Sugarbeet Germplasm Lines Selected from Crosses between Cultivated Sugarbeet and Wild *Beta vulgaris* subsp. *maritima* from the United Kingdom

L.G. Campbell and K.K. Fugate

USDA-ARS, Northern Crop Science Laboratory, 1605 Albrecht Blvd, Fargo, ND 58102-2765

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

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ABSTRACT

It is generally acknowledged that sugarbeet has a narrow genetic base and that genetic diversity is necessary for continued improvement. However, introgression of wild beet germplasm into sugarbeet requires considerable time and resources to recover the root shape, yield, and sucrose concentration of current adapted cultivars. This report describes nine sugarbeet germplasm lines that were selected from crosses between a sugarbeet breeding line and nine *Beta vulgaris* subsp. *maritima* accessions originating from England, Wales, and the Channel Islands. The sucrose concentration of the nine germplasm lines ranged from 131 g kg^{-1} to 143 g kg^{-1}, compared to 148 g kg^{-1} for an adapted hybrid. The 4-year average recoverable sucrose concentration of the nine germplasm lines was 89% of the recoverable sucrose concentration of an adapted hybrid. The average root yield of the nine germplasm lines was approximately 75% of the root yield of two adapted hybrids. When used in the development of advanced breeding populations, these lines will contribute to increased genetic diversity within the commercial sugarbeet crop. The infusion of genes from these and other exotic sources into breeding populations may expand the limits of improvement through selection and produce parental lines with enhanced combining ability.

Additional Keywords: *Beta vulgaris*, crop wild relatives, exotic germplasm, introgression of wild relatives, pre-breeding.

Prior to becoming a source of sugar, *Beta* species had a long history of providing food for human consumption and feed for livestock (Biancardi, 2005). The breeding efforts of Achard and the Vilmorins, in the late eighteenth and early nineteenth century (Francis, 2006) are credited with the transformation of fodder beet into sugarbeet (*Beta vulgaris* subsp. *vulgaris* L.), which now supplies approximately 20% of the sucrose consumed by humans worldwide (FAO, 2009). The recognition that a few early sugarbeet cultivars provided and continue to provide the genetic foundation of cultivated sugarbeet suggests that the crop might benefit from an infusion of additional genetic diversity. However, the acknowledgement that the crop itself remains the most important genetic resource for the development of improved varieties indicates that the genetic basis of sugarbeet may not be as narrow as its history suggests (McGrath, et al., 1999; Frese, et al., 2001). Average sugarbeet root yields in the U.S. have increased from approximately 22 Mg ha-1 in the early twentieth century to 56 Mg ha^{-1} or more between 2006 and 2014 (USDA, National Agricultural Statistics Service, 2015). A substantial portion of the increased productivity in the U.S. (Panella, et al., 2014) and similar increases in Europe (Loel et al., 2014) has been attributed to varietal improvement. Hence, while it generally is recognized that the introgression of unique genetic sources into elite populations enhances long-term breeding progress, there is little urgency attached to these endeavors (Hjerdin, et al., 1994; Ober and Luterbacher, 2002).

Beta vulgaris subsp. *maritima* provides a readily available resource for broadening the genetic base of sugarbeet (Panella and Lewellen, 2007; Biancardi et al., 2012). Because *B. v.* subsp. *maritima* is closely related to sugarbeet, crossing or fertility problems among hybrid progeny are rare. *B. v.* subsp. *maritima* is distributed over a large geographic area and therefore exposed to a wide range of environmental conditions and disease organisms (Luterbacher et al., 2000; Frese, et al., 2001) and resistance genes from exotic sources have been successfully introduced into elite populations and lines (Lewellen, 1992; Panella and Lewellen, 2005; Panella and Lewellen, 2007). However, the need for cytoplasmic-nuclear male-sterility, monogerm seed, some disease resistance genes from single sources, and the undesirable traits frequently introduced from wild relatives has limited utilization of exotic germplasm to broaden the genetic base or enhance the inherent productivity of the crop (Doney, 1993; Stander, 1993; Frese, et al., 2001; Panella and Lewellen, 2005). Four lines, y317, y318, y322, and y387, selected from a cross between an annual *B.v.* subsp. *maritima* from Greece, PI 546420, and a sugarbeet germplasm line, L53, were characterized by Doney (1995) as unique sources of genetic variability for combining ability and root yield. To ameliorate the relatively low sucrose concentration often associated with populations derived from crosses between sugarbeet and its wild relatives, y318, y322, and y387 were backcrossed to sugarbeet germplasm line L19, noted for its high sucrose concentration, and after selection of progeny from this cross, lines were released as F1030, F1031, and F1032, respectively (Campbell 2015). Seven additional germplasm

lines selected from crosses between wild *Beta* species and a sugarbeet breeding line from California also will expedite the introduction of genetic diversity into the commercial crop (Campbell, 2009).

This report describes nine sugarbeet germplasm lines - F1033 (PI 676962), F1034 (PI 676963), F1035 (PI 676964), F1036 (PI 676965), F1037 (PI 676966), F1038 (PI 676967), F1039 (PI 676968), F1040 (PI 676969), and F1041 (PI 676970) - that will facilitate the introduction of additional genetic diversity into elite breeding populations and eventually commercial varieties. All nine lines were selected from crosses between a sugarbeet breeding line developed by USDA-ARS in California, R376-43, and nine *B. v.* subsp. *maritima* accessions originating from England, Wales, and the Channel Islands (Doney et al., 1990). The infusion of genes from these and other exotic sources into elite breeding populations may expand the limits of improvement through selection and produce parental lines with enhanced combining ability.

MATERIALS AND METHODS

Population Formation and Line Development

The cultivated parent, R376-43, used to form the populations from which F1033 – F1041 were selected, was a breeding line developed by USDA-ARS, Salinas, CA. R376-43, a self-incompatible inbred, which can be traced to a broad-based population that included all available virus yellows resistance sources in the USA and Europe. This population was released as C31 (Lewellen et al., 1978). Resistance to rhizomania (causal agent, *Beet necrotic yellow vein virus*; BNYVV) was introduced into C31 and the resulting population designated R76. R376-43 is a full-sib family selected for its combining ability for sugar and root yield and disease resistance from R76. Genetic male-sterile segregates of R376-43(Aa) were pollinated with nine wild *B. v.* subsp. *maritima* accessions that originated in England, Wales, and the Channel Islands (Table 1 and Fig. 1). Eight of the nine wild accessions were described as annuals or mixtures of annuals and biennials. PI 540643 was characterized as biennial. Ten plants from each *B.v.* subsp. *maritima* accession were crossed with ten R376-43(aa) plants. Ten F_1 plants from each cross (100 plants) were randomly intercrossed to produce the F_2 generation. Equal numbers of seeds from each F_2 plant were grown and intercrossed to produce F_3 seed. Selection for non-bolting plants with single non-protruding crowns and fleshy non-branching dominant taproots began in the F_3 generation.

The population that became F1040 was subject to nine cycles of selection for visual plant and root characteristics; all the other populations were subjected to eight cycles prior to beginning selection for sucrose concentration. For each cycle, approximately 650 plants of each line were grown in a single block (160 row m). Plants that produced seed stalks or had extremely high crowns were rogued throughout the season. During the late summer, all remaining plants were dug and visually examined. To the extent possible, roots with single crowns, minimal branching, and

Table 1. Accession (PI) number, site information, and location of original collection, for the nine wild *Beta vulgaris* subsp. *maritima* populations from England, Wales, and the Channel Islands that were crossed with a cultivated sugarbeet breeding line from California (R376-43) and subsequently released as F1033, F1034, F1035, F1036, F1037, F1038, F1039, F1040, and F1041.

