British Beet Research Organisation

10/99 **FINAL REPORT** **2012**

Assessment of genotypic differences in susceptibility to frost damage

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Executive Summary

- Temperatures during the 2010/11 campaign caused significant levels of frost damage to beet still in the ground, resulting in major financial losses to growers and the processor.
- A trial at Broom's Barn that was established to test the relative rust susceptibility of 18 RL varieties and 45 year2 and year3 entries was used to assess differences between these varieties in the degree of frost damage to the roots.
- Varieties differed in frost damage as a result of cold temperatures followed by warm January temperatures. There were large contrasts: a small number of varieties showed greater than 32% damage while five others exhibited less than 12% damage.
- Variety effects may have been confounded by differences between varieties in rust susceptibility; however, differences in frost damage between vareties could not be attributed solely to indirect effects of rust on canopy size prior to the first frosts.
- If differences between varieties in resilience to frost prove robust (this was one trial on one field in one season), then this should guide decisions on which varieties to schedule for early vs. late lifting.
- Breeders may find these data useful so that an extra measure of winter hardiness can be built into future varieties.

Objectives

- To determine the extent of differences between genotypes in the amount of frost damage
- To determine what characteristics of the varieties could be related to differences in frost damage

Background

The 2010/11 campaign ran into a very cold period in late November and December with ground temperatures reaching -12°C in some regions. This caused significant frost damage in beet crops and those stored in clamps. The damage was heightened by a period of warm weather in January. An important question is whether varieties differ in susceptibility to frost damage. If so, this would give growers, particularly those with fields targeted for late delivery, a potential management option in the future. In addition, by including NL entries, breeders may be able to make associations between genotypes with similar genetic backgrounds and freeze damage tolerance. This will be important for future breeding, almost exclusively for the UK market.

A trial was conducted at Broom's Barn in 2010 included as part of the BBRO project (08/01; sugar beet variety trial programme) investigating variety responses to levels of rust. This included 18 RL varieties and 45 year2 and year3 entries. These plots were for disease assessment only, and were not scheduled for yield measurements. However, the plots remained in the ground over winter, and by January 2011 there were obvious differences in the degree of frost damage. Thus, the trial was harvested and assessed to determine the degree to which varieties showed frost damage.

Materials and Methods

Field Experiment

The trial consisted of 64 genotypes planted in a randomised complete block design with four replications. Plots were drilled at 18.4 cm spacing in 3 rows x 12 m with 50 cm row spacing. Rows of an extremely rust susceptible variety were planted between each block to act as rust spreader plants. The entire trial was sprayed with Fortress (quinoxyfen) on 25 Jul 2010 to control powdery mildew, but did not receive any other fungicide treatments. Plots were assessed for rust incidence (as % leaf area affected) on 6 Sep 2010, 29 Sep, and 27 Oct. Plots were further visually assessed for the amount of erect green leaf remaining on tops on 18 Jan 2011.

Plots were harvested on 25 Jan 2011 by hand-lifting 8 m of the central plot row. In cases when a wheeling was adjacent to the central plot row, one of the side rows was lifted to avoid a possible effect of more crown being exposed next to a wheeling. Roots were topped at the lowest leaf scar in the field and counted. Roots were bagged and stored outdoors until processed in the tarehouse on 26, 27, 28 and 31 January, 2011 (one block per day).

Visual assessment of frost damage

In the tarehouse, roots were washed and weighed. Random samples each of 20 roots were sliced longitudinally using a mounted guillotine knife and visually

assessed for frost damage by judging the percentage of root that was translucent and glassy in appearance, which was almost always clearly demarcated from white, opaque, sound tissue (see photos). In addition, each root was scored according to whether roots had become 'gummy', characterised by soft, oozing tissue. Roots were given a score of 0 if no gumminess was evident, 1 if crowns were gummy, or 2 if the gumminess extended beyond the crown into the root. A total of 5,120 roots were manually sliced and assessed and the average percentage of the damaged surface area was calculated for each variety. Photographs were taken of example plots that contrasted for damage.

Sugar assays-polarimetry
Roots were washed, weighed and passed over a set of saws to produce brei. Samples of brei were taken from each plot, frozen in trays, sealed and stored at -20°C until analysed for sucrose concentration and impurities using UK industry standard methods (Jaggard et al. 1999). One tray of frozen brei was shipped on dry ice to the British Sugar Wissington factory laboratory for analysis. A further fresh brei sample was placed in 15 mL tubes, immediately frozen in liquid N_2 and stored at -20°C until analysis for hexose sugars (glucose and fructose) and sucrose using enzyme-based assays.

In the tarehouse, another set of tissue samples were taken, but from longitudinal slices of damaged beet. The slices revealed sectors of the root that were damaged or undamaged, and a disc of tissue (14 mm x 5 mm thick) was removed from each sector, placed in a tube and frozen at -20°C.

Sugar was extracted from frozen and thawed brei and clarified in a basic lead acetate solution (20°C) and analysed by polarimetry (Optical Activity, Ltd, Cambridge, UK). The polarimeter was calibrated using a quartz crystal standard. The α–amino N concentration in the filtrate was analyzed by the 'blue-point method' with an amino N analyzer (ChemLab, Cambridge, UK) that was calibrated daily using a sodium glutamate standard. Sample aliquots were diluted in a buffer containing 27 mM $Cu(NO_3)_2$ and 1.8 M sodium acetate (pH 6.0). Potassium and sodium concentration in the filtrate was analysed by atomic absorption (Varian, Palo Alto, CA, USA). Root water content was determined as the difference between brei fresh weight and the weight after drying to a constant weight at 80°C.

Sugar assays-enzyme-linked colorimetric assay
Approximately 150 mg of frozen brei was extracted in 1 mL ethanol (80%, v/v) for 2 h at room temperature. This ensured solubilisation of the sugars and denatured native sugar metabolism enzymes. Tubes were centrifuged and 800 μL aliquots were removed and frozen at -20°C. For the assay, the extracts were thawed, and an aliquot ws removed and diluted in water. For glucose and fructose, the dilution was 1:3.3; for sucrose, extracts were diluted 1:100. The ethanol in the diluted samples had no effect on the assay. Aliquots of diluted sample or standard sugar solutions were added to wells on a microtitre plate.

The assay was based on the method described by Cairns et al. (1987). Related assays have been used before in sugar beet (Spackman and Cobb, 2002) and sugar cane (Campbell et al., 1999). Briefly, glucose in the sample was converted to glucose-6-phosphate with hexokinase and ATP, and fructose was likewise converted

to fructose-6-phosphate, and then to G-6-P with phosphoglucoisomerase. $NADP^+$ was then reduced to NADPH in the presence of G-6-P and glucose-6-phosphate dehydrogenase. The transfer of electrons in this reaction was coupled to thiazolyl blue (MTT), a tetrazolium salt, which when reduced becomes a bright blue formazan dye. The production of blue colour is directly proportional to the amount of glucose in the sample as all other reactants are supplied in excess. The absorbance of the blue dye was measured at 595 nm using a microtitre plate reader (Anthos 2001, Anthos Labtec Instruments, Austria). For sucrose analysis, a second plate was prepared as above, but with a sample dilution of 1:100. Sucrose was converted to glucose and fructose by invertase, then the procedure was followed as above for glucose and fructose. Sucrose concentration was determined by subtracting the background concentration of glucose measured in the first plate. Sugars were quantified using standard curves created using sugar standards of known concentration.

Sugar assays-HPLC

Samples of frozen brei were shipped on dry ice to the Wissington factory tarehouse laboratory. Frozen brei was extracted in deionised water according to standard tarehouse procedure (26 g beet material plus water to a total weight of 200 g). Samples were mixed with a Grindomix laboratory food processor and filtered through filter paper. Filtrate was kept on ice, then divided into aliquots; two were frozen for repeat analyse and one was analysed immediately. Samples were first tested for degradation during the sampling process by a rapid biosensor assay for glucose and lactose (Super GL Ambulance, Dr. Müller Gerätebau GmbH, Germany*)*, and any samples with high values were discarded.

Samples were assayed using a Dionex HPLC by injecting 10 μL onto a Carbopak column (4 mm x 30 cm) preceded by a PA1 guard column. The column temperature was held at 30°C, and samples were eluted with degassed water at high pH (85:15 water: 1M NaOH) at flow rate of 1.0 mL min-1. Concentrations of glucose, fructose, sucrose and raffinose were quantified using pulsed electrochemical detection and comparing peak heights with known standards using Chromeleon 6.6 software.

Statistical analysis

Data were analysed by ANOVA using Genstat v.12 (VSN International, Ltd., Oxford, UK). For all the analyses of the percentage data, the residual diagnostic plots indicated that a logit transformation should be applied to make the variance more constant and treatment effects more additive. After the analysis, the table of means on a natural scale was created from back-transformed data. Within each experiment, Pearson product-moment correlation coefficients between all variables were calculated using Excel (Microsoft) software and tested for significance at the level indicated in the Tables or in the text (*, P = 0.05; **, P = 0.01). Regression coefficients were calculated and lines fitted using SigmaPlot software (SPSS, Inc.). Because of a weak correlation between frost damage and rust scores, a covariate analysis was also performed for each rust score date. The principle underlying covariate analysis is that by accounting for variability using the covariate, the experiment is more precise as the residual variance should be smaller. Statistical analyses were performed and checked by the Rothamsted Statistics Department (R. White).

Results

Weather
Temperatures at Broom's Barn on several occasions in late November and December fell to -5°C or colder for two consecutive nights, when soil temperatures were already colder than 1°C: these conditions appear to define a threshold for risk of frost damage (Ref. 1, 2). Subsequently, a warm period of five consecutive days in mid-January when temperatures reached 11-12 °C then induced roots to deteriorate rapidly in many plots (Figs. 1, 3). These temperatures were well below and above the long-term averages for those periods. The temperatures recorded on grass were colder than air temperatures, but soil temperatures at 5 cm below the soil surface were insulated from subzero temperatures of the air (Fig. 2). This suggests that heat loss was transferred through root and crown tissues into the air, rather than via conductance to the soil, which would tend to buffer changes in root temperatures.

Variety differences in frost damage
The trial that was used for the frost damage assessments was originally a trial designed to test varietal differences in susceptibility to rust. However, levels of rust were not high and at the end of October the canopy appeared similar to those in commercial fields (Fig. 4). In January, 2011, following the frost events, it was noticed that certain plots had a greater survival of young leaves, and these were scored for "% erect green leaves". A few test digs revealed that the roots of these plots were also less damaged than others. It was decided to lift the entire trial and assess the degree of damage on all varietes.

Examination of the results from all varieties showed a large contrast: a small number of varieties showed greater than 32% damage while five others exhibited less than 12% damage. In the latter group the root tissues remained mostly white, opaque and solid (Fig. 5). The other varieties showed intermediate amounts of damage (Fig. 6). Statistical analysis of the damage scores showed that differences between the varieties at the extremes were unlikely to have occurred by chance ($P< 0.001$). The mean damage scores of the 18 RL varieties are shown in Fig. 7, and that of all genotypes in Fig. 8. The 18 RL varieties comprised most of the extreme contrasts in frost damage within the entire set, and therefore were a useful subset to study in greater detail.

