraclostrobin treatment harvested on 31 October (Table 5). Prior to storage (0 DAH), harvest date, the treatment X harvest date interaction, and contrasts between the raffinose concentration of crowns of roots harvested before a frost and the average of roots harvested after a frost and the difference in the concentration of raffinose in the crowns of roots from the pyraclostrobin treatment and the no pyraclostrobin treatment harvested before a frost were significant in 2008 (Table 3). With the exception of the treatment X harvest date interaction, the same sources of variation were significant for raffinose concentration of true roots 0 DAH (Table 3). Harvest date raffinose concentration means of crowns 0 DAH in 2008 ranged from 0.11 g (100 g S)<sup>-1</sup> for roots harvested before a frost (27 October 2008) to 0.27 g (100 g S)<sup>-1</sup> (Table 5) for roots harvested on 29 October (LSD<sub>0.10</sub> = 0.08). For five of seven harvest dates, the raffinose concentration in the crowns of the pyraclostrobin treatment was greater than the concentration in crowns of roots from the no-pyraclostrobin treatment 0 DAH (Table 5). However, the difference between the two treatments was significant for only two harvest dates, 28 and 29 October. Crowns of roots harvested before a frost had 0.12 g (100 g S)<sup>-1</sup> (SE = 0.04) less raffinose than the average of crowns sampled after a frost, 0 DAH. Crowns from the pyraclostrobin treatment harvested before a frost had 0.12 g (100g S)<sup>-1</sup> (SE = 0.07) less raffinose than roots from the no-pyraclostrobin treatment harvested before a frost, 0 DAH. True roots harvested before a frost in 2008 had 0.11 g (100 g S)<sup>-1</sup> (SE = 0.04) more raffinose than the average of root tissue sampled after a frost, 0 DAH. True root tissue from the pyraclostrobin treatment harvested before a frost had 0.15 g (100 g S)<sup>-1</sup> (SE = 0.08) more raffinose than roots from the no-pyraclostrobin treatment harvested before a frost, 0 DAH.

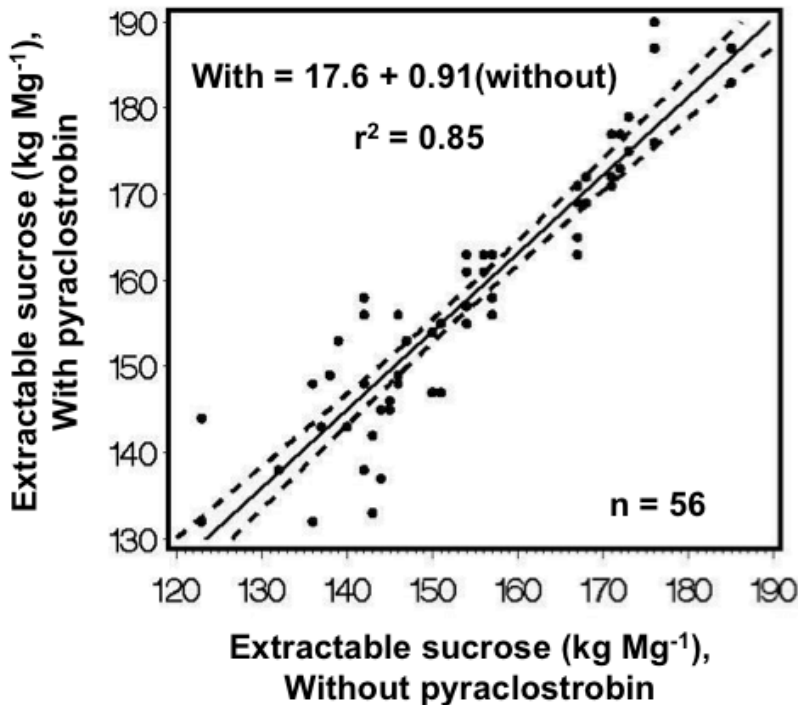
## DISCUSSION AND CONCLUSIONS

In general, the analysis of variance provided only limited insight into what, and to what extent, the variables measured were impacted by pyraclostrobin. Furthermore, there were no apparent consistent relationships among pairs of variables in either trial. However, the frequency of significant differences among traits of interest was greater than would be expected by chance alone. Sixteen of the 32 tests of significance (F-tests) in the application-timing trial (Table 1) were significant at the 10% probability level. Included in this 16 were seven of the 20 comparisons that included some variability due to treatment (treatment main effect, interactions that included a treatment effect, and contrasts that were linear combinations of treatments). Seventeen of 104 tests of significance (F-tests) that included some variability due to treatment were among the 35 tests that were significant in the date-of-harvest trials (Table 3).