Released germplasm	PI-number of wild parent	Site	Locale	Country
F1033	518313	Coarse gravel, beach	Church Norton	England
F1034	518344	Silt soil and cobbles, cliffs overlooking sea	Land's End	England
F1035	518354	Coarse gravel, near beach	Blue Anchor Bay	England
F1036	518357	Marshy beach above high tide	Severn Beach	England
F1037	518371	Mud and gravel just above high tide	Talacre	Wales
F1038	518374	On beach in grass underlain with gravel	Cemlyn Bay	Wales
F1039	518429	Coarse gravel	Hollesley	England
F1040	540628	Soil with cobbles	St. Brelade Bay	Jersey. Is.
F1041	540634	Rocks around cove	Rocquaine Bay	Guernsey, Is.

Figure 1. Collection locale of the nine wild *Beta vulgaris* subsp. *maritima* populations from England, Wales, and the Channel Islands (Google Earth®) that were crossed with a cultivated breeding line (R376-43) and subsequently released as F1033, F1034, F1035, F1036, F1037, F1038, F1039, F1040, and F1041.

a dominant taproot were selected for increase. Each cycle, 40 to 60 selected roots of each line were planted in the greenhouse and randomly pollination to produce seed for the next cycle.

Subsequently, each of the nine populations was subjected to three additional selection cycles based upon the sucrose concentration of individual roots relative to other roots within a single cell of a 10-cell grid. Individual cells of the grid were 10 m long and two rows wide with a row-spacing of 56 cm. Plants at the ends of the rows were not sampled. Moderate-size roots were chosen for the individual sucrose measurements. Samples for analysis were obtained by collecting the tissue removed diagonally from the taproot with a 3.2 cm wood bit (~ 10 cm long) and an electric drill. Sampled roots remained viable and were used as mother roots to produce seed for additional selection cycles. Forty plants were selected from each population and each selection cycle. Selected roots from the third selection cycle provided seed for replicated field trials between 2012 and 2015.

Experimental Procedures and Analysis

The experimental design for the 2012 to 2015 field evaluations was a randomized complete block design with four replicates. Individual experimental units were two-row by 10-m plots with rows 56 cm apart. Trials were planted near Fargo, ND during the first two weeks of May and harvested during the last two weeks of September. Root yield was the weight of all roots from a single plot at harvest expressed as Mg ha^{-1}. Sucrose concentration, and the sodium, potassium, and amino-nitrogen concentrations that were used to calculate recoverable sucrose per ton (Dutton and Huijbregts, 2006) were determined using the brei of a composite random sample of 10 - 12 roots from each plot.

The brei from each field-plot sample or individual root was mixed and a portion was quickly frozen for later analysis. Sucrose was determined polarimetrically (Autopol 880, Rudolph Research Analytical, Flanders, NJ) using aluminum sulfate-clarified brei samples (McGinnis, 1982). The aluminum sulfate-clarified filtrate used to determine sucrose concentration also was used to measure sodium, potassium, and amino-nitrogen concentrations. Sodium and potassium concentrations were determined by flame-photometry (Corning 410C, Cole-Parmer Instrument Co., Chicago, IL). Amino-nitrogen concentration was determined with a spectrophotometer (Spectronic-21D, Milton Roy Co., Ivyland, PA) using the copper method and a wavelength of 610 nm (International Commission on Uniform Methods of Sugar Analysis, 2007).

The F1033 to F1041 populations, were screened in specialized disease nurseries to assess disease development when exposed to *Cercospora beticola* Sacc. (Cercospora leaf spot), *Fusarium* spp., (Fusarium root rot), and *Aphanomyces cochlioides* Drechsl. (Aphanomyces root rot). The Cercospora leaf spot (CLS) nurseries were in southern Minnesota (Betaseed, Inc, Shakopee, MN) in 2014-2016. Aphanomyces root rot and Fusarium root rot were evaluated in nurseries near Shakopee, and Sabin, MN (Be-

taseed, Inc.), respectively, in 2015 and 2016. These nurseries were located and managed with the objective of providing a reliable indication of the response to a single disease organism with minimal complications due to other diseases. Each nursery included resistant and susceptible check cultivars selected by nursery managers. Sugarbeet root aphid (*Pemphigus* sp.) damage was assessed by Betaseed, Inc. in a greenhouse assay (Panella et al., 2008) in 2014 and 2015. The root aphid evaluations were not randomized, so statistical analysis was not appropriate. However, comparisons between lines and with checks provide insight into the relative performance of lines when challenged by sugarbeet root aphid.

The SAS GLM procedure (ver. 9.4, SAS Institute, Inc., Cary, NC) was used for the analysis of variance. Years were assumed to be random effects and genotypes fixed effects (McIntosh, 1983). Fisher's Protected LSD was used to determine when differences among means were significant (P=0.05).

RESULTS

F1033, F1034, F1035, F1036, F1037, F1038, F1039, F1040, and F1041 are multigerm diploid biennial lines that produce roots with white skin and flesh (Fig. 2). All nine lines have tapered roots with a relatively shallow grove. During the initial selection-cycles, selection was for root size, minimal branching, and non-protruding single crowns. Consequently, some root shape variation within lines remains. However, F1034, F1036, and F1040 can generally be characterized as having broad-elliptical roots, somewhat similar to ACH-817, and the remaining six germplasm lines as having narrow-triangular roots (Fig. 2). No bolters were observed and plants with multiple crowns were infrequent in the trials that were the basis of the data for Table 2. Hypocotyls of F1033, F1039, and F1041 are red. The hypocotyls of F1035, F1036, and F1038 are predominately red (> 90%) with the remaining being green. The ratio of green to red hypocotyls for F1034, F1037, and F1040 is 50(G):50(R), 30(G):70(R), and 40(G):60(R), respectively.

Root Yield and Quality

The 4-year average sucrose concentration of the nine germplasm lines ranged from 131 g kg^{-1} for F1041 to 143 g kg^{-1} for F1035, compared to 148 g kg^{-1} for the adapted hybrid with the higher sucrose concentration, ACH-817 (Table 2). The only significant difference between the sucrose concentration of F1035 and ACH-817 was the 15 g kg^{-1} difference that occurred in 2014. Except for F1035 and the 140 g kg^{-1} of F1038, differences between the 4-year sucrose concentration means of F1041, the line with the lowest sucrose concentration, and the other seven germplasm lines were not significant. The 4-year average sucrose concentration of the nine germplasm lines was 92% of the sucrose concentration of ACH-817. The average sucrose concentration of the germplasm lines ranged from 99% of the sucrose concentration of ACH-817 in 2012, the year with

Table 2. Sucrose and recoverable sucrose concentration and root yield of F1033, F1034, F1035, F1036, F1037, F1038, F1039, F1040, F1041, and two adapted hybrids (ACH-817 and ACH-R716), Fargo, ND, 2012-2015.