In most plots there was large variability from plant to plant within the harvested row, with some roots showing 60% damage, while a nearby root might show none (Fig. 9). The factors contributing to this variability are not known. In general, small roots (e.g. crown diameters of approximately 5 cm) tended to show less damage than typical roots, but in badly-affected varieties even small roots were damaged. Likewise, in varieties with little damage, most of the roots within the plot were uniformly in good condition. Thus, the categorisation of 'susceptible' and 'resistant' varieties is unlikely due to random error. The partitioning of variance within the ANOVA shows that the variety accounted for 75% of the total variation within the data and the trial precision was good ($R^2 = 0.75$).

Variety aspects that may have contributed to frost resilience
There are many factors that could help explain both the variability within a plot and the differences between varieties. For instance, root size, root shape, crown height

above the soil surface, root sugar or water content, and canopy size could affect susceptibility to frost damage. Root shape did not appear to have a strong effect. In gappy fields, large roots with substantial root material above the soil surface could be particularly susceptible to damage. In this trial, however, plant populations were relatively uniform and there was no relationship between plant populations, root size and frost damage (Table in Fig. 16). In some cases, the degree of damage corresponded to the appearance of the remaining tops (Fig. 4), as shown by the negative correlation between the percentage of tops with erect, green leaves and damage score (Table in Fig. 16).

Adjacent to the trial area was a crop of Bullfinch that had received a regular fungicide programme, but was also heavily damaged by frost. Several roots were dug that varied in the amount of root exposed above the root surface. The damage extended several cm below the soil surface level (Fig. 19). Thus, it was not only 'proud' roots that were damaged by frost, and this did not appear to explain the plant-to-plant variability within plots. It is interesting that the insulating properties of the soil due to its thermal mass—soil temperatures at 5 cm below the surface did not go much below 0°C, which is above the damage threshold for beet roots—did not protect the roots, suggesting that heat was conducted out of root tissues through the root itself and not the soil.

There was a weak but positive correlation between susceptibility to rust and frost damage (Figs. 10, 11, 16). A covariate analysis conducted with frost damage score and rust scores from the three assessment dates did not show any significant effect of the covariate. The ANOVA, taking into consideration the rust score as covariate, did not lead to a different interpretation or ranking of varieties for frost damage. Therefore, varietal differences in frost damage were likely due to factors other than the level of rust infection. However, the weak association suggests that the size and integrity of the canopy may have played a role in protecting roots from frost, as varieties with greater levels of rust-infection probably had smaller leaf cover. A similar relationship between rust score and frost damage was observed in 2009/2010 in a similar trial at Broom's Barn (Ref. 6). Unfortunately no measurements of canopy cover were made in either of these trials. An older study conducted in the Netherlands indicated benefits of foliage cover to protect roots from an initial frost, but little protection against subsequent frosts once the leaves were killed (Ref. 4).

It is unlikely that rust infection, or related effects on canopy size, can explain all of the differences in frost damage, since some of the varieties that showed very little frost damage had greater than average levels of rust infection (Figs. 10,11). For example, Carissima had 18% of its leaf area infected by rust (scored on 29/9/10), while Bullfinch, which had more frost damage, had only 7.5% infection by rust (LSD = 9%). Furthermore, adjacent to this trial, a crop of Bullfinch that received two fungicide applications was also badly damaged by frost. More information is needed about the role of the canopy in frost protection, but it remains good practice to maintain a healthy and efficient canopy as long into the winter as possible.

A correlation analysis showed that only small amounts of the variation in frost damage could be explained by variation in factors such as yield potential, root size and sugar concentration (data collected on varieties in RL trials prior to any frosts). The contribution of physiological properties of the root tissue to resilience to frost is

not known. Also we do not know the conditions which allow beet to recover or 'grow out' of damage: certain fields that showed evidence of damage immediately after a frost at a later stage appeared healthy and fit for processing, according to many anecdotal reports. It is unlikely that any 'growth' occurs at these temperatures, but it may be possible for transiently cold-stressed root cells to leak their contents into intercellular spaces (causing the glassy appearance) then re-absorb the water later (Ref. 2).

Survey data
Any variety, however, will succumb to temperatures that are low enough to overcome any protective aspects, whether from the foliage or from the root. Survey data collected by British Sugar of 126 crops harvested after the warm January temperatures indicated that even the variety Carissima, which had very little damage in the Broom's Barn trial, was heavily damaged in other growing areas where the weather was colder. In future, comparison of accurate weather records with amounts of frost damage could provide additional clues about varietal differences.

Sugar concentrations
The sugar concentration values in this dataset are those from roots exposed to frost, and are of little use in determining the role of sucrose concentration in root tissues prior to frost. Similarly, this is true for dry matter content, as varietal differences in these variables are most likely a consequence of damage. However, varietal differences in sugar concentration and root yield measured before plants experienced freezing temperatures (in the five RL trials in 2010) showed little relationship with damage score (Table in Fig. 17).

It is well-known that sucrose is degraded by microbes acting on frost-damaged tissue, and that this results in increased levels of the invert sugars glucose and fructose. We compared different zones of frost damaged tissues, and these findings were confirmed (Fig. 12). Glucose concentrations, in particular, were strongly associated with damage score (Fig. 13, 14), and sucrose concentrations were smaller in the varieties with greater damage (Fig. 14). There was poor correlation between sucrose measured by polarimetry vs. HPLC (Table in Fig. 18). As shown previously by Shore et al., polarimetry over-estimates sucrose concentrations when invert levels are high, perhaps due to interference in the light rotation by elevated levels of fructose.

The accumulation of levan gums by microbes feeding on damaged tissue were evident in many roots, and bubbles were also visible, perhaps due to CO2 production by root cells and microbes (Fig. 20).

Conclusions
We observed clear differences between varieties in their susceptibility to frost damage; however, there are several caveats. Firstly, this was a rust trial, and there was a positive association between the percentage of foliage affected by rust and frost damage, although it is unlikely that this was a major contributing factor to frost susceptibility. Secondly, this was one trial on one field in one season: it is well known that varieties change rankings from one location or environment to another,

particularly when ranked for traits with low heritability such as yield. It is not known how consistent are the varietal rankings for frost susceptibility.

There is anecdotal evidence that the size of the canopy may afford some degree of protection from frost, and therefore the frost 'resistant' varieties simply may have had better canopies due to greater rust resistance. Therefore, the genetic component may be related to rust resistance, not to frost resistance *per se*. Nevertheless, it is unlikely that rust is the entire explanation of varietal differences in frost damage. For instance, the variety with the smallest amount of damage was not very resistant to rust, and the varieties with the most damage did not show the greatest levels of rust. A photo of the trial shows that overall, the canopy was reasonably full and healthy at the end of October (Fig. 4). Adjacent to this trial was a commercial crop of Bullfinch that received a full two-spray fungicide programme, showed little rust, but was substantially damaged by frost.

It remains unclear what causes such large differences between plants within the same row of the same variety, but clues to understanding this would be extremely helpful in making genetic improvements in new varieties, or perhaps identify ways to manage late lifted crops differently.

There are many hypotheses that can be posited to explain varietal differences in frost damage. It is worthwhile to explore these methodically. Better understanding of these differences could enable breeders to develop more frost resistant varieties in the future.

Future directions

There has been an expression of interest by Dr. Andrew Kniss, University of Wyoming, to collaborate on further chemical and physiological analyses. Beet production in Wyoming is also susceptible to early season frosts.

Further work can be done in collaboration with breeders to examine pedigree effects that could explain differences in susceptibility to frost. For instance, one breeder's preliminary analysis revealed that some pollinators appeared more frequently in frost tolerant varieties, and certain female components were more common in the susceptible genotypes. However, the sample size was too small for a thorough analysis.

A new BBRO-sponsored project "Optimising fungicide use for improving the canopy in relation to harvest date (12/06), will aim to assess differences in frost damage in relation to variety and canopy management in late-harvested trials, in the advent of sufficient cold temperatures to cause damage.

After two consecutive cold winters, 2009/10 and 2010/11, is there any indication that mild winters of the recent past are no longer the norm? Weather records from Broom's Barn since 1964 do not indicate trends towards colder minimum air temperatures during December and January, but do show a slight warming trend in minimum air temperatures in November (Fig. 22). However, the number of ground frosts in November shows no trend, but varies from one frost (2009) to 19 (in 1985; 16 in 2010) (Fig. 23). It is clear that we have no way to predict what weather holds in store for the next campaign.

References

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Acknowledgements

Rodger White (Rothamsted Statistics Department); Solange de Andrade (visiting scientist from EMBRAPA, Brazil) for the sugar assays; Christopher Clark for help with the frost assessments and data analysis; Anne Perry for handling the samples; Karen Pardo (British Sugar) for the HPLC analyses; Ed Burkes and Paul Bee (British Sugar) for survey data.

Knowledge and technology transfer

Findings from the research have been communicated through articles in the popular and farming press, and in talks given at meetings and conferences. The data have been purchased by a leading seed company; perhaps this information will help guide future selections in breeding programmes to avoid frost susceptible varieties.

Project résumé: Expenditure and scientific staff input

What was the planned expenditure?	£ 13,000
What was the actual expenditure?	£ 13,000
What was the planned scientific input in staff years?	0.15
What was the actual scientific input in staff years?	0.15
Have there been any outputs this year?	YES
Have any opportunities for IP rights been identified?	NO
Are there any scientific opportunities arisen not mentioned?	NO

Project résumé: Publications, presentations and dissemination of results

- Ober ES, Stevens M, Clark CJAC (2011) Do sugar beet varieties differ in their resilience to frost damage? British Sugar Beet Review 79: 18-23.
- BBRO Advisory Bulletins, February and June, 2011
- Four Seasons Sugar Beet Flyer, Issue 17, Spring, 2011. (Bayer Crop Science).
- Farmers Guardian , 11/2/11, p. 20; 25/3/11, p 20.
- Farmers Weekly, 11/2/11, p.47; 25/3/11, p57.
- Crop Production Magazine, April, 2011, p 64.
- Talks given at BBRO Winter meetings, Feb, 2011
- Presentation given to BSPB beet group
- Interview given to BBC Radio Lincolnshire, Feb, 2011

