Suitable Biomass for a Sustainable Sugarcane Industry

This project is supported by CRC for Developing Northern Australia, Far Northern Milling Pty Ltd, Sugar Research Australia, and The University of Queensland.

Yield and composition of the sugarcane plant crop

Introduction

A challenge facing all sugarcane mills that wish to diversify their income streams is to use the processing capacity for most of the year. The crushing season in Australia generally lasts about 22 weeks. For the Far Northern Milling Company, the problem is exaggerated by the fact that there is already a shortfall in available biomass during the short crushing season. For year-round operation, and to address the current shortfall in total biomass availability two options should be considered. Firstly, if sucrose is no longer the main emphasis then alterations to the sugarcane cropping cycle can be considered [4]. This approach led to a farming system aimed at maximum biomass mass production i.e., "Energy canes". Alternative sugarcane varieties can be developed that have superior growth rates and biomass yield [7, 6, 10]. Energy canes might also be the ideal feedstock for a farming system aimed at biomass yield rather than sucrose yield.

In this Update

Worldwide there is an interest in further developing the energy cane concept. Broadly sugarcane can be classified into three groups [10] :

- Traditional sugarcane varieties containing about 75% water, 12% fibre, and 13% sugar. This raw material provides juice for sugar and ethanol production, and fibre for electricity production,
- Type I energy cane—cane varieties bred to maximise sugar and fibre yield. This type of cane, conceptualised by [3, 4], has lower water content (65%), fibre ranging from 13% to 17% and small reduction of the sugar content. This raw material, in addition to providing juice for sugar and ethanol production, contributes with more fibre for the greater production of electricity, lignocellulosic ethanol, as well as other derivatives of economic value for the sugarcane industry,
- Type II energy cane variety selected to maximise fibre yield (fibre> 30%), with insignificant sugar content and lower water content (60%). This raw material is not of interest to the current sugarcane industry, being required by other agro-industry sectors that need biomass for the energy generation.

In Update 3 we presented the crop composition of all the sugarcane varieties in the two trials at Mossman and the Tablelands. This update describes the plant crop performance of the sugarcane genotypes at the two trail sites.

Biomass yield

There was more than a 45 TCH difference between the varieties in the Mossman trial (Fig 1). However, this can be largely attributed to the poor establishment and slow growth of QN12-512 and QN12-520 (Update 3). Three of the other varieties, Q240, QS08-7370 and QN13-609, also performed worse than Q208 at Mossman.

The top four clones for TCH were experimental clones QN12-512, QN13-609 and QS08-7370 also performed poorly at the Tablelands site. At this site three clones had yields significantly higher than Q208 namely, QS10-8770, WSRA24 and QS08-8662. Unfortunately the yield of four clones were extremely variable and this confounded statistical analysis.

Noteworthy is that this variation was not observed at the Mossman site and probably reflect site rather than

Frikkie Botha, Crop Science, Queensland Alliance for Agriculture & Food Innovation The University of Queensland, St Lucia QLD 4072
☎ (+61) 048 840 0074 ✉ f.botha@uq.edu.au

genotype variability.

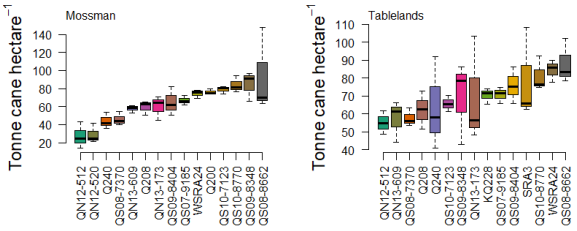

Figure 1: *Crop yield (TCH) of the cane genotypes included in the field trials in Mossman and Tablelands. The crop was harvested 10 months after planting. Genotypes were sorted with the highest value on the right.*

These reported yields reflect cane mass at the time of harvest. As typical energy canes have a lower water content than Q208 [10, 3]. However, this pattern does not seem to hold for most of the experimental clones that were included in the trial as potential type I energy canes (fig. 2). Seven of the "energy canes" in the Mossman trial had a water content higher than the commercial standards. In the tablelands trial 6 of the experimental "energy canes" had a water content higher than Q208 (Fig 2)