Released germplasm	Year				4-year Mean
	2012	2013	2014	2015	
	---------- Sucrose, g kg^{-1} ----------				
F1033	154 ab†	114 bc	131 d	133 c	133 CD
F1034	155 ab	121 ab	135 b-d	136 bc	137 B-D
F1035	157 ab	124 ab	143 b	150 ab	143 AB
F1036	144 b	117 a-c	143 b	137 bc	135 CD
F1037	162 a	117 a-c	135 b-d	139 bc	138 B-D
F1038	156 ab	119 ab	141 bc	142 a-c	140 BC
F1039	148 ab	129 a	134 b-d	135 bc	136 B-D
F1040	150 ab	118 a-c	135 b-d	132 c	134 CD
F1041	147 ab	104 c	132 cd	141 a-c	131 D
ACH-817	153 ab	127 ab	158 a	156 a	148 A
ACH-R761	152 ab	125 ab	132 cd	137 bc	137 B-D
Mean	152 A	120 C	138 B	140 B	137
	----------Recoverable sucrose, kg Mg^{-1} ----------				
F1033	132 ab	83 bc	105 de	108 b	107 CD
F1034	132 ab	90 ab	108 b-e	115 b	111 BC
F1035	133 ab	97 ab	120 b	126 ab	119 AB
F1036	118 b	86 bc	118 bc	111 b	108 CD
F1037	141 a	84 bc	106 c-e	113 b	111 BC
F1038	134 ab	82 bc	115 b-d	119 ab	112 BC
F1039	122 ab	106 a	110 b-e	111 b	112 BC
F1040	126 ab	88 bc	106 c-e	108 b	107 CD
F1041	120 b	74 c	102 e	110 b	101 D
ACH-817	128 ab	92 ab	136 a	135 a	123 A
ACH-R761	128 ab	90 ab	103 de	110 b	108 CD
Mean	128 A	88 C	112 B	115 B	111

Table 2 *(Con't)*. Sucrose and recoverable sucrose concentration and root yield of F1033, F1034, F1035, F1036, F1037, F1038, F1039, F1040, F1041, and two adapted hybrids (ACH-817 and ACH-R716), Fargo, ND, 2012-2015.

Released germplasm	Year 2012	2013	2014	2015	4-year Mean
	----------Root yield, Mg ha^{-1} ----------				
F1033	37.2 a	45.0 b-d	36.6 cd	43.6 cd	40.6 CD
F1034	35.5 a	42.8 b-e	31.4 fg	37.6 e	36.8 DE
F1035	28.4 a	35.5 e	31.9 e-g	40.1 de	34.0 E
F1036	28.1 a	39.2 c-e	36.1 c-e	46.4 bc	37.4 DE
F1037	29.3 a	47.1 bc	36.2 c-e	42.7 c-e	38.8 DE
F1038	36.2 a	36.9 de	35.0 d-f	42.6 c-e	37.7 DE
F1039	41.7 a	47.8 bc	40.0 c	47.7 bc	44.3 BC
F1040	37.3 a	36.4 de	29.2 g	39.3 de	35.6 DE
F1041	41.0 a	39.9 c-e	36.3 c-e	44.6 b-d	40.5 CD
ACH-817	43.3 a	50.9 b	47.1 b	49.6 b	47.7 B
ACH-R761	46.8 a	63.9 a	59.8 a	63.4 a	58.5 A
Mean	36.8 B	44.1 A	38.2 B	45.2 A	41.1

†Differences among genotypes within a year followed by the same lower case letter year are not significant, based upon Fisher's protected $LSD_{0.05}$; Differences among main-effect means followed by the same uppercase letter are not significant (P = 0.05).

the highest average sucrose concentration, to 86% of ACH-817 in 2014, a year with intermediate sucrose concentrations.

Differences in recoverable sucrose concentration, a function of sucrose concentration and, to a lesser extent, the concentration of sodium, potassium, and amino-nitrogen, followed a pattern similar to that observed for sucrose concentration (Table 2). The 4-year average recoverable sucrose concentration of all the germplasm lines, except F1035, was lower than the recoverable sucrose concentration of ACH-817 (P = 0.05). The 4-year average recoverable sucrose concentration of F1035 was 97% of the average recoverable sucrose concentration of ACH-817; ranging from 93% in 2015 to 105% in 2013. Differences in recoverable sucrose concentration between F1041, the line with the lowest recoverable sucrose con-

Figure 2. Roots of F1033, F1034, F1035, F1036, F1037, F1038, F1039, F1040, F1041, and an adapted cultivar, ACH-817, Fargo, ND, 2015.

centration, and F1033, F1036, and F1040 were not significant. The 4-year average recoverable sucrose concentration of the nine germplasm lines was 89% of the recoverable sucrose concentration of ACH-817.

Root yields of the germplasm lines ranged from 28.1 Mg ha^{-1} for F1036 in 2012 to 47.8 Mg ha^{-1} for F1039 in 2013. Although within-year differences among the nine lines frequently were not significant, F1039 had the highest root yield in all four years and the highest 4-year average root yield, 44.3 Mg ha^{-1}. F1035, the line with the highest sucrose and recoverable sucrose concentration had an average root yield of 34.0 Mg ha^{-1}, the lowest among the nine lines. Average root yield differences between F1035 and F1034, F1036, F1037, F1038, and F1040 were not significant. All of the lines had lower average root yields than the two adapted hybrids, ACH-817 and ACH-R716. The 4-year average root yield of the nine germplasm lines was 65% of the root yield of ACH-R716, the adapted hybrid with the higher yield, and 85% of the root yield of ACH-817, the lower yielding hybrid.

Disease and Root Aphid Resistance

None of the nine germplasm lines had CLS ratings equal to or lower (more resistant) than ($P = 0.05$) the ratings for the resistant check (Table 3). However, in all but three comparisons (the final ratings for F1039 in 2014 and the last ratings for F1033 and F1035 in 2015) the CLS ratings of the nine lines were lower (more resistant) than the rating of the susceptible check. In all comparisons among the nine germplasm lines, F1036 had the lowest or second lowest (2014 mean) CLS rating. Differences between F1036 and F1034 or F1041 were relatively small and, except for the last 2015 ratings, not significant ($P \leq 0.05$). F1033, F1035, and F1039 frequently had relatively high CLS ratings, compared to the other germplasm lines.

None of the nine germplasm lines had Aphanomyces root rot ratings equal to or lower (more resistant) than the resistant check in 2015 (Table 4). Furthermore, F1034 and F1040 had higher root ratings than the other seven lines and all nine lines had lower root ratings than the susceptible check ($P = 0.5$), in 2015. With the exception of the relatively high ratings for F1036 and F1039 differences between the germplasm lines and the resistant check were not significant in 2016, the year with the more severe disease. In 2016, six of the nine lines had Aphanomyces ratings higher than F1041, the line with the lowest rating ($P \leq 0.05$). Differences between F1041, F1033, and F1035 were not significant in 2016.

With one exception, Fusarium ratings for the nine germplasm lines were lower than the ratings for the resistant check (Table 4); the 2015 rating for F1041 was 2.8, compared to 2.7 for the resistant check. Furthermore, the ratings for all the germplasm lines were lower than the moderate check ($P \leq 0.05$) in both years. Ratings for F1035 and F1041 were higher than the ratings for F1037 ($P \leq 0.05$) in 2015, in which F1037 was the line with the lowest rating. Differences among the germplasm lines were not significant in 2016.

Table 3. Final and mean Cercospora leaf spot (CLS) ratings for F1033 – F1041, a CLS susceptible check, a moderately susceptible check and a resistant check during 2014 to 2016.