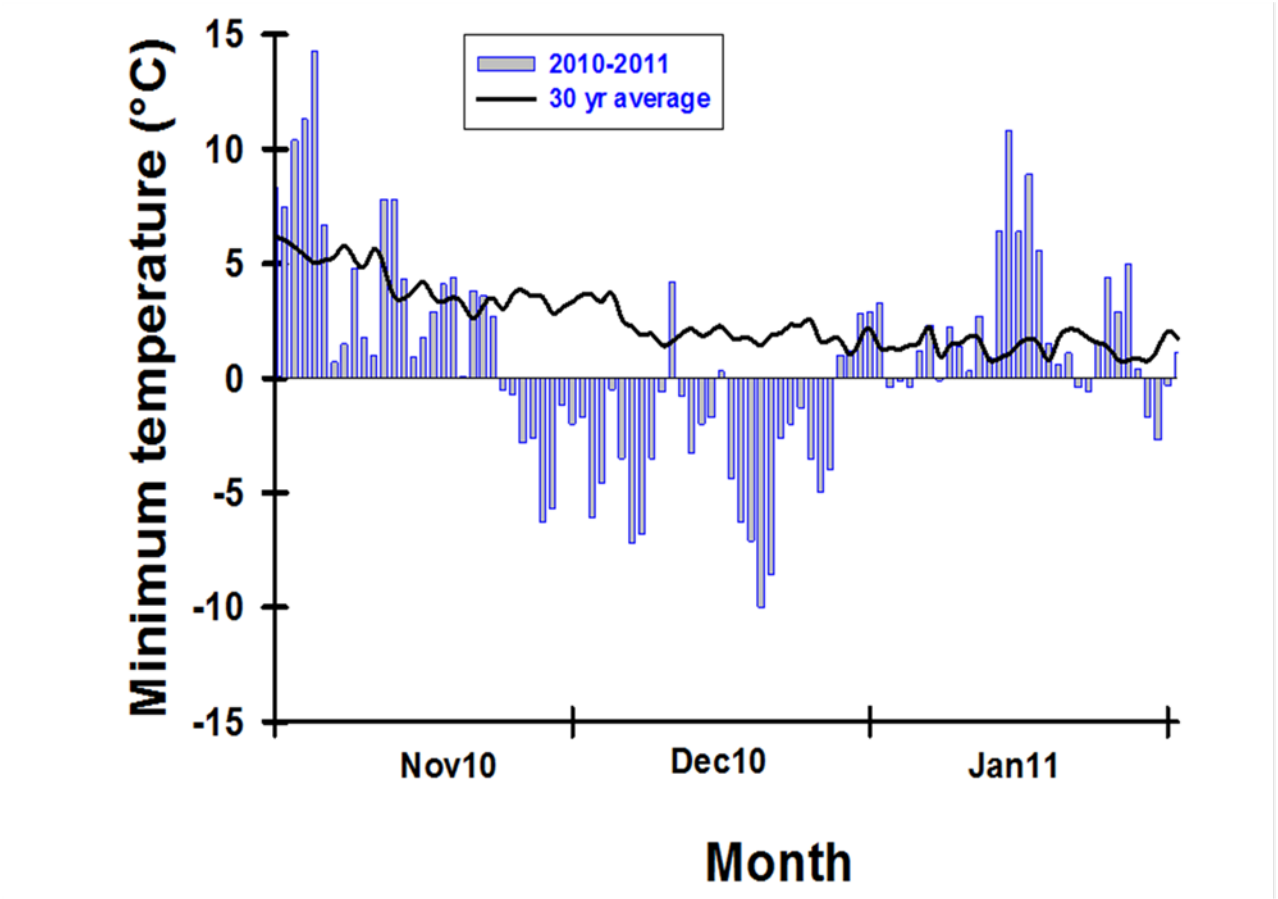


Figure 1. Minimum air temperatures recorded at Broom's Barn during the 2010/2011 campaign, compared with the 30-yr average. The cold temperatures in December, followed by warm temperatures in January were critical.

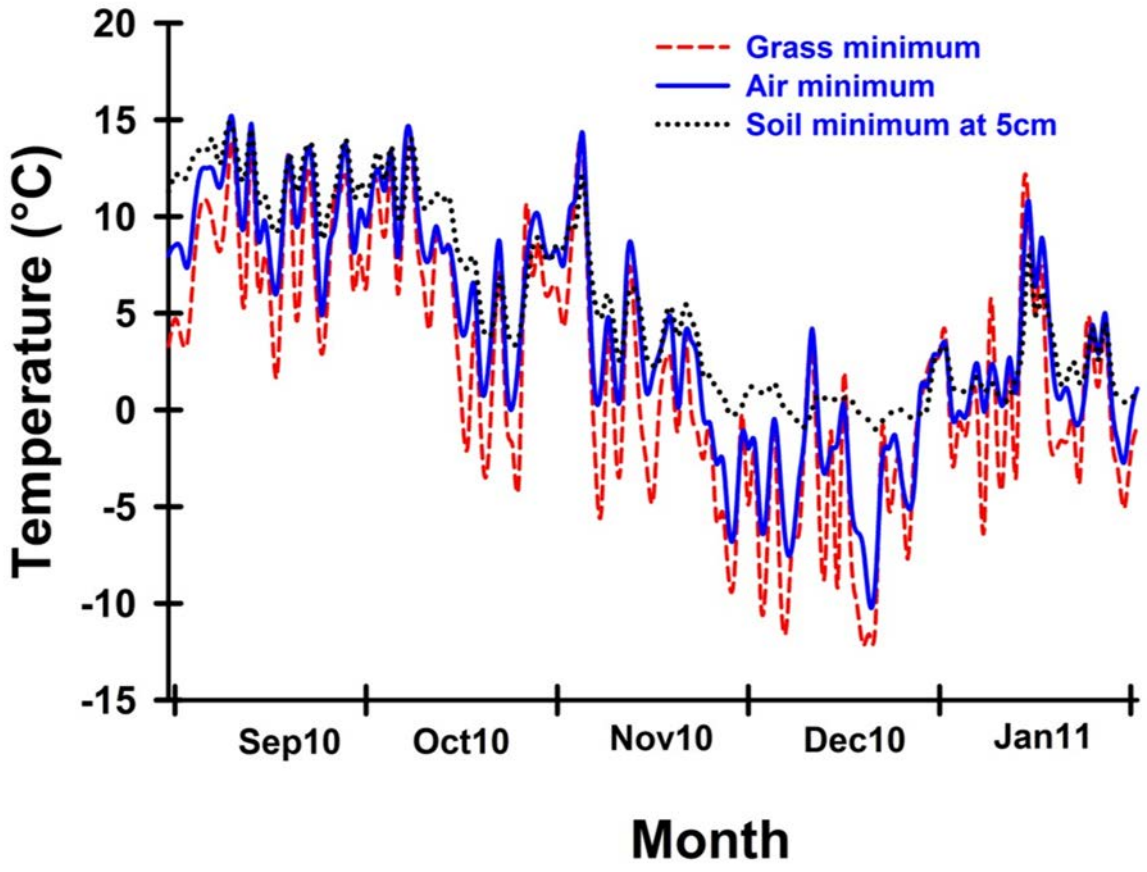


Figure 2. Minimum daily grass, air a soil temperatures at a depth of 5cm from Sep, 2010 to Jan 2011 recorded at Broom's Barn. Note that soil temperatures are insulated from air temperatures, and did not reach the critical level of -2.5°C assumed to cause damage to root tissues. Therefore, heat loss was probably conducted through the root and crown tissues to the air.

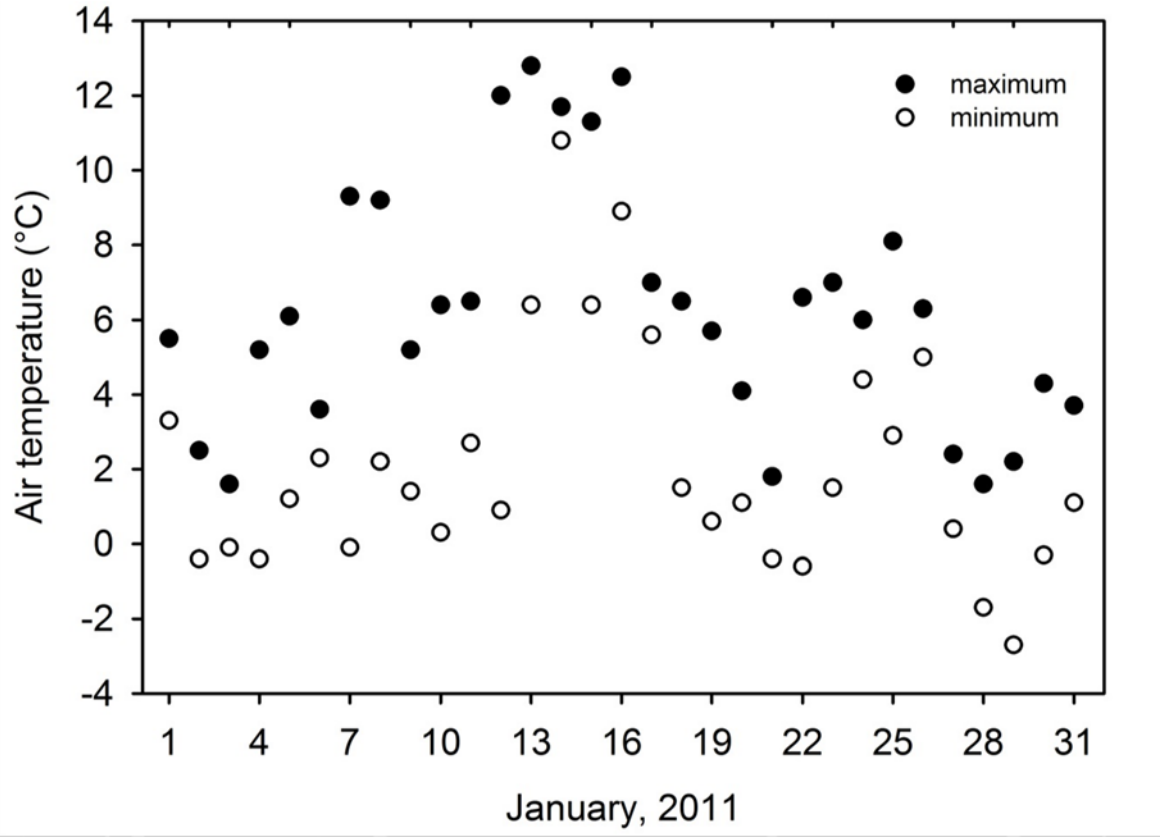


Figure 3. Minimum and maximum air temperatures during January, 2011 recorded at Broom's Barn.

Figure 4. Appearance of plots on 27 October, 2010 (upper panel) and on 19 January, 2011 (lower panel). The same plot is not shown on the two dates. Plots with less green leaf visible in January had roots that showed greater levels of frost damage (sample roots are shown with their corresponding plots (separated at the blue stake).

Figure 5. Four representative roots from plots of Carissima (top) and Bullfinch (bottom) that illustrate the contrasting responses. Labels are plot numbers for reference.