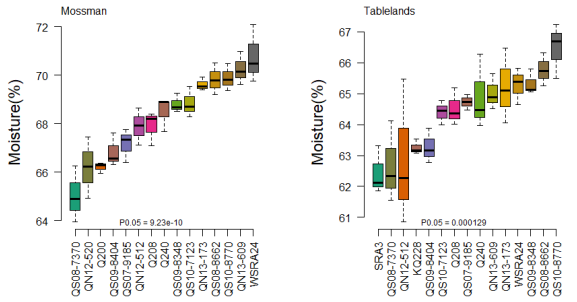

Figure 2: *Moisture content at harvest of the cane genotypes included in the field trials in Mossman and Tablelands. The crop was harvested 10 months after planting. Genotypes were sorted with the highest value on the right.*

Evidently much of the apparent biomass gain observed (Fig. 1) is merly due to a larger water content in the tissue. It is also evident that cane produced at Mossman has a higher water content than in the Tableland production system. For this reason all further yield data in the project will be presented as TCH on a dry weight basis.

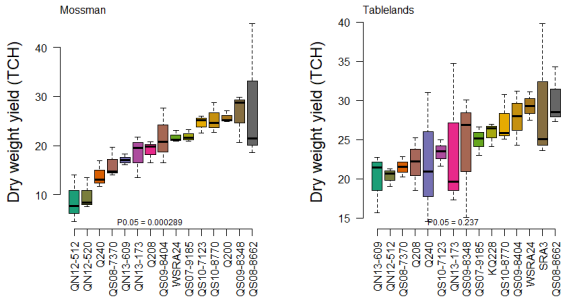

Figure 3: *Biomass yield on a dry weight basis of the cane genotypes included in the field trials in Mossman and Tablelands. The crop was harvested 10 months after planting. Genotypes were sorted with the highest value on the right.*

When expressed on a dry weight basis yields between Mossman and Tablelands are similar. There seven and six of the experimental clones had a higher biomass yield than the commercial standards at Mossman and Tablelands respectively (Fig. 3). Noteworthy is the very good biomass yield of Q200 at Mossman.

Primary Quality Traits

In a breeding program focused on the development of type I sugarcane [10], Brix, Pol, purity and fibre content are routine measured as the primary quality components of sugarcane [5].

Six culm samples were taken from the field plots at two time points during the season approximately six and twelve months from planting or ratooning (see data for details). Samples were analysed with a modified method [5]. Culm samples were disintegrated using a Dedini laboratory disintegrator and then processed using the SpectraCane™ automated NIR-based system [5]. At the end of each harvesting season, SpectraCane™ is re-calibrated against the conventional laboratory data. In addition, every tenth sample through SpectraCane™ is automatically saved and processed through the conventional laboratory where juice is squeezed from the shredded cane using a hydraulic press. The remaining fibre is then dried and weighed to calculate the fibre content.

There is a highly significant variation ($p<0.001$) in fibre content between the genotypes at both locations (Fig. 4). Seven of the genotypes in the Mossman, and 8 genotypes in the Tablelands trails have fibre levels indicative of type I energy canes [10]. It is important to note that for most of the tested genotypes higher fibre levels were recorded at Tablelands than Mossman. For example compare the fibre content of WSRA24 grown at the two different locations.