	2014		2015		2016	
Germplasm	**Final**	**Mean (4)[†]**	**Final**	**Mean (6)**	**Final**	**Mean (5)**
	----------Disease rating, 1 – 9[‡] ----------					
Lines						
F1033	7.5	4.4	7.8	4.3	6.7	4.7
F1034	6.0	3.5	6.5	3.6	5.3	3.6
F1035	7.3	4.1	7.7	4.5	7.2	4.8
F1036	4.8	3.1	4.2	2.7	4.3	3.1
F1037	6.3	3.8	7.2	3.9	6.7	4.5
F1038	6.0	3.6	6.5	3.8	5.8	4.4
F1039	8.2	4.7	7.3	4.1	7.0	4.9
F1040	5.2	3.0	6.0	2.9	5.0	3.4
F1041	5.0	3.3	6.7	4.1	6.3	4.3
Checks						
Susceptible	9.0	5.9	9.0	6.8	9.0	7.8
Moderate Susc.	8.0	4.4	8.7	4.7	8.0	5.1
Resistant	2.3	1.6	1.7	1.3	1.0	1.2
$LSD_{0.05}$	1.3	0.6	1.5	0.7	1.3	0.8

[†]Number in parenthesis indicates the number of observation dates included in the mean. The Final reading is almost always the highest (most severe Cercospora leaf spot ratings) recorded for the season.

[‡]Higher Cercospora leaf spot ratings are indicative of increases severity (1 = no visible symptoms).

There is no indication that utilizing any of the nine germplasm lines to introduce genetic diversity into an elite population would contribute root aphid resistance to the population (Table 4). The 2.3 average root aphid rating for F1034 was the lowest among the nine lines; however, this rating was conspicuously higher than 1.1 average rating for the resistant check. All disease ratings should be regarded as preliminary and confirmed before being considered a source of resistance.

Table 4. Aphanomyces root rot and Fusarium root rot ratings for F1033 – F1041 and corresponding resistant checks, moderately susceptible checks, and susceptible checks, 2015 – 2016, and root aphid ratings during 2014 to 2015.

Germplasm line	Aphanomyces		Fusarium		Root aphid	
	2015	2016	2015	2016	2014	2015
	----- Disease rating, 0 to 9[†] -----				- Rating, 1 to 4 -	
F1033	3.3	3.8	2.2	2.5	2.8	3.5
F1034	4.3	5.1	2.0	2.7	1.9	2.6
F1035	2.5	3.1	2.5	2.7	2.5	3.3
F1036	2.8	5.5	1.8	3.5	3.0	3.7
F1037	2.8	4.1	1.7	3.7	2.9	3.8
F1038	3.2	4.8	2.2	2.7	3.1	3.0
F1039	2.8	5.5	2.2	3.8	2.9	3.5
F1040	4.2	4.3	2.2	2.8	2.8	3.6
F1041	3.3	2.8	2.8	3.2	2.8	3.6
Resistant check	1.0	4.1	2.7	4.0	1.1	1.1
Moderate check	4.2	5.8	5.0	5.3	- - -	- - -
Susceptible check	6.3	8.5	7.8	8.7	2.8	3.7
$LSD_{0.05}$	1.1	1.2	0.7	1.3	- - -	- - -

[†]Higher ratings are indicative of increased severity of disease damage; Aphanomyces ratings are based upon root symptoms, Fusarium ratings on foliar observations.

DISCUSSION

Selection for sugarbeet plant type and sucrose concentration likely favored genes from the cultivated parent used in the initial cross. Relatively low selection intensities combined with an adequate number of individuals to advance each generation should have minimized the elimination of favorable donor alleles from the exotic parents. Each of the nine segregating populations was subjected to 11 or 12 selection cycles and the effective population size for advancement of each cycle was ≥ 40 (Fehr, 1987). The nine *B v.* subsp. *maritima* accessions used in the original crosses represent distinct collection sites (Figure 1). Although a small sample, the accessions contributing to the nine released

germplasm lines would appear to be representative of the wild *B.v.* subsp. *maritima* populations of the UK south of 54° N latitude. When used in the development of advanced breeding populations, these germplasm lines will contribute to a broadening of the genetic diversity within the commercial sugarbeet crop.

The nine germplasm lines will be maintained by USDA-ARS, Fargo, North Dakota and freely distributed in quantities sufficient for reproduction. Requests for seed should be directed to USDA-ARS-NCSL, Sugarbeet and Potato Research Unit, 1605 Albrecht Blvd. N., Fargo, ND 58102. Seed samples also have been deposited with the USDA National Plant Germplasm System where they will be available for research purposes, including the development of new varieties. Plant Variety Protection will not be pursued for any of the lines.

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Agronomic Potential of Using Precipitated Calcium Carbonate on Early Plant Growth and Soil Quality in the Intermountain West - Greenhouse Studies

Gary W. Hergert[1], Murali K. Darapuneni[2], Abdullah M. Aqeel[3], Robert G. Wilson[1], Robert M. Harveson[1], Jeff D. Bradshaw[1] and Rex A. Nielsen[1]

[1]University of Nebraska-Lincoln, Panhandle Research & Extension Center, Scottsbluff, NE 69361. [2]Department of Plant and Environmental Science and Agricultural Science Center-Tucumcari, New Mexico State University, Tucumcari, NM 88401. [3]Adjunct Faculty, Bergen Community College, Paramus, NJ 07652.

Corresponding author: Gary Hergert (ghergert1@unl.edu)

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ABSTRACT

Storage and management of large piles of precipitated calcium carbonate (PCC) from sugarbeet processing are a challenge in the western US. Potential uses of this product on surrounding agricultural lands in western NE, eastern WY and northeast CO requires an evaluation of chemical and agronomic impacts of PCC on soils and crop growth. A preliminary greenhouse study was conducted in Scottsbluff, NE using 10 soils from the 3 states. Soils were mixed with 11, 22, 33 and 44 Mg ha^{-1} rates of PCC to test the early plant growth of sugarbeet, corn, and dry bean in addition to determining soil chemical characteristics. Chemical analysis of PCC from the three processing factories indicates that PCC provides some nitrogen and phosphorus, in addition to some iron, depending on rate. Application of four rates of PCC to neutral to slightly alkali soils neither improved nor negatively impacted the soil chemical characteristics. Dry matter of the three crops after 7 weeks showed no significant effects of PCC. Future utilization of PCC in this region will require further research based on long-term investigations of possible effects of PCC on soil chemical characteristics and plant growth under field conditions.