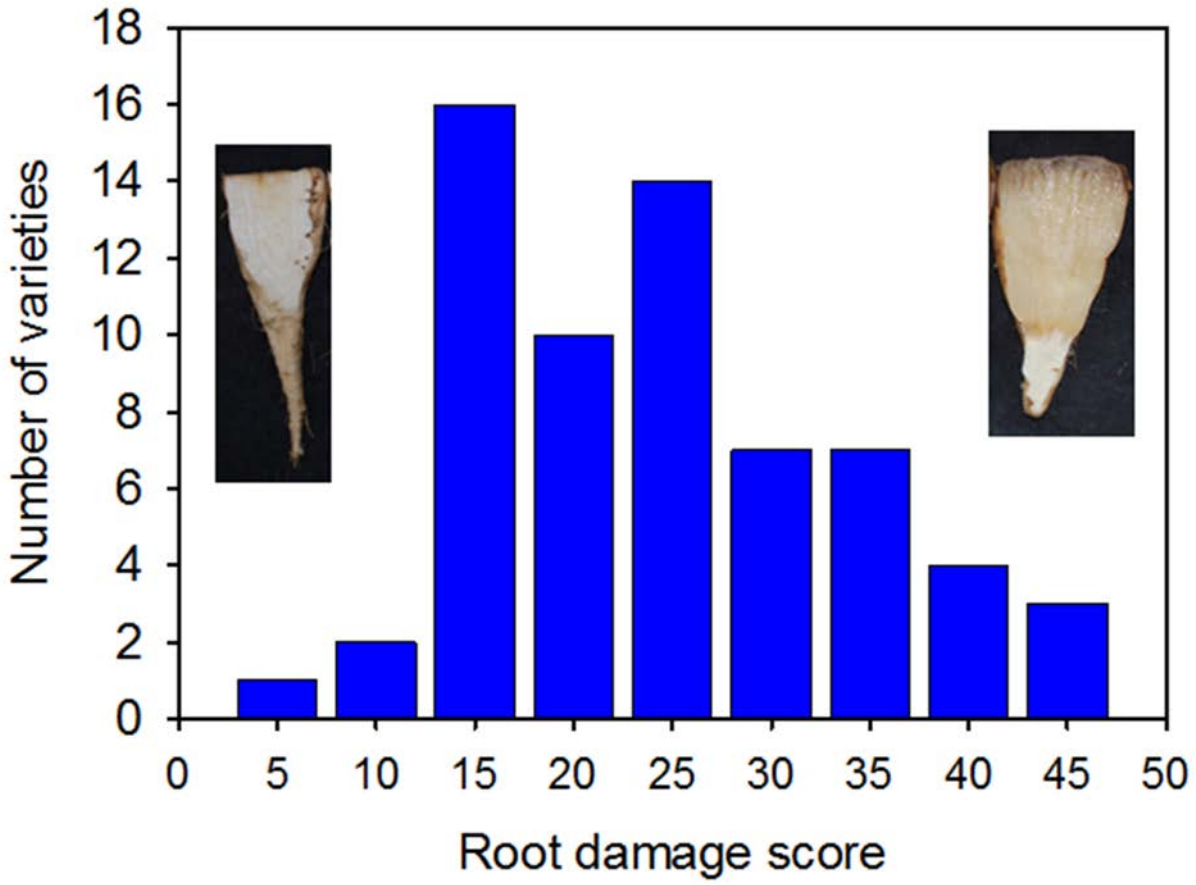


Figure 6. A histogram showing the distribution of varieties according to the root damage score. Examples are shown of roots with low and high damage scores.

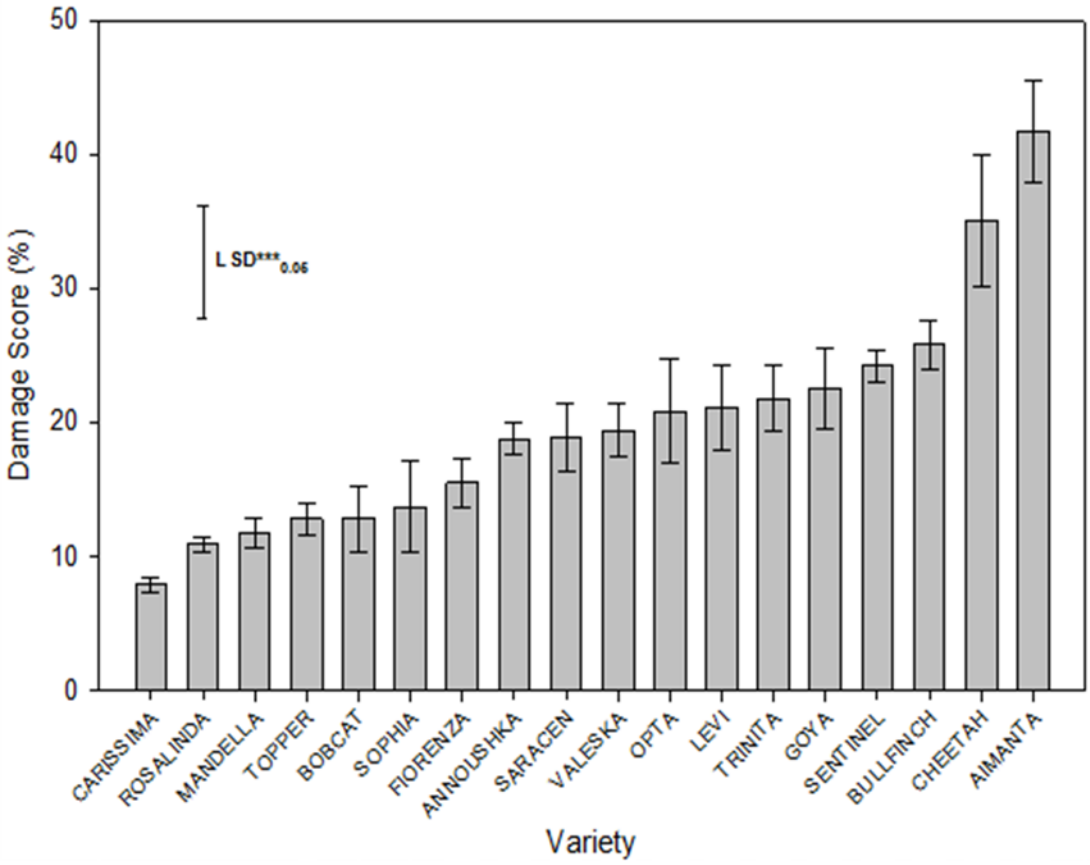


Figure 7. The mean frost damage scores of the 18 Recommended List varieties. The error bars indicate the plot-to-plot variability within a variety, and the LSD bar indicates the variance within the entire trial. Variety means can be compared using the LSD bar: bar height differences smaller than the LSD bar are not significantly different.

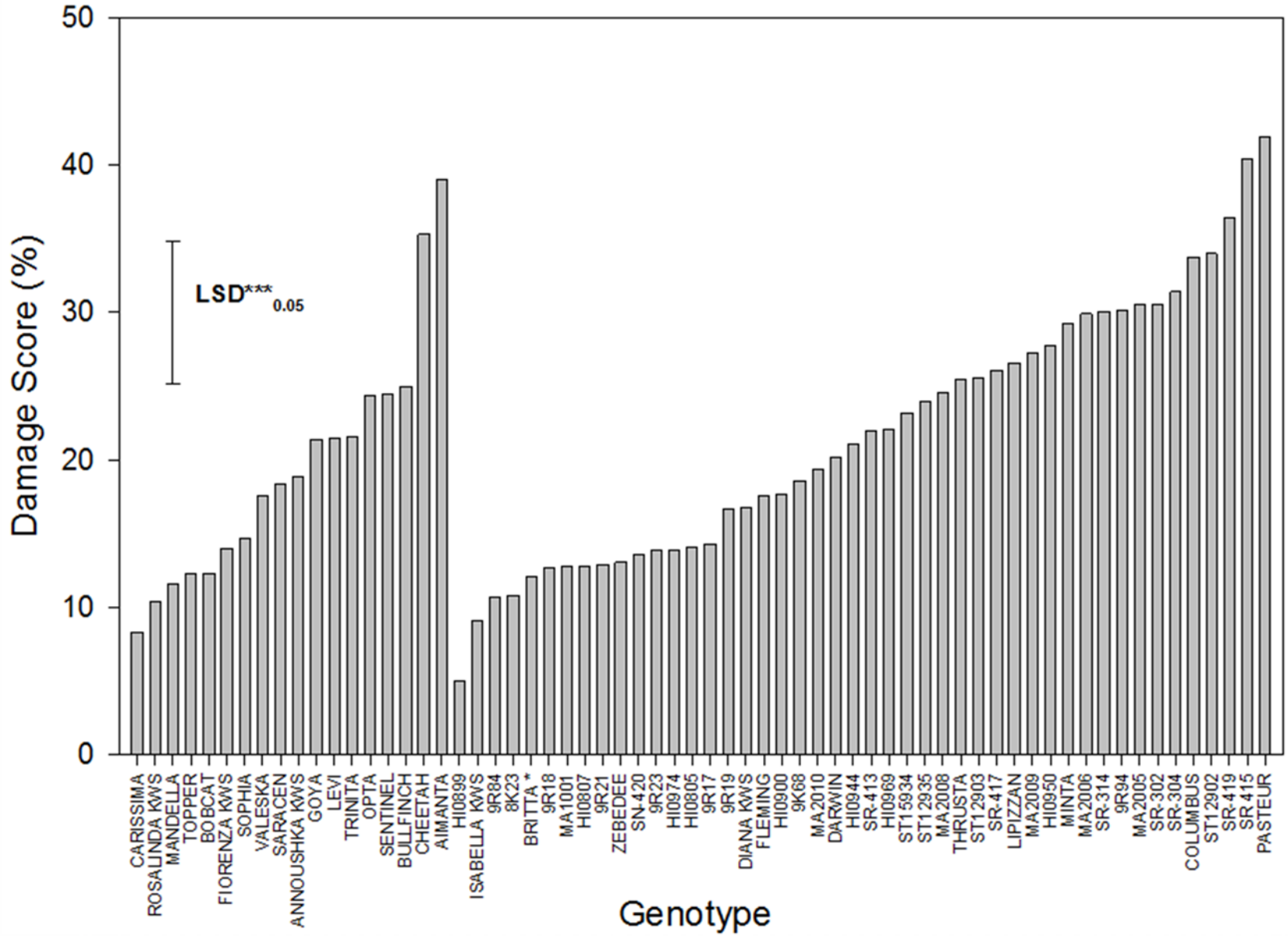


Fig.8. The mean frost damage scores of all tested genotypes, with the the 18 Recommended List varieties grouped to the left. The RL varieties include most of the extreme contrasts in frost damage within the entire set. The LSD bar indicates the variance within the trial. Variety means can be compared using the LSD bar: bar height differences smaller than the LSD bar are not significantly different.