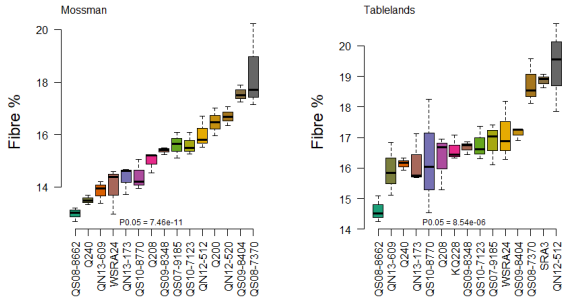

Figure 4: *Fibre content (% FW) of the cane genotypes included in the field trials in Mossman and Tablelands. The crop was harvested 10 months after planting. Genotypes were sorted with the highest value on the right.*

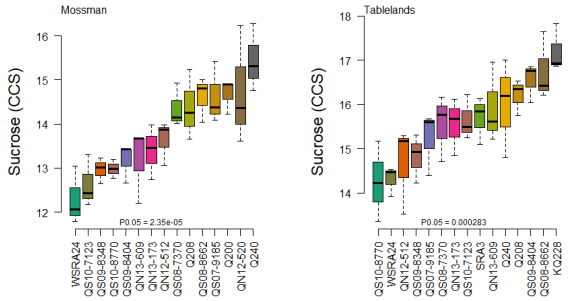

Figure 5: *Sucrose content (CCS) of the cane genotypes included in the field trials in Mossman and Tablelands. The crop was harvested 10 months after planting. Genotypes were sorted with the highest value on the right.*

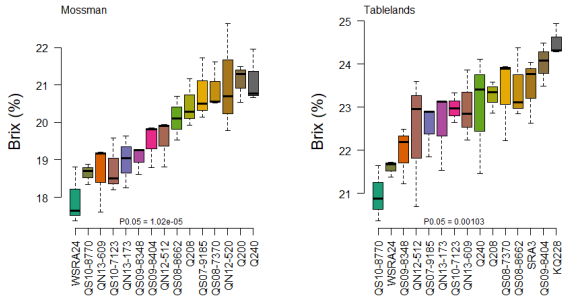

Figure 6: *Brix (%) of the cane genotypes included in the field trials in Mossman and Tablelands. The crop was harvested 10 months after planting. Genotypes were sorted with the highest value on the right.*

When variation between genotypes is due to a range of variables it becomes very difficult to visualise and interpret the data. A principal component analysis can be used to extract the important information from a multivariate data table and to express this as a set of few new variables called principal components. These new variables correspond to a linear combination of the original variables [1].

In order to do a PCA analysis of the performance and composition of the genotypes in the trial variables were standardised by normalising parameters against that of variety Q208.

Principle component (PC)1 explains 65% of the variation between the genotypes in the Mossman trial (Fig. 7A), and the three most important factors in PC1 is Brix, purity and water content (Fig. 7C). PC2 explains >25% of the variation and the main factor in this component is fibre content. PC3 describes 11% of the total variation and the main factor is TCH ($p<0.001$). The three main principle components describe more than 98% of the total variation in the Mossman trial.

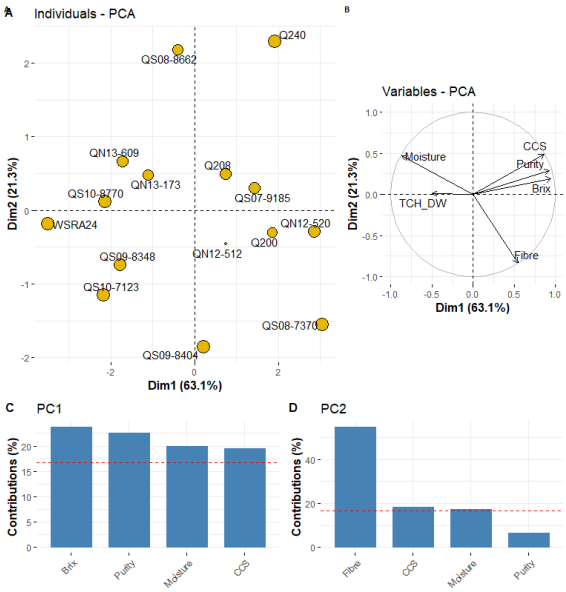

Figure 7: *Principal component analysis (PCA) of the yield and mill room components of the 15 varieties in the Mossman trial. Principal component analysis (PCA) of the observation (A) and the variables main variables (B). The three main contibutors to PC1 (C) and PC2 (D).*

Evidently, the three main factors differentiating the genotypes are fibre, sucrose and moisture. Most of the experimental genotypes fall well into the definition of Type I energy canes [10].