Additional Key words: spent lime, sugar processing, plant growth, soil chemical properties

Abbreviations: ***PCC= Precipitated Calcium Carbonate***

Precipitated calcium carbonate (PCC) is a by-product of the sugar clarification process when sugarbeet (*Beta vulgaris* L.) is processed. This sugar processing step has been around for many years and has not changed significantly (Dedek, 1952; McGinnis, 1971). Calcium oxide and carbon dioxide are injected into extracted juice and calcium carbonate reforms, precipitating impurities to produce the thin juice from which sugar is extracted. Dutton and Huijbregts (2006) identified removal of impurities including organic molecules plus inorganic forms of phosphorus (P), magnesium (Mg) and calcium (Ca) with limited removal of potassium (K), sodium (Na) and nitrates. Western Sugar factories at Fort Morgan, CO, Scottsbluff, NE and Torrington, WY produce approximately 100,000 tons of PCC per year (Jerry Darnell, Western Sugar Cooperative, personal communication) . Historically, PCC has been stockpiled near the factory site and the amount continues to grow. PCC piles can be a management problem as they grow weeds, produce dust during wind storms, and continue to require additional land for storage (Jerry Darnell, Western Sugar Cooperative, personal communication). This is a concern with current EPA regulations on particulate matter as part of the National Ambient Air Quality Standards (https://www.epa.gov/pm-pollution/table-historical-particulate-matter-pm-national-ambient-air-quality-standards-naaqs).PCC has been effectively used in production agricultural as a liming source replacement for agricultural lime. In the Red River Valley (RRV) and southern MN it has generally had a beneficial effect on growth and yield (Brantner et al., 2015a; Bredehoeft et al., 2013; Giles and Smith, 2005; Windels et al., 2008). It has also been shown to reduce *Aphanomyces* severity (Brantner et al., 2015b; Lien et al., 2015; Windels et al., 2006). Most MN soils near sugar beet factories are neutral to above pH 7 except in northern and eastern MN (Sims, et al., 2010). In MI, PCC significantly increased sugarbeet yield (Clark et al., 2015). A large field study in MI (Christenson, et al., 2000) including sugar beet, soybean (*Glycine max* L.) corn (*Zea mays* L.), field (dry) bean (*Phaseolus vulgaris* L.) and wheat (*Triticum aestivum* L.) showed limited effects of PCC on yield up to 5.6 Mg ha-1. Research in California showed a significant effect of PCC on controlling soil-borne pathogens (Campbell and Greathead, 1989). Similar research on field pea (*Pisum sativum* L.) in ND showed disease reduction from PCC (Chittem et al., 2016). In Great Britain, PCC is classified as a specialty lime with 14% less acid neutralizing capacity than pure lime. It is currently marketed as a liming material in parts of Great Britain (Windels et al., 2008).

Most of the soils in the Western Sugar production area (CO, NE, MT and WY) have pH levels that are neutral to basic and many are calcareous and do not require liming. Considering the large quantity of PCC available in the region and the economics associated with transporting PCC, Western Sugar was interesting in exploring potential local uses of PCC from factory sites. Our question was that since the soils were already high pH or calcareous, would additional PCC cause any production problems or could there be some possible benefits.

A multiphase research project was designed using greenhouse,

growth chamber and field plots to evaluate PCC effects on crops and soils. Factors considered were PCC characteristics, soil characteristics, problems that might be associated with high Kochia (*Kochia scoparia* L.) seed levels in PCC, and determining PCC effects on plant growth including dry weight, nutrient deficiencies and vigor. Greenhouse studies were proposed as a first step to determine appropriate rates and protocols for field experiments. This paper will discuss the greenhouse research. The objective of this research was to determine the effect of PCC on early plant growth of sugarbeet (*Beta vulgaris* L.), corn (*Zea mays* L.), and dry bean (*Phaseolus vulgaris* L.) and to test the change in soil characteristics of 10 soils collected from western Nebraska, eastern WY , and northeast CO .

MATERIALS AND METHODS

The PCC used in the study was collected from Western Sugar factories at Scottsbluff, NE, Ft. Morgan, CO, and Torrington, WY. Samples from each location were taken by compositing random core samples from different areas in PCC piles from areas where some PCC is currently being taken for other industrial uses. 12 inch deep samples were taken horizontally into the faces of open areas that were 10 to 15 feet tall and composited. The chemical characteristics of PCC collected at different locations are shown in the Table 1.

Soil samples were collected from 1 acre areas in ten farmer field sites in western NE, eastern WY, and northeast CO, usually

Table 1. Chemical characteristics of Precipitated Calcium Carbonate (PCC) obtained from three sugar factories.

Location	Moisture	CCE†	pH	Soluble Salts	SAR§	Total N	P	K	Mg	S	Zn	Fe	Mn	Cu
	g kg^{-1}			dS m^{-1}										
Scottsbluff, NE	153	72.3	8.8	4.8	0.30	2860	4585	1362	6765	5266	45	6765	7	0.22
Torrington, WY	82	70.1	9.4	8.7	0.34	4630	5176	2270	8444	5766	45	8444	9	0.11
Ft. Morgan, CO	141	69.4	9.6	4.1	0.29	3768	4676	2315	7854	5539	45	7854	10	0.38

† Calcium carbonate equivalency (Mamo et al., 2015).
§ Sodium absorption ratio

Table 2. Chemical characteristics of soils from three western states (0 to 20 cm) used in the greenhouse study.

Location	Soil	CCE[†]	pH	OM[§]	Nitrate-N	P	K	Zn	Fe
NE soils		%		%	---------- mg kg^{-1} ----------				
A1	Tripp very fine sandy loam	1.4	7.9	1.3	7.9	10	427	1.2	3.9
A2	Otero-Bayard fine sandy loam	2.0	7.9	1.5	9.1	11	358	1.6	3.5
A3	Mitchell silt loam	1.7	7.8	1.9	22.3	39	755	1.3	2.2
A4	Alliance loam	0.5	6.9	1.8	12.6	34	516	1.8	4.4
WY soils									
B1	Bankard loamy fine sand	5.4	8.1	1.4	16.1	24	325	1.9	7.8
B2	Mitchell silt loam	7.7	7.9	2.3	22.6	17	627	1.5	2.5
B3	Kim clay loam, alkali	3.1	7.8	1.6	21.6	10	407	0.5	2.8
CO soils									
C1	Heldt-Limon assoc.	2.8	7.5	2.8	24.1	130	843	3.8	5.3
C2	Rago and Kuma silt loams	0.4	6.3	2.1	13.7	50	625	2.2	18.3
C3	Weld silt loam	0.8	7.5	2.5	27.2	89	1317	3.3	4.4

[†]Calcium carbonate equivalency (Mamo et al., 2015).
[§]OM is soil organic matter.

within a 40 to 50 km radius of a factory site. Eight soil cores from 0 to 20 cm were taken and composited. Soil was analyzed for standard soil test parameters including pH, salinity, organic matter, Olsen P, nitrate-N and DTPA-extractable Zn, Mn, Cu and Fe (Table 2). Analytical methods used were: organic matter (Nelson and Sommers, 1996), nitrate-N (Mulvaney, 1996), water pH (Thomas 1996), Olsen P (Olsen et al. 1954), ammonium acetate-extractable Ca, Mg, K, and Na (Chapman 1965) and DTPA-extractable Zn, Fe, Mn and Cu (Lindsay and Norvell, 1978). For the greenhouse study soils and PCC were mixed using a standard hand cement mixer. The PCC rates were 0, 11, 22, 33 and 44 Mg ha^{-1}. The experiment was designed as a split-split design with 4 replications. Soil type was the main factor with crop and PCC rate as sub-factor and sub-sub factor, respectively. After mixing with PCC, the different soils were filled into 15 cm diameter plastic pots with 3 drain holes. The crops used were sugarbeet, corn, and dry bean. Five seeds were planted per pot and thinned to three plants after emergence. The plants were not supplemented with additional fertilizer due to the short growing period. Soil moisture was maintained by weekly watering as needed to maintain a stress-free environment for 7 weeks. Greenhouse temperature ranged from a low near 20 to 22 °C at night up to 30 to 32°C during the day. The light source was LU250S/HTL/EN (30500 lumens) bulbs. Spectrum characteristics are at http://www.eyehortilux.com/products/htl-hps/PerformanceSpecs/lu250shtlen/66640. Light was on for 16 h per day. Plants were harvested at 7 weeks after planting to measure early growth. All three plants in each pot were harvested and dried in an oven at 80°C for 48 hours to determine dry weight. Soil samples were collected before planting (before and after PCC was applied) and after dry matter harvest to determine changes in chemical characteristics. The soil samples were dried, ground, and analyzed for pH, soluble salts, and selected macro and micro nutrients as above.