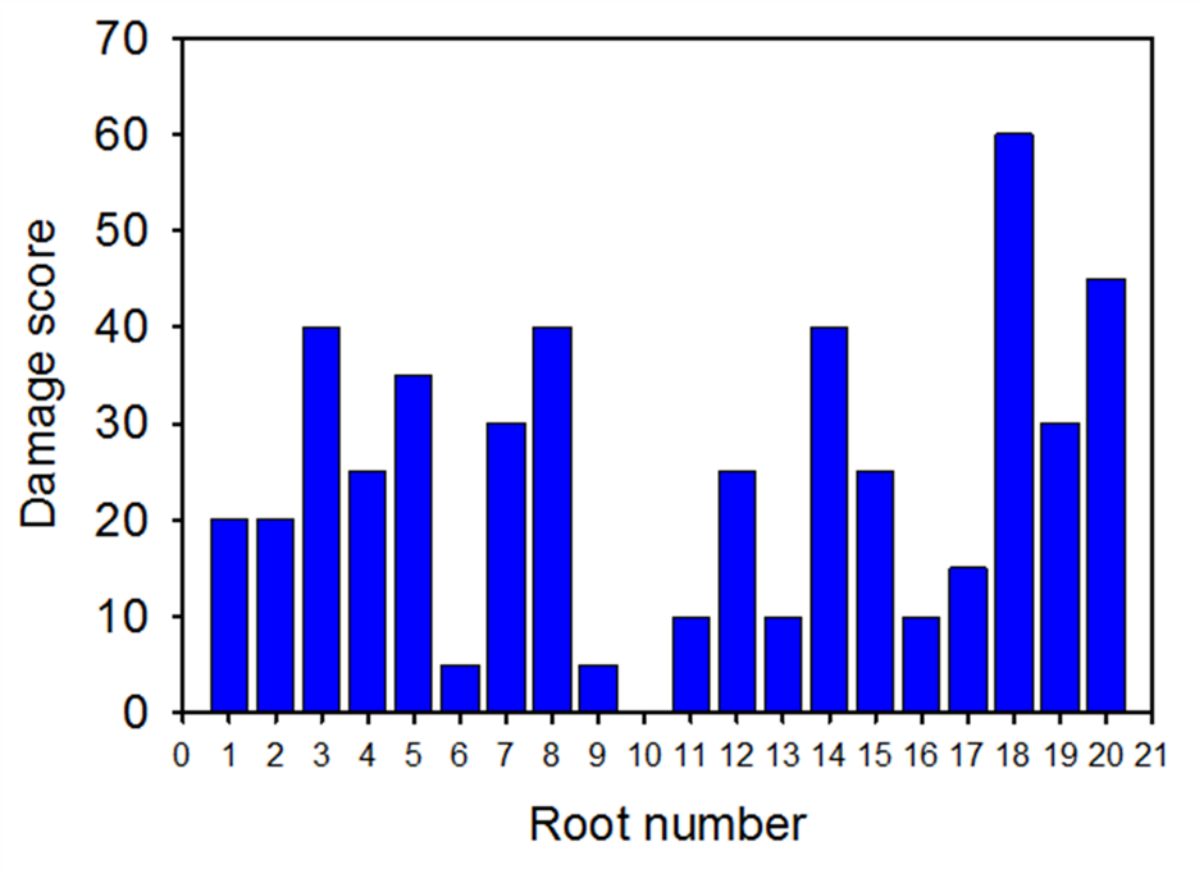


Figure 9. A typical example of the variation in root-to-root frost damage within 8 m of a single plot row.

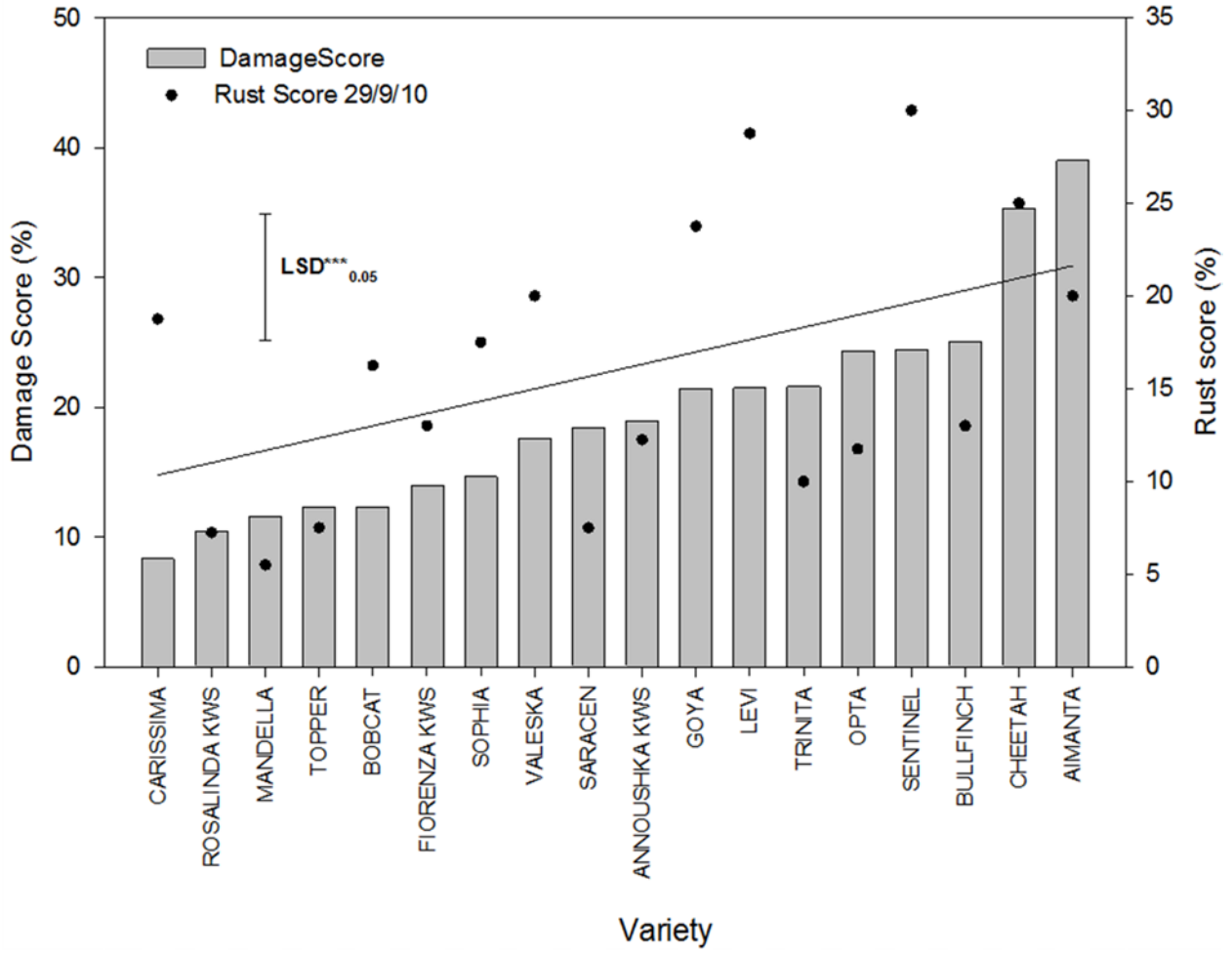


Figure 10. . The mean frost damage scores of the 18 Recommended List varieties (as in previous Fig), but with rust level scores indicated by the black dots for each variety. There is a general positive association between rust susceptibility and frost damage, but note that some varieties with low frost damage showed relatively high rust scores, and vice versa.

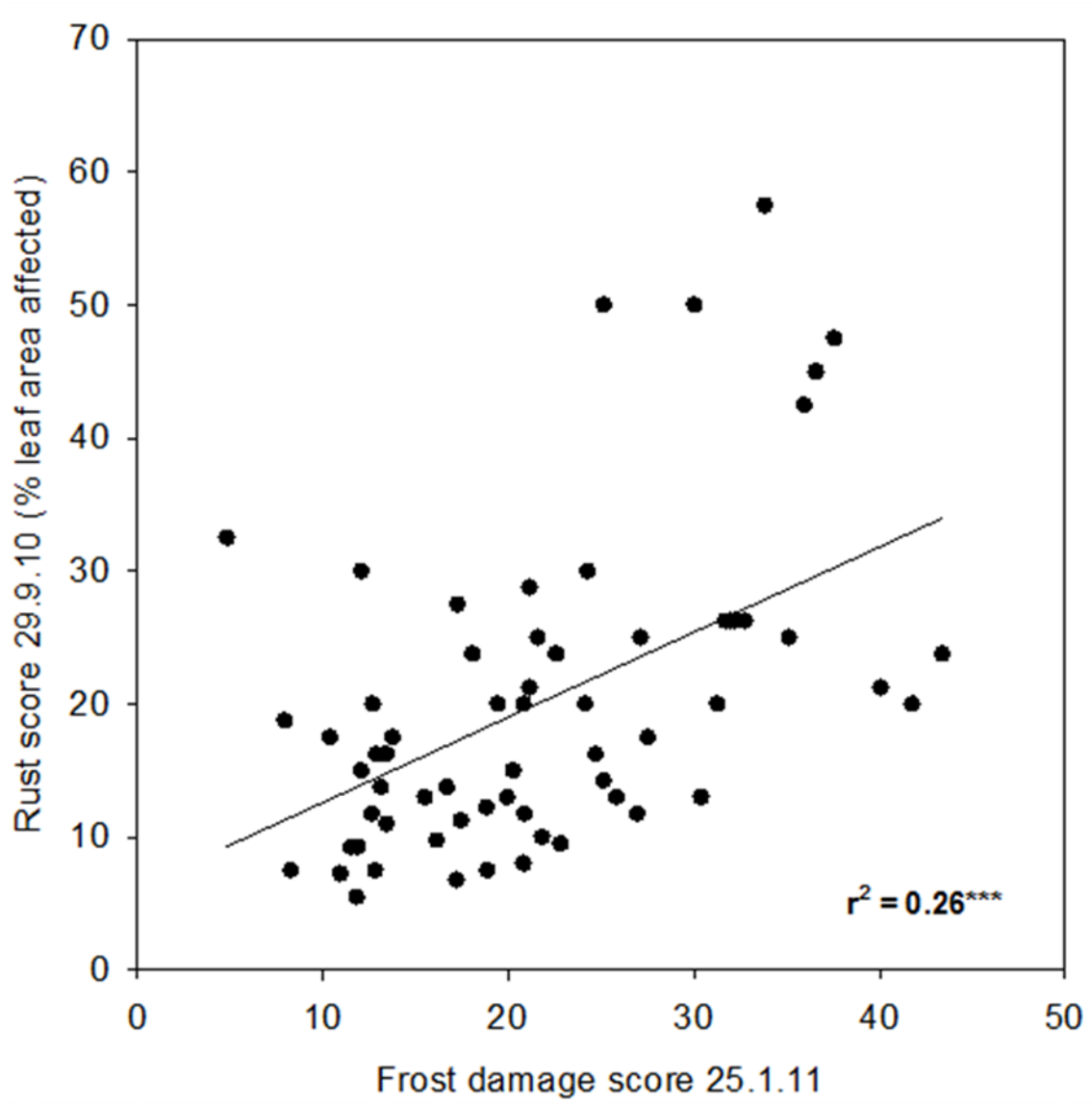


Figure 11.The association between the percentage of leaf area affected by rust on 29/9/10 and frost damage score assessed at the end of January, 2011. Each dot represents the mean values for a variety. The correlation is statistically significant, but note the large amount of scatter. The correlation coefficient indicates that 26% of the variation in frost damage score can be explained by the variation in rust score; the remaining variation is unexplained.