Biomass composition

A major challenge for the effective utilisation of total biomass is the great innate variability between different biomass types and within individual species and varieties. This inconsistency arises from genetic variability, varied growth, and harvesting conditions. This variability in biomass composition presents major challenges for processors as conversion processes require physically and chemically uniform materials.

In contrast to the extensive knowledge base about sucrose, reducing sugar and bagasse content of commercial sugarcane varieties, little is know about the chemical composition, and variability in chemical profiles within

sugarcane germplasm.

There has been considerable interest in the sugarcane lignocellulosic fraction which is typically rich in cellulose (44%) and hemicellulose (28%), lignin (21%) and ashes (5%) [2, 8, 9].

A major objective of the current research project is to develop a comprehensive chemical profile of sugarcane tissues for both current commercial varieties and atypical germplasm that are well suited for biomass production and diversification opportunities.

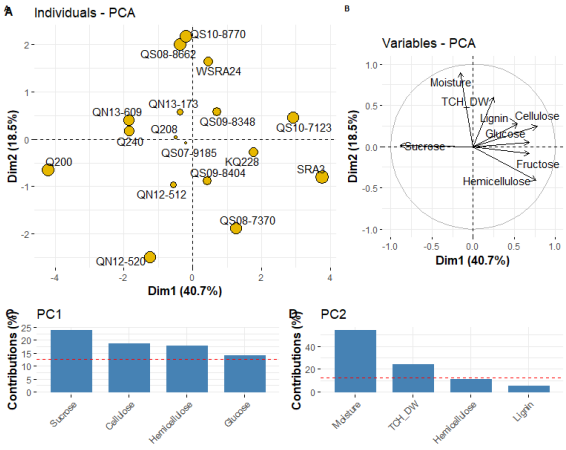

Figure 8: *Principal component analysis (PCA) of the yield and biomass composition components of the 15 varieties in the Tablelands trial. Principal component analysis (PCA) of the observation (A) and the variables main variables (B). The three main contibutors to PC1 (C) and PC2 (D).*

Table 1: *Composition of the lignocellulosic fraction of 15 sugarcane genotypes grown in the Mossman mill production area.*

Genotype	Fibre	Glucan	Xylan	Other sugar[1]	Uronic acid[2]	Lignin[3]
	% of total biomass (Dry weight)					
KQ228	44.1 - 45.4	16.7 - 18.2	11.5 - 10.2	1.2 - 1.4	0.4 - 0.5	10.3 - 11.0
Q208	46.7 - 48.7	18.9 - 20.6	11.0 - 11.4	1.2 - 1.4	0.6 - 0.8	10.4 - 11.4
Q240	42.9 - 45.9	15.7 - 17.9	9.2 - 10.8	1.0 - 1.3	0.4 - 0.5	8.8 - 10.2
QN12-512	46.1 - 49.2	17.2 - 18.6	10.2 - 10.9	1.4 - 1.5	0.4 - 0.8	9.9 - 11.2
QN12-520	47.8 - 52.2	16.3 - 18.4	10.2 - 10.7	1.2 - 1.6	0.5 - 0.7	9.8 - 12.1
QN13-173	47.2 - 49.4	17.8 - 18.4	10.5 - 11.6	1.2 - 1.4	0.7 - 0.9	10.8 - 11.5
QN13-609	44.5 - 46.5	17.6 - 18.3	10.9 - 11.1	1.3 - 1.4	0.7 - 1.0	10.7 - 11.0
QS07-9185	45.4 - 46.9	16.6 - 17.8	9.7 - 9.8	1.1 - 1.2	0.4 - 0.5	10.6 - 11.4
QS08-7370	47.8 - 52.2	18.2 - 21.1	12.2 - 13.6	1.5 - 1.5	0.6 - 0.8	11.3 - 11.7
QS08-8662	43.0 - 50.2	16.5 - 18.5	10.0 - 11.1	1.2 - 1.5	0.6 - 0.7	9.6 - 11.1
QS09-8348	42.2 - 49.4	15.7 - 19.6	10.2 - 12.7	1.3 - 1.3	0.4 - 0.6	10.2 - 11.7
QS09-8404	48.1 - 52.9	17.5 - 19.3	12.3 - 12.7	1.4 - 1.5	0.5 - 0.6	11.0 - 12.2
QS10-7123	46.2 - 50.7	17.3 - 19.7	10.5 - 11.5	1.3 - 1.5	0.4 - 0.6	9.9 - 10.9
QS10-8770	46.1 - 48.2	17.0 - 18.1	9.5 - 12.1	1.1 - 1.3	0.4 - 0.7	11.4 - 11.5
SRA3	48.5 - 50.4	18.5 - 19.5	10.1 - 11.4	1.3 - 1.4	0.5 - 0.8	10.6 - 12.1
WSRA24	47.8 - 49.3	18.7 - 19.7	11.0 - 11.6	1.1 - 1.5	0.7 - 0.9	11.0 - 12.1