Data was analyzed in SAS 9.4 (SAS Institute, Cary, NC) using the Proc GLM procedure. Data was not combined for any of the yield and soil characteristics due to heterogeneity of variance and thus the results for each soil type were reported separately. After the examination of main and interaction effects, a mean separation tests were carried out using Fisher LSD at 0.05 probability level for all analyses.

RESULTS AND DISCUSSION

Chemical analysis of PCC and soils used in the greenhouse

The PCC collected at different locations contained varying amounts of plant essential nutrients, including nitrogen (N), phosphorus (P), sulfur (S) and iron (Fe) (Table 1). On average, each metric ton of PCC contains about 3.8 kg of total N, 4.8 kg of P, 5.5 kg of S, and 1.4 kg of Fe. These nutrients are added to the PCC during the sugar clarification process as small suspended beet tissue (Dutton and Huijbregts, 2006). PCC collected from the three factory sites may have varied due to site-specific variation in the sugar extraction process, differences in grower

nutrient management or spatial variability of different parts of the pile due to mixing of PCC over time. PCC piles are often reworked and shaped over time and some PCC is sold and used (Jerry Darnell, Western Sugar Cooperative, personal communication). We took samples from areas where PCC was currently being taken from the pile for other uses. The variability in nutrient content is common with PCC (Sims et al., 2010). The question is whether the rate of mineralization of these nutrients would be sufficient to provide additional nutrients over time. Other research has shown slight increases in soil N and P (Sims et al., 2010) indicating some mineralization to no effect on N or P uptake by plants (Christenson et al., 2000) to slight decreases in Zn and Mn in sugar beet and dry bean and soybean (Christenson et al., 2000).

The calcium carbonate equivalency of the PCC from the three locations ranged between 69 to 72%, meaning that this PCC would be as effective or more than regular agricultural lime (60% ECC) for changing soil pH (Mamo et al, 2015).

Chemical characteristics of the different soils (Table 2) showed that soil texture ranged from sandy loam to clay. The pH ranged from slightly acidic to slightly alkaline and organic matter ranged from 1.3 to 2.8%. The nutrient status of CO soils was higher than western NE and eastern WY soils.

Table 3. ANOVA for main and interaction effects of treatments on dry matter content of sugarbeet, corn, and dry bean tested in the greenhouse analyzed by PROC GLM.

Source of variation	DF	Type III SS	Mean Square	F value	Pr>F
Replication (R)	3	2.3075	0.7692	3.15	0.026
Crop Species (CS)	2	302.1259	151.0629	350.55	<.0001
Error (A) (R*CS)	6	2.5856	0.4309	1.76	0.1079
Soil Type (S)	9	172.3629	19.1514	97.65	<.0001
Error (B) (R*S)	27	5.2956	0.1961	0.8	0.7463
Lime rate (L)	4	6.7731	1.6933	12.68	0.0003
Error (C) (R*L)	12	1.6028	0.1336	0.55	0.8823
CS*S	18	32.4624	1.8035	7.89	<.0001
Error (D) (CS*S*R)	54	12.3455	0.2286	0.94	0.6041
S*L	36	16.0998	0.4472	2.49	0.0002
Error (E) (S*L*R)	108	19.3797	0.1794	0.73	0.9639
CS*L	8	4.6690	0.5836	2.37	0.0490
Error (F) (CS*L*R)	24	5.9168	0.2465	1.01	0.4561
CS*S*L	72	19.8157	0.2752	1.13	0.2564
Error (G) (CS*S*L*R)	216	52.7856	0.2444	—	—
Total	599	656.5279	—	—	—

Dry matter accumulation

Statistical analysis of dry matter accumulation as affected by PCC is summarized in Table 3. The ANOVA considered soil type and lime rate as main effects for each crop species. Appropriate error factors were considered for the calculation of F value and probability level for both main and interaction effects. Because the crop by soil interaction was significant, the analysis was rerun by crop (analysis not shown). Soil type had a significant effect on dry weight of all three crops at Pr>F <0.0001. The effect of PCC on dry weight of all three crops was significant at the <0.05 probability level. The soil by PCC rate interaction was also significant for both corn and sugarbeet. Soils significantly interacted with PCC rate because of the varied characteristic pH and calcium carbonate content. Crop species responded differently to various soil types and PCC rate in producing dry matter, which is expected given the different physical and chemical characteristics of the soils and crop species as affected by higher soil lime content (Hoeft and Sorensen, 1969; McLean and Brown, 1984).

Mean separation test results were presented in the Table 4. The mean separation in the dry weight accumulation of dry bean showed little to no statistical significance among different PCC rates for all soil types, which confirms the lack of significance of PCC rate and interaction effects as shown in the ANOVA table (Table 3.) However, as there were significant soil type and PCC rate interactions for corn and sugarbeet, means were compared among four levels (0, 11, 22, 33 and 44 Mg ha^{-1}) of PCC rates by each soil type. Although the effect of PCC rate on dry weight accumulation of both crops for most soils was apparent, the trend was not consistent. In one or two soils, PCC application slightly decreased dry weight accumulation in early growth compared to the control, however the cause is unknown and the effect was not shown for all soils. Mean separation of most other soils indicated that the effect of PCC rate on dry weight was not significantly different from the control. A possible reason for lack of response of dry matter to PCC rate may be the short duration of the study (7 weeks) for significant mineralization to occur. Under normal field conditions, mineralization is a growing season-long process that occurs over 3 to 4 months, depending on the crop. Assessing mineralization is difficult and in spite of numerous attempts over the years, no standardized soil test is in use today to measure it (Stanford, 1982). However, it was evident in almost all soils that there were no significant negative effects of PCC application on early plant growth and dry matter accumulation of the three crop species, even at the maximum application rate.

For different comparisons, there was not a consistent significant increase or decrease in dry matter for the check versus different PCC rates or low versus high PCC rates. The results were encouraging as we interpreted these results to indicate that very high PCC rates could be used. If PCC were to be applied to fields, the highest rates would probably not be above 22 Mg ha^{-1} due to the limitations of current lime spreading equipment.

Table 4. Effect of precipitated calcium carbonate (PCC) mixed with ten different soils on dry weight of sugarbeet, dry bean, and corn at seven weeks after planting in a greenhouse experiment.