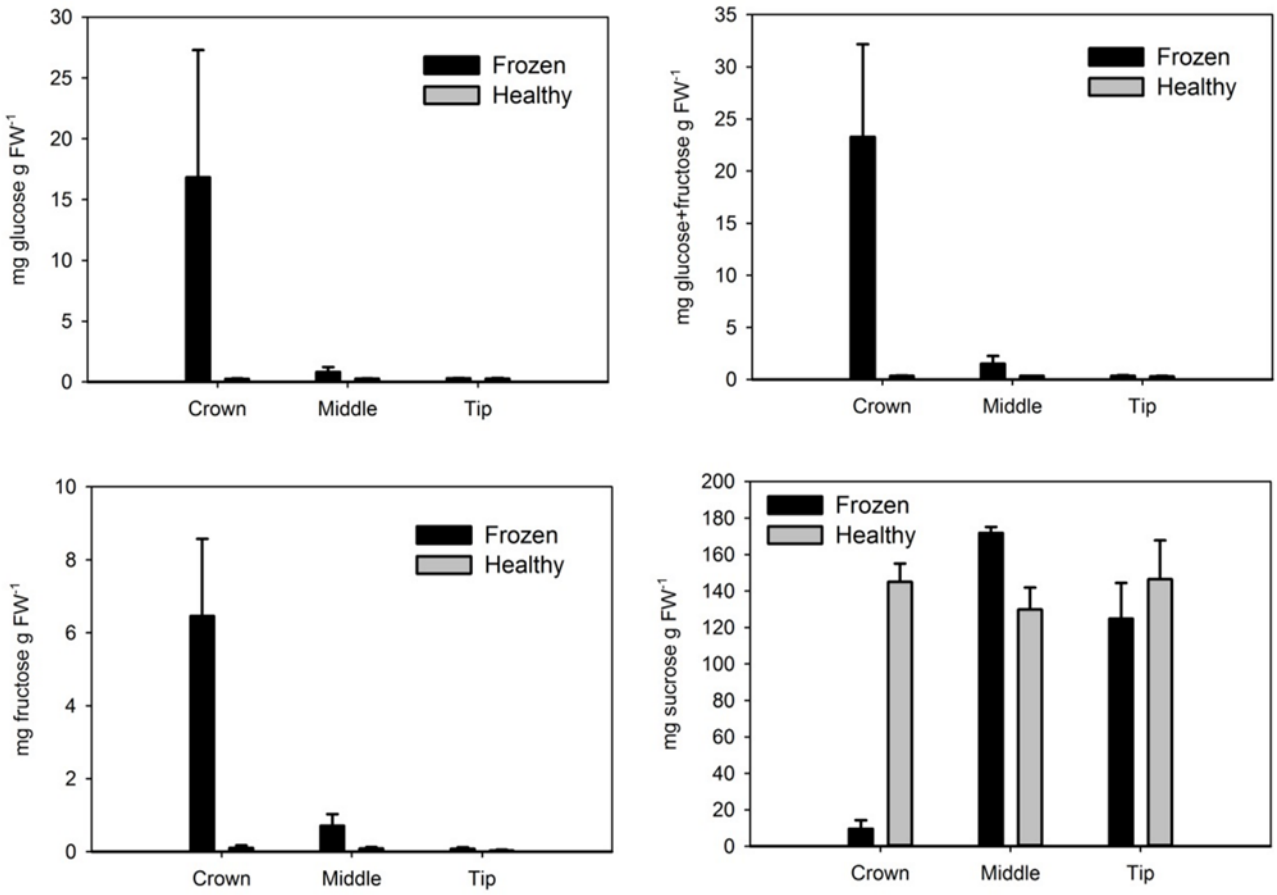
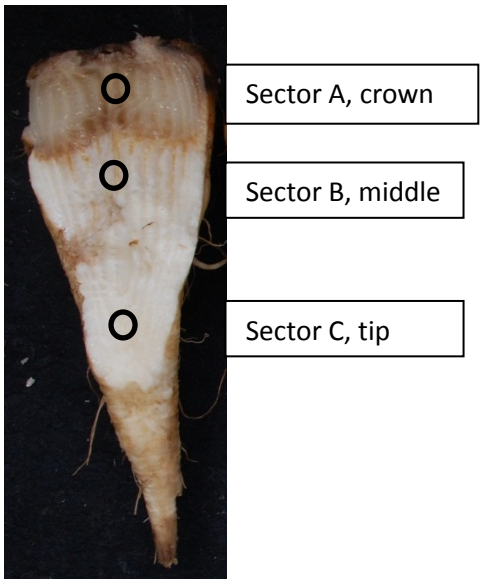


Figure 12.Comparison of sugar concentrations in tissue sampled from different zones of frost-damaged roots. Sugars were measured by enzymatic colorimetric assay. The different sectors and illustrated below. As expected, hexoses were higher, and sucrose was lower in the most heavily damaged part (crown). Concentrations can be converted to % on a fresh weight basis by dividing the values by ten. Bars represent the mean ± se (n = 4).



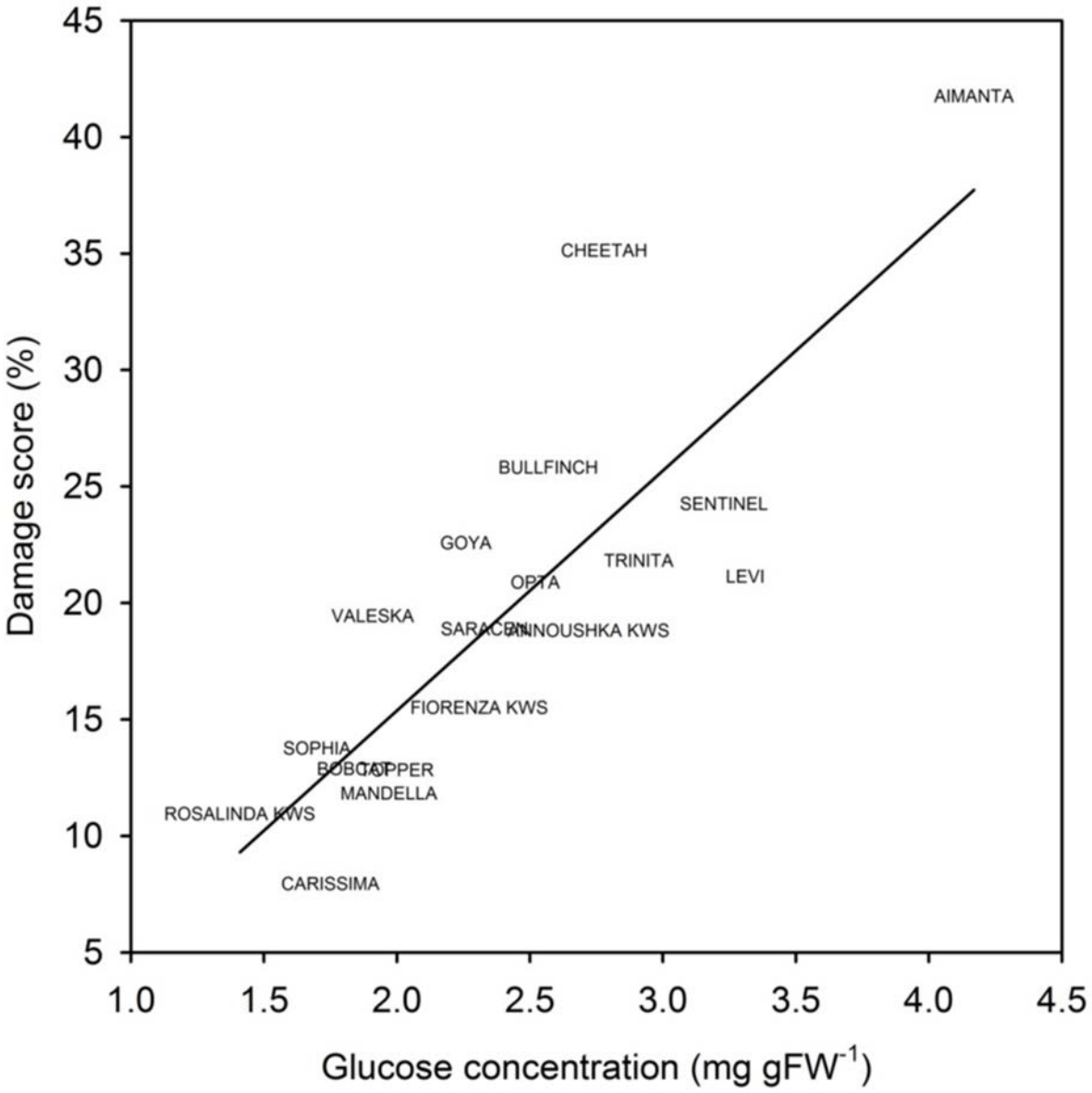


Figure 13. Correlation between glucose (an invert sugar) concentration and frost damage. As expected in deteriorated tissues , greater glucose levels probably results from increased sucrose degradation. Glucose was measured by an enzyme-based coloriometric assay.

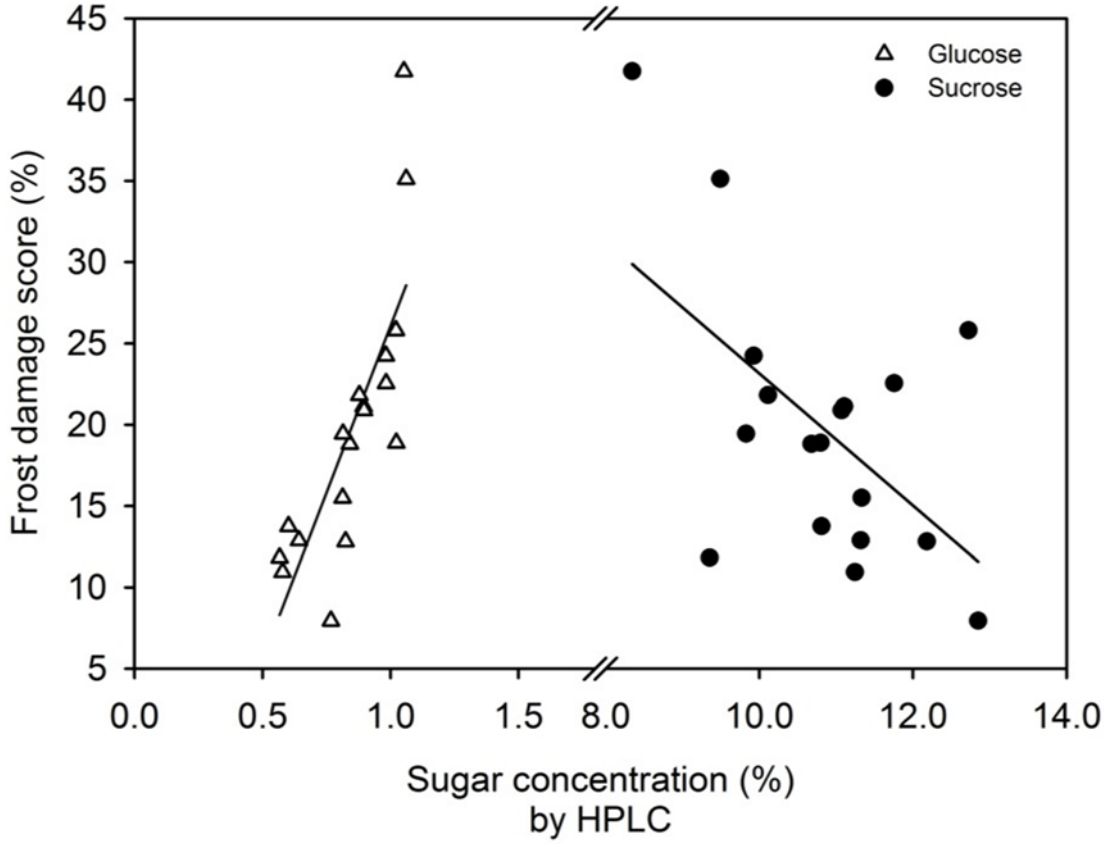


Figure 14. Correlation between glucose (an invert sugar) concentration, sucrose concentration and frost damage. As expected in deteriorated tissues , greater glucose levels probably results from increased sucrose degradation. Sugars were measured by HPLC by British Sugar.