Based upon the relatively low frequency of significant treatment main effects or interactions including a treatment effect on extractable sucrose concentration (Tables 1 & 3) and other reports (Khan and Carl-

son 2009; Hubbell et al., 2009), a reluctance by agriculturalists and consultants to recommend an application of pyraclostrobin when a fungicide is not needed for disease control is understandable. However, upon close examination, there appeared to be a disproportionate number of paired comparisons between a pyraclostrobin treatment and the corresponding untreated check in which the extractable sucrose concentration of the pyraclostrobin treatment was, at least slightly, greater than that of the untreated check (Tables 2 & 4). The extractable sucrose concentration of roots from the pyraclostrobin treatments (early and/or late application) was greater than the concentration in roots from the untreated check in 23 of the 32 possible paired comparisons in Table 2 (before and after frost harvest dates). In the 2007 date-of-harvest trial, the extractable sucrose concentration of the pyraclostrobin treatment exceeded that of the untreated check six of 10 times; in 2008, the pyraclostrobin treatment was greater than

**Fig. 2.** Regression of extractable sucrose concentration of roots that received a foliar pyraclostrobin application on extractable sucrose concentration of roots that did not receive a foliar pyraclostrobin application in field trials from Crookston, Minnesota and Prosper, North Dakota in 2007 and 2008.



the untreated check in 13 of 14 paired comparisons (Table 4). If applying pyraclostrobin had no effect on extractable sugar concentration, one would expect it to exceed the untreated check approximately half the time. However, the pyraclostrobin treatment exceeded that of the untreated check in 42 of the 56 (75%) paired comparisons in Tables 2 and 4. There is a high probability that this deviation from the 50% expected with no treatment effect is significant (Chi-square = 14;  $P < 0.001$ ;  $n = 56$ ).

A paired t-test including all 56 paired comparisons (Table 2 & 4) between roots from a pyraclostrobin treatment with untreated roots indicated that the extractable sucrose concentration of roots from a pyraclostrobin treatment had  $3.7 \text{ kg Mg}^{-1}$  ( $CI_{90} = 2.4 - 5.0$ ;  $P < 0.01$ ) more extractable sucrose than their untreated counterparts. The 20 comparisons that included only roots harvested before a frost indicated a  $2.1 \text{ kg Mg}^{-1}$  ( $CI_{90} = 0.3 - 3.9$ ;  $P = 0.05$ ) increase due to a pyraclostrobin application. The  $4.6 \text{ kg Mg}^{-1}$  ( $CI_{90} = 2.8 - 6.4$ ;  $P < 0.01$ ) difference observed in the 36 pairs of samples harvested after a frost suggested that the increase in extractable sucrose associated with a pyraclostrobin treatment may be greater in roots harvested after a frost than in those harvested before a frost.

Regression of the extractable sucrose concentration of roots from a pyraclostrobin application as a function of the extractable sucrose concentration of the corresponding untreated roots (Fig. 2) suggested that the magnitude of the increase in extractable sucrose attributable to a pyraclostrobin treatment decreased as the extractable sucrose concentration of the untreated roots increased (slope = 0.91). Based upon the regression equation, there would be no benefit from a pyraclostrobin application under conditions that resulted in extractable sucrose concentrations equal to or greater than  $196 \text{ kg Mg}^{-1}$  without a pyraclostrobin treatment (a concentration beyond the range of the data used in the regression analysis). In contrast, conditions that produced extractable sucrose concentrations of  $120 \text{ kg Mg}^{-1}$  without pyraclostrobin would produce an average of  $6.8 \text{ kg Mg}^{-1}$  more extractable sucrose with a pyraclostrobin application. The average extractable sucrose concentration of roots from a pyraclostrobin treatment was  $158.1 \text{ kg Mg}^{-1}$ , compared to an average of  $154.4 \text{ kg Mg}^{-1}$  without pyraclostrobin.

Environmental conditions and date of harvest in relation to a damaging frost frequently overshadowed smaller differences due to pyraclostrobin treatments. The interval between a pyraclostrobin application and the beginning harvest date (scheduled prior to a forecasted frost) in the environments sampled was longer than would usually occur in production fields. In practice, a pyraclostrobin application would be scheduled in anticipation of an early October harvest, approximately two weeks earlier than the harvest dates in these trials. The longer time between the pyraclostrobin application and harvest may have reduced the impact of the pyraclostrobin applications, compared to an earlier harvest.

In conclusion, the trials summarized in this report and others suggest that the positive plant health benefits realized from a pyraclostrobin application may be relatively small and frequently not detectable in standard agronomic trials. However, the frequency of having a higher extractable sucrose concentration in roots that had a pyraclostrobin application relative to untreated roots was too high to assume pyraclostrobin had no positive effect and that no further evaluations are needed. There were no detectable relationships between extractable sucrose concentration and the other storage properties measured that might provide insight into a physiological basis for any increase in extractable sucrose associated with a pyraclostrobin application. Knowledge of the physiological basis of the observed and reported benefits of pyraclostrobin would be beneficial in establishing the circumstance in which a late-season pyraclostrobin application would increase yield and/or reduce frost damage.

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