Location	Soil	PCC Rate (Mg ha^{-1})				
		0	11	22	33	44
		Average Dry Weight (gm plant^{-1})				
Corn						
Scottsbluff, NE	A1	2.52 ab†	2.33 bc	2.58 ab	1.77 c	3.07 a
	A2	3.24 a	3.24 a	3.05 a	2.92 a	3.38 a
	A3	3.57 a	3.57 a	3.02 ab	2.25 b	3.43 a
	A4	3.25 a	2.57 b	2.58 b	2.22 b	2.53 b
Torrington, WY	B1	2.34 ab	2.21 b	2.88 a	2.88 a	2.74 ab
	B2	2.68 a	2.06 a	2.71 a	2.32 a	2.33 a
	B3	2.21 a	2.14 a	2.40 a	2.55 a	2.52 a
Ft. Morgan, CO	C1	4.62 a	4.14 ab	4.24 ab	3.91 ab	3.58 b
	C2	3.55 a	3.20 ab	2.82 b	3.08 ab	3.23 ab
	C3	2.43 a	2.40 a	2.43 a	2.53 a	2.36 a
Dry bean						
Scottsbluff, NE	A1	1.37 a	1.77 b	1.06 a	1.26 a	1.34 a
	A2	1.95 a	1.74 ab	1.63 ab	1.15 b	2.02 a
	A3	1.82 a	2.27 a	1.76 a	2.05 a	1.71 a
	A4	1.59 a	1.02 b	1.26 ab	1.03 b	1.23 ab
Torrington, WY	B1	1.53 ab	1.86 a	1.98 a	1.40 b	1.88 a
	B2	1.80 a	1.77 a	1.92 a	1.93 a	1.72 a
	B3	1.65 a	1.30 ab	1.07 ab	0.85 b	1.37 ab
Ft. Morgan, CO	C1	3.29 ab	4.39 a	3.30 ab	2.93 b	3.19 ab
	C2	3.84 a	3.58 a	2.76 ab	2.51 b	2.91 a
	C3	2.02 b	2.38 ab	2.60 ab	1.83 b	2.84 a
Sugarbeet						
Scottsbluff, NE	A1	0.83 ab	0.41 c	0.60 c	0.61 c	1.00 a
	A2	0.83 a	0.80 a	0.82 a	0.67 b	0.84 a
	A3	1.61 a	1.65 a	1.20 b	1.61 a	1.55 a
	A4	0.78 a	0.68 ab	0.53 b	0.68 ab	0.62 ab
Torrington, WY	B1	0.61 bc	0.51 c	1.10 a	0.75 b	0.93 ab
	B2	1.15 a	0.63 b	0.78 b	0.76 b	0.87 ab
	B3	1.33 a	0.37 d	0.46 cd	0.93 bc	0.81 bc
Ft. Morgan, CO	C1	2.52 a	1.99 b	2.46 ab	1.75 b	2.31 ab
	C2	1.63 a	1.07 b	1.13 b	1.59 a	1.51 a
	C3	1.22 a	1.63 a	1.54 a	1.49 a	1.52 a

†Numbers with different letters represent statistical significance at 0.05 level (12 plants per treatment).

Note: Mean separation was performed for four PCC rates by soil type

Soil characteristics

The effect of PCC rates on selected chemical characteristics of ten soil types at the end of study are shown in Figs. 1 to 4. Soil pH increased in most samples from before to after the study in all PCC application rates and soil types (Fig 1.). Since repeated analysis of the soils before adding PCC was not done, it was not possible to perform a statistical analysis. Initial soil pH of the 10 sites ranged from 6.3 to 8.1 before PCC and from 7.6 to 8.4 after 7 weeks. There was also a significant increase in most of the soils that did not receive PCC. Drinking water (Scottsbluff municipal supply) from the tap was used to water pots. The water was analyzed after the experiment and found to have pH 7.8, salinity of 0.78 dS m^{-1}, 92 mg l^{-1} Ca and 27 mg l^{-1} Mg.

PCC analysis showed it had about 5.5 kg metric ton^{-1} of sulfur as sulfate (Table 1). Presence of such high proportion of S would contribute to the relative stability in the change of soil pH for higher rates of PCC (data not shown). In addition, the presence of sulfate-sulfur in PCC is a likely reason for the antifungal activity that can influence the control of

Fig 1: Effect of precipitated calcium carbonate (PCC) rates on soil pH of ten soil types before and after seven weeks of plant growth with one of three crop types, dry bean, corn, or sugarbeet, under greenhouse conditions in 2012. Rate effects of PCC on pH are statistically not significant at 0.05 level in all soil types.

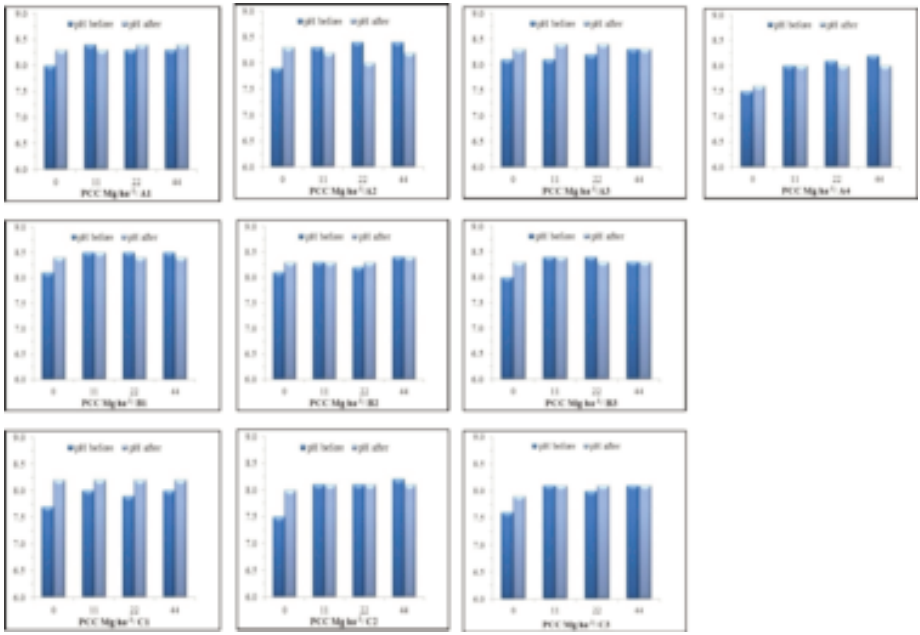
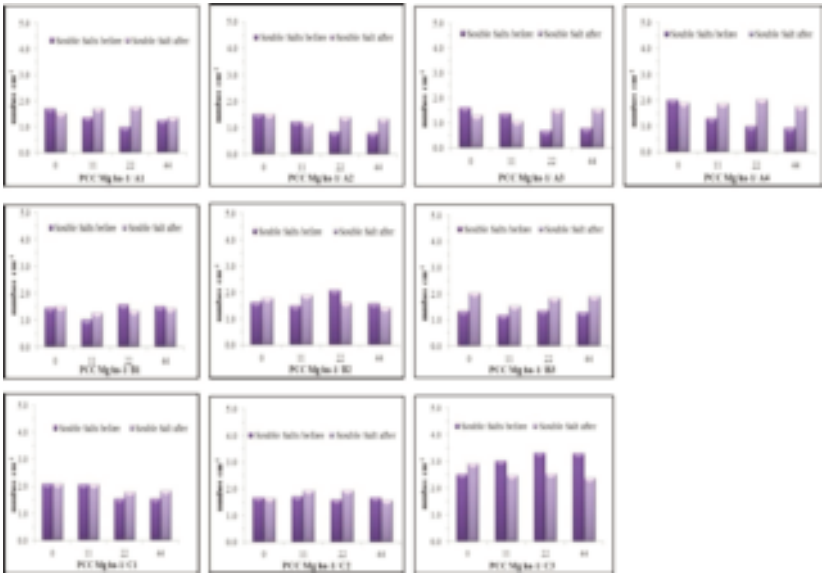


Fig 2. Effect of precipitated calcium carbonate (PCCP rates on soluble salts of ten soil types before and after the study under greenhouse conditions in 2012. Rate effects of PCC soluble salts are statistically not significant at 0.05 probability level in all soil types.