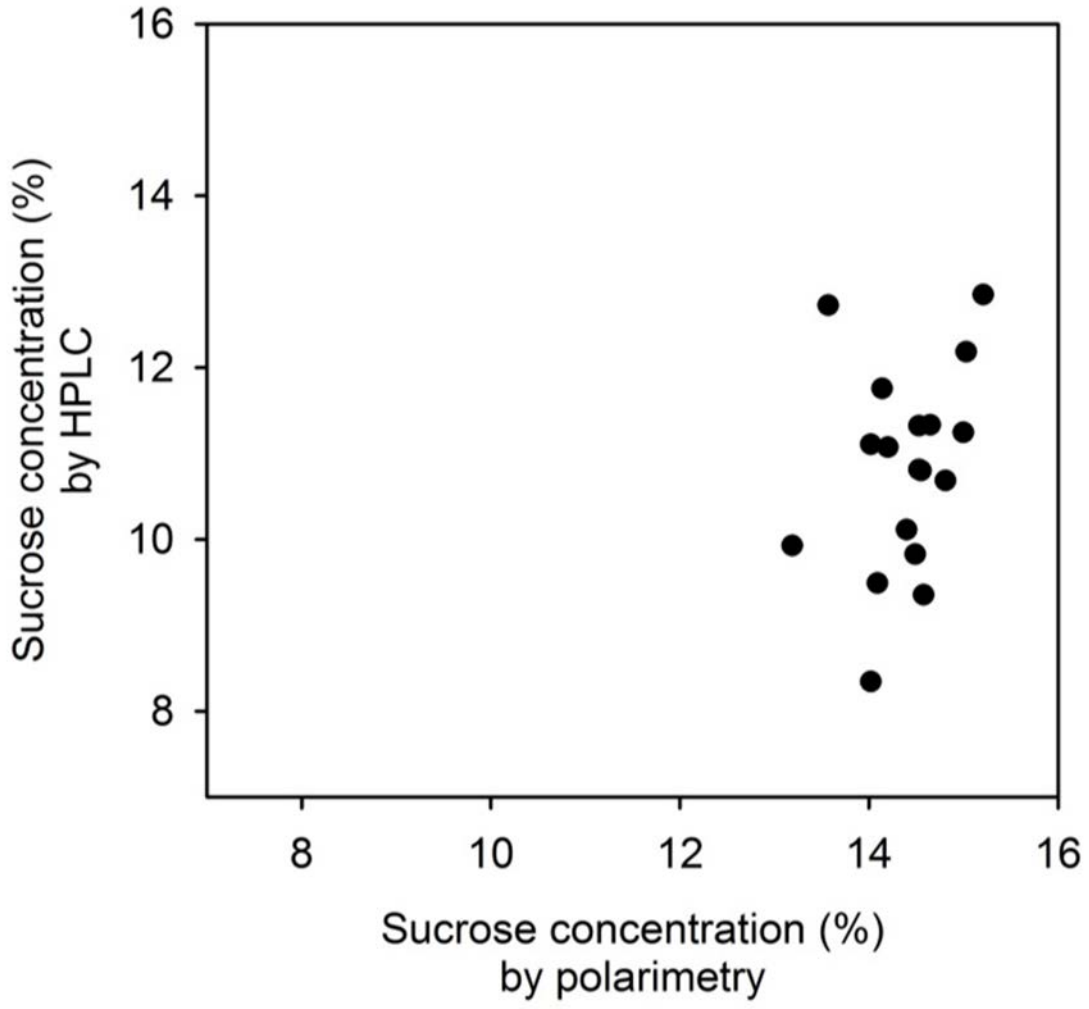


Figure 15. Correlation between sucrose measured by polarimetry vs. HPLC. As shown previously by Shore et al., polarimetry over-estimates sucrose concentrations when invert levels are high, perhaps due to interference in the light rotation by elevated levels of fructose.

	DamageScore	SugarYield	RootYield	AdjYield	RootSize	Popul	DryMatter%	Sugar%	Na	K	AminoN	Rust 06.09	Rust 29.09	Rust 27.10	YellowingScore 27.10
DamageScore	**1.00**														
SugarYield	-0.26	**1.00**													
RootYield	0.13	**0.84**	**1.00**												
AdjYield	**-0.43**	**0.96**	**0.66**	**1.00**											
RootSize	0.15	**0.70**	**0.84**	**0.54**	**1.00**										
Popul	-0.01	**0.36**	**0.40**	0.29	-0.16	**1.00**									
DryMatter%	**-0.56**	0.15	-0.31	**0.38**	-0.27	-0.10	**1.00**								
Sugar%	**-0.71**	**0.42**	-0.13	**0.66**	-0.12	-0.01	**0.84**	**1.00**							
Na	0.24	-0.20	-0.02	-0.26	-0.02	-0.03	-0.31	**-0.33**	**1.00**						
K	0.24	-0.27	-0.13	-0.31	-0.12	-0.05	-0.07	-0.26	**0.35**	**1.00**					
AminoN	-0.06	-0.12	0.00	-0.17	0.06	-0.13	-0.21	-0.20	0.21	0.10	**1.00**				
Rust 06.09	**0.37**	**-0.38**	-0.15	**-0.46**	-0.09	-0.12	**-0.54**	**-0.47**	**0.43**	0.24	0.23	**1.00**			
Rust 29.09	**0.48**	-0.29	-0.02	**-0.39**	0.01	-0.05	**-0.58**	**-0.50**	**0.46**	0.28	0.23	**0.82**	**1.00**		
Rust 27.10	**0.36**	-0.28	-0.05	**-0.36**	-0.02	-0.08	**-0.57**	**-0.45**	**0.59**	0.25	0.31	**0.68**	**0.84**	**1.00**	
YellowingScore 27.10	0.12	**-0.53**	**-0.44**	**-0.52**	**-0.44**	-0.08	-0.14	-0.25	0.07	0.14	0.25	0.09	0.10	0.16	**1.00**
erect leaves%	**-0.63**	0.12	-0.27	0.31	-0.24	-0.10	**0.58**	**0.69**	-0.15	-0.23	0.09	-0.23	-0.22	-0.16	0.02

Fig. 16. Correlation matrix of variables measured on all 64 genotypes. Values are Pearson correlation coefficients. Boldface indicates statistical significance ($P < 0.05$). The intensity of the colouring is related to the strength of the correlation (red, strong positive correlation; blue, strong negative correlation; white, no correlation). As with all correlations, values do not imply cause and effect.

	Damage Score	Gum Score	Sugar %	DryMatter%	Root Yield	Sugar Yield	Root size	Popul	Rust 29.09	Yellowing Score 27.10	erect leaves%	NIAB Sugar Yield	NIAB Root Yield	NIAB sugar%	%G+F/Suc	mg Glu+Fru gFW	mg Glu gFW	mg Fru gFW	mg Suc gFW	HPLC Raffinose (%)	HPLC Glucose (%)	HPLC Fructose (%)	HPLC Sucrose (%)
Damage Score	1.00																						
Gum_Score	0.83	1.00																					
Sugar_%	-0.67	-0.66	1.00																				
DryMatter%	-0.58	-0.47	0.70	1.00																			
Root_Yield	0.08	-0.02	-0.04	-0.46	1.00																		
Sugar_Yield	-0.13	-0.25	0.33	-0.20	0.93	1.00																	
Root_size	0.26	0.07	-0.15	-0.46	0.77	0.67	1.00																
Plant Popul	-0.24	-0.16	0.14	-0.09	0.52	0.54	-0.13	1.00															
Rust 29.09	0.45	0.39	-0.59	-0.62	0.23	0.01	0.36	-0.13	1.00														
YellowingScore 27.10	0.28	0.26	-0.09	0.17	-0.51	-0.49	-0.59	-0.01	-0.18	1.00													
erect leaves%	-0.56	-0.67	0.43	0.26	-0.33	-0.15	-0.49	0.15	-0.39	0.07	1.00												
NIAB SugarYield	0.34	0.18	-0.30	-0.66	0.63	0.49	0.70	0.07	0.45	-0.40	-0.18	1.00											
NIAB RootYield	0.34	0.18	-0.41	-0.81	0.67	0.49	0.65	0.19	0.42	-0.39	-0.13	0.94	1.00										
NIAB % sugar	-0.25	-0.16	0.44	0.82	-0.55	-0.38	-0.37	-0.37	-0.24	0.27	0.01	-0.59	-0.83	1.00									
%G+F/Suc	0.82	0.83	-0.70	-0.56	-0.18	-0.39	0.03	-0.35	0.53	0.29	-0.45	0.17	0.22	-0.26	1.00								
mg Glu+Fru gFW	0.77	0.81	-0.66	-0.48	-0.20	-0.40	-0.05	-0.28	0.46	0.37	-0.45	0.08	0.15	-0.24	0.96	1.00							
mg Glu gFW	0.83	0.87	-0.65	-0.53	-0.14	-0.33	-0.01	-0.25	0.46	0.41	-0.51	0.17	0.23	-0.28	0.93	0.97	1.00						
mg Fru gFW	-0.04	-0.02	-0.21	0.06	-0.27	-0.33	-0.18	-0.20	0.11	-0.05	0.09	-0.29	-0.24	0.09	0.34	0.37	0.12	1.00					
mg Suc gFW	-0.30	-0.28	0.35	0.47	-0.02	0.12	-0.18	0.19	-0.32	0.10	0.01	-0.20	-0.24	0.21	-0.38	-0.14	-0.12	-0.12	1.00				
HPLC Raffinose (%)	-0.53	-0.50	0.10	0.05	0.04	0.05	-0.12	0.22	0.17	-0.36	0.55	0.13	0.11	-0.06	-0.36	-0.34	-0.45	0.31	0.04	1.00			
HPLC Glucose (%)	0.79	0.86	-0.60	-0.34	0.01	-0.20	-0.03	0.03	0.42	0.31	-0.62	0.09	0.06	0.00	0.68	0.67	0.72	-0.02	-0.12	-0.40	1.00		
HPLC Fructose (%)	0.62	0.77	-0.55	-0.18	0.03	-0.17	-0.04	0.08	0.23	0.20	-0.69	-0.01	-0.03	0.06	0.52	0.56	0.58	0.07	0.05	-0.38	0.93	1.00	
HPLC Sucrose (%)	-0.57	-0.34	0.29	0.36	0.09	0.16	-0.09	0.23	-0.17	-0.35	-0.01	-0.25	-0.29	0.29	-0.52	-0.47	-0.51	0.05	0.32	0.35	-0.13	0.10	1.00

data:	all data (18 RL vars only)	
α level	r (test)	r^2
0.05	0.47	0.22
0.01	0.59	0.35
0.001	0.71	0.50
df = 16		

Fig. 17. Correlation matrix of variables measured on 18 RL varieties. Note the Strong association between invert sugar concentration and damage score. There is poor correlation between sucrose measured by HPLC and polarimetry, presumably because of interference caused by invert sugars on polarimetry (Shore, Dutton and Houghton) Values are Pearson correlation coefficients. Boldface indicates statistical significance (P <0.05). The intensity of the colouring is related to the strength of the correlation (red, strong positive correlation; blue, strong negative correlation; white, no correlation). As with all correlations, values do not imply cause and effect.