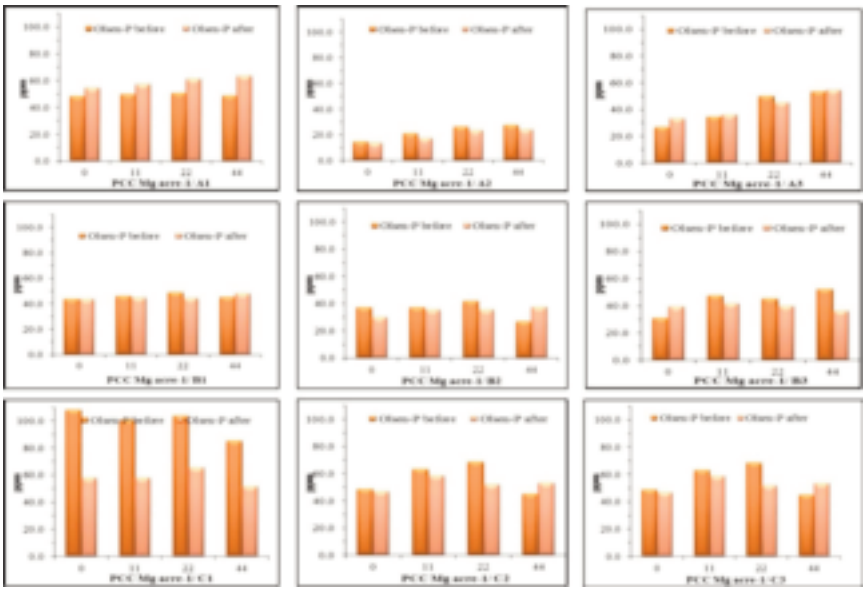


root rot fungi in sugarbeet as indicated by Campbell and Greathead (1989) and Windels et al. (2008).

Salinity (Fig. 2) of the 0 PCC rate plots increased from an average of 1.27 dS m^{-1} to 1.82 dS m^{-1} during the greenhouse study. With the high evaporative demand and hot temperatures in the greenhouse during this study, we hypothesize that there was sufficient drying to provide some salt and lime accumulation from the tap water which may have affected the pH of the check samples. In retrospect, distilled water should have been used. Change in soil pH among different PCC rates (excluding the check) was not significant. The average salinity level of all the PCC rates averaged over soil type (excluding the check) was 1.76 dS m^{-1} which was not much different than the check.

In most of the soils, an increase in Olsen-P was observed for all PCC rates compared to the control (Fig. 3), except for soil C1. The increased Olsen-P was attributed to addition of P from PCC. However, there was no particular trend of Olsen-P among different PCC rates. As the rate of PCC increased from 11 to 44 Mg ha^{-1}, the Olsen-P content was increased in the Scottsbluff soils. However, this trend was not apparent in the Wyoming and Colorado soils. In most of the Colorado soils with soil pH around 7.5, a higher rate of PCC application (44 Mg ha^{-1}) decreased Olsen-P content. The possible reason for the decreased trend of P can be

Fig 3. Effect of precipitated calcium carbonate (PCC) rates on soil Olsen-P of ten soil types before and after the study under greenhouse conditions in 2012. Rate effects of PCC on Olsen-P are statistically not significant at 0.05 probability level in all soil types, except C1.



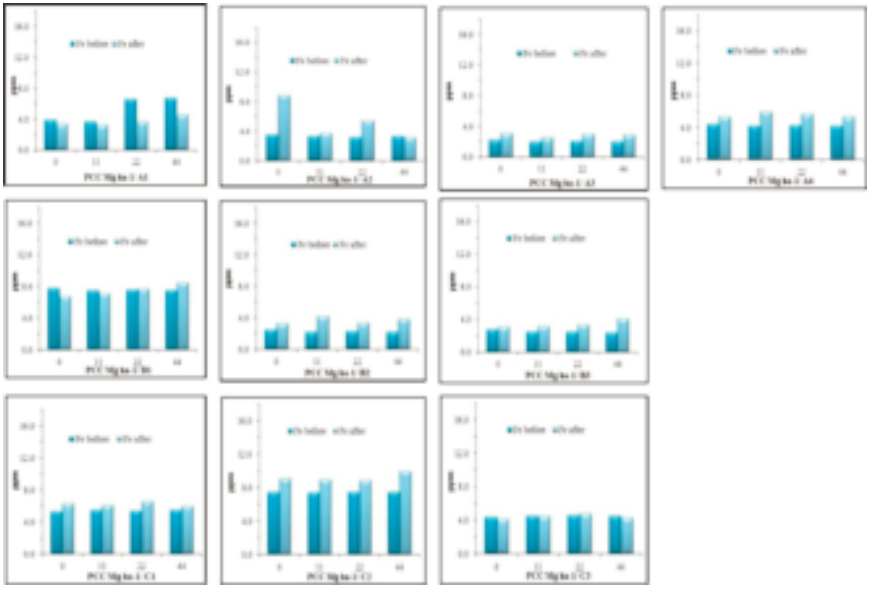
explained by potential fixation of P. P fixation generally peaks around pH 8 to 8.5 (Lindsay, W. L. 1979). which was also a possible factor for the decrease in P content with increase in the PCC rate.

The effect of PCC rate on Fe content is shown in Fig. 4. PCC did not significantly affect the DTPA-Fe level. Before the experiment, the average of the 10 soils was 5.5 mg kg^{-1} and it was 5.6 mg kg^{-1} after the experiment (Fig. 3). This was considered to be a positive result as higher lime and pH can often lead to lower Fe levels. With a few exceptions, the PCC effect on DTPA-Zn was inconsistent in different soil types (data not shown). Overall, the response of different PCC application rates in improving the plant nutrient availability depended on the soil type and soil test level at the time of PCC application.

CONCLUSIONS

Using PCC on grower fields should be a win-win proposition. The greenhouse studies provided sufficient information to test the agronomic potential for further research in field plots. We concluded from the greenhouse study that application of PCC did not significantly change soil chemical characteristics. Dry matter accumulation of sugarbeet, corn,

Fig 4. Effect of precipitated calcium carbonate (PCC) rates on soil Fe of ten soil types before and after the study under greenhouse conditions in 2012. Rate effects of PCC on Fe are statistically not significant at 0.05 probability level in all soil types.



and dry bean was not significantly increased or decreased in most of the soils. Part of the reason for the non-responsiveness may be lack of enough time for PCC mineralization to occur to enhance the soil chemical characteristics. Some of the effect may have been the tap water used to water pots. In order to recommend the agronomic utilization of PCC material in neutral to alkali soils, long term effects on plant growth and soil characteristics need to be examined under field conditions. In addition to that, future evaluation of PCC for the control of diseases and pests would also justify the cause of PCC application for potential agronomic and yield benefits.

Based on the results of greenhouse studies, it is evident that application of up to 44 Mg ha^{-1} PCC did not benefit nor harm the plant yield and soil characteristics. Applying agricultural lime or PCC above rates of 10 to 15 Mg ha^{-1} would be difficult and expensive. Based on this data and other reports (Brantner et al., 2015; Bredehoeft et al., 2013; Christenson et al., 2000; Giles and Smith, 2005; Sims et al., 2010; Windels et al., 2008), we concluded that rates for future field studies should be less than 25 to 30 Mg $ha^{-1.}$ Moreover, optimization of PCC application rate would help producers reduce application costs and possibly increase the overall profitability.

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