	Damage Score	*Gum_Score*	*BB Sugar_%*	*NIAB % sugar*	*%G+F/Suc*	*mg Glu+Fru gFW*	*mg Glu gFW*	*mg Fru gFW*	*mg Suc gFW*	*HPLC Raffinose (%)*	*HPLC Glucose (%)*	*HPLC Fructose (%)*	*HPLC Sucrose (%)*
Damage Score	**1.00**												
Gum_Score	**0.83**	**1.00**											
Sugar_%	**-0.67**	**-0.66**	**1.00**										
NIAB % sugar	-0.25	-0.16	0.44	**1.00**									
%G+F/Suc	**0.82**	**0.83**	**-0.70**	-0.26	**1.00**								
mg Glu+Fru gFW	**0.77**	**0.81**	**-0.66**	-0.24	**0.96**	**1.00**							
mg Glu gFW	**0.83**	**0.87**	**-0.65**	-0.28	**0.93**	**0.97**	**1.00**						
mg Fru gFW	-0.04	-0.02	-0.21	0.09	0.34	0.37	0.12	**1.00**					
mg Suc gFW	-0.30	-0.28	0.35	0.21	-0.38	-0.14	-0.12	-0.12	**1.00**				
HPLC Raffinose (%)	**-0.53**	**-0.50**	0.10	-0.06	-0.36	-0.34	-0.45	0.31	0.04	**1.00**			
HPLC Glucose (%)	**0.79**	**0.86**	**-0.60**	0.00	**0.68**	**0.67**	**0.72**	-0.02	-0.12	-0.40	**1.00**		
HPLC Fructose (%)	**0.62**	**0.77**	**-0.55**	0.06	**0.52**	**0.56**	**0.58**	0.07	0.05	-0.38	**0.93**	**1.00**	
HPLC Sucrose (%)	**-0.57**	-0.34	0.29	0.29	**-0.52**	-0.47	**-0.51**	0.05	0.32	0.35	-0.13	0.10	**1.00**

data:	**all data (18 RL vars only)**	
α level	**r (test)**	r^2
0.05	**0.47**	0.22
0.01	**0.59**	**0.35**
0.001	**0.71**	**0.50**
df = 16		

Fig.18. Correlation matrix showing relationships between different sugar assay methods.

BB sugar%: polarimetry on roots exposed to frost
NIAB sugar%: polarimetry on roots harvested prior to frost (avg value from five trials in 2010)
mg Glu g FW: sugar analysis by enzymatic colorimetric assay at Broom's Barn
HPLC: sugar analysis done by British Sugar

Fig.19. A photograph of roots of Bullfinch harvested 25 Jan, 2011 from two neighbouring rows in a field adjacent to the rust trial plots reported here. In this field, the crop was sprayed twice with a standard fungicide programme; however, note the similar level of frost damage as observed in the rust trial. The red labels indicate the soil level in situ. Some crowns extended further from the soil than others, sometimes due to the presence of a wheeling. In all cases, note that the level of tissue damage extended several cm below the soil surface. It was not just ‘proud’ roots that were damaged.

Fig.20. A close-up of a damaged root showing the accumulation of gums, and bubbles presumably as a result of microbial activity. Note also the clear demarcation between damaged and healthy tissue: cells are either killed by the low temperature, or remain intact and viable.

Fig.21. A green, healthy side shoot emerging from an extensively frost-damaged crown. This suggests that this young, cold-hardened leaf tissue is more resistant to frost than the subtending root tissue. Viable shoots may go on to form bolters in the summer following frost-damaged crops that are not disced and incorporated.

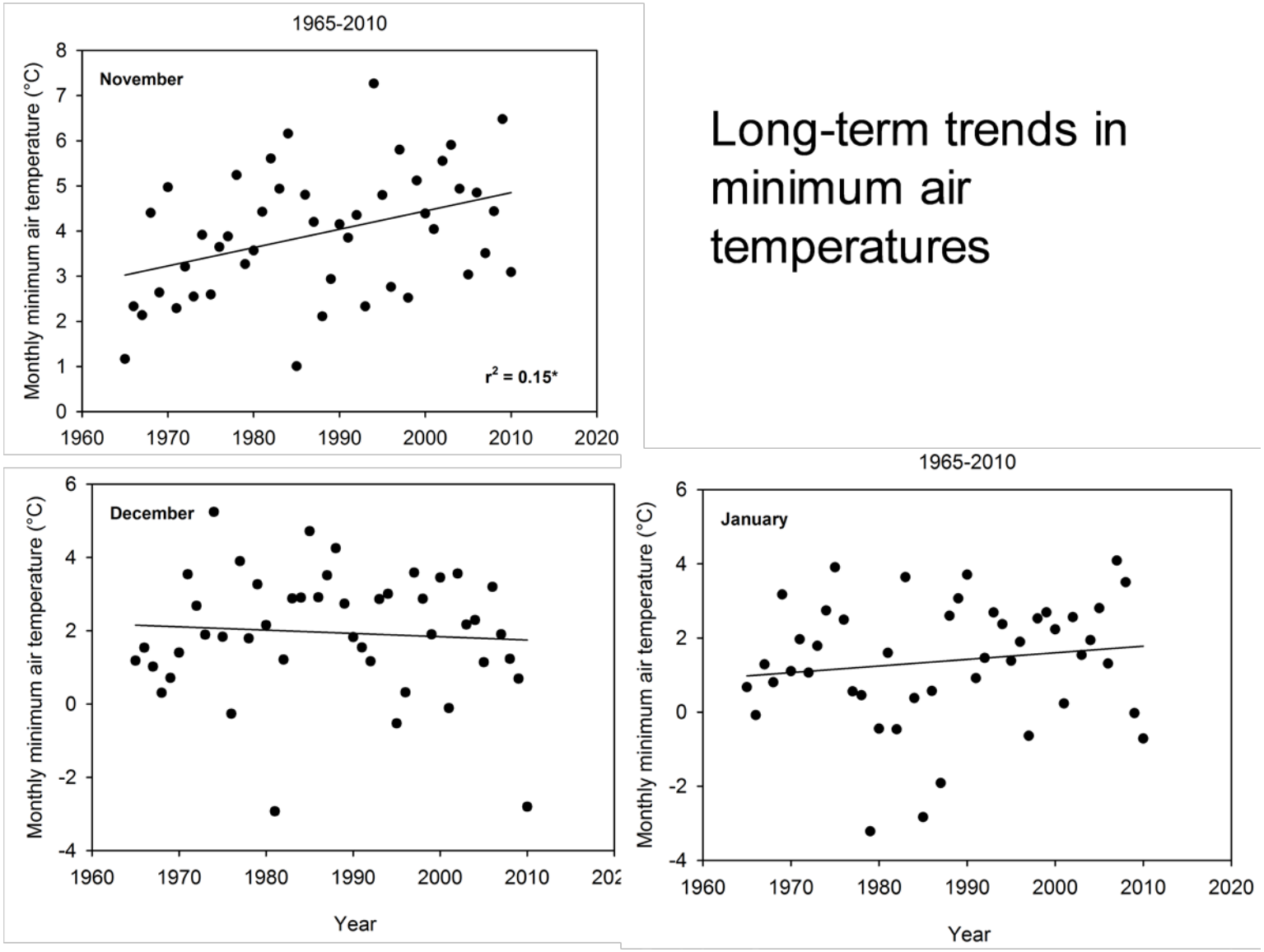


Fig. 22. Long-term trends in minimum air temperatures recorded at Broom's Barn. There appears to be a significna warming trend for November temperatures, but no apparent trends in December or January. The risk of frost damage in winter months appears to have unchanged.

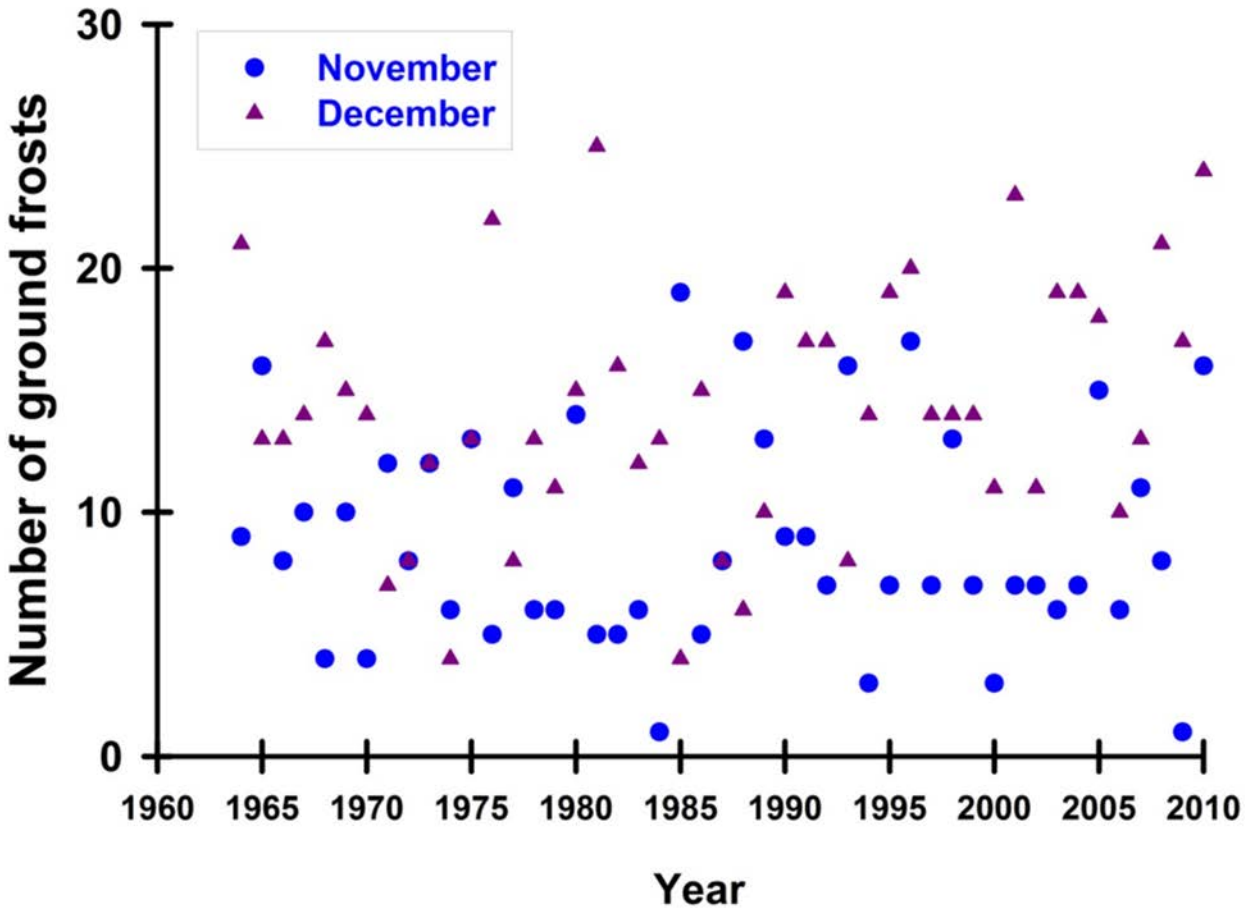


Fig. 23. The number of November and December ground frosts recorded at Broom's Barn over 30 years. The risk of frost damage is present in almost every winter, and does not appear to be changing.