[1]Reducing sugars, glucose and fructose
[2]Organic acids, amino acids, protein and lipid
[3]Total mass per internode

Metabolome

To determine the polar metabolite profile of sorghum tissue was sequentially extracted with methanol and then water. The resulting extract was dried under vacuum and the pellet methoximated and trimethylsilylated prior to GC-MS analyses. Two internal standards were included in all analyses ($^{13}C_5$,^{15}N Valine and $^{13}C_6$ Sorbitol. We routinely identified 135 metabolites in the sugarcane tissue.

Of these 24 metabolites are present at levels > than

Table 2: *Soluble sugars of 15 sugarcane genotypes grown in the Mossman mill production area.Values represent the minimum and maximum levels present in the culm tissue.)*

Genotype	Total sugar	Sucrose	Glucose	Fructose	Other[1]
	% of total biomass (Dry weight)				
KQ228	42.9 - 42.4	25.6 - 26.2	7.8 - 8.6	3.7 - 4.8	0.2 - 0.5
KQ228	42.4 - 42.9	25.6 - 26.2	7.8 - 8.6	3.7 - 4.8	0.2 - 0.5
Q200	40.3 - 42.2	22.0 - 29.0	5.4 - 6.8	2.7 - 4.6	0.3 - 0.6
Q208	42.3 - 43.1	23.0 - 26.5	7.0 - 8.4	3.9 - 5.8	0.2 - 0.5
Q240	39.7 - 40.6	25.3 - 26.7	6.2 - 7.2	2.1 - 4.6	0.2 - 0.5
QN12-512	39.3 - 41.1	20.5 - 26.5	5.3 - 7.2	4.1 - 6.1	0.2 - 0.5
QN12-520	36.7 - 40.5	19.4 - 26.3	7.3 - 7.7	3.8 - 5.4	0.2 - 0.5
QN13-173	37.6 - 42.4	16.8 - 25.9	6.6 - 8.7	4.0 - 6.2	0.1 - 0.4
QN13-609	36.0 - 42.3	17.8 - 27.0	5.5 - 7.6	4.5 - 5.9	0.2 - 0.5
QS07-9185	35.4 - 39.0	18.0 - 23.3	6.2 - 7.8	3.1 - 5.4	0.1 - 0.5
QS08-7370	32.9 - 37.5	16.6 - 22.1	5.3 - 7.5	3.3 - 5.8	0.1 - 0.4
QS08-8662	43.0 - 44.6	23.5 - 27.3	7.0 - 8.6	3.3 - 5.7	0.2 - 0.5
QS09-8348	37.0 - 39.3	18.5 - 22.7	5.5 - 7.8	3.3 - 5.7	0.2 - 0.5
QS09-8404	40.2 - 42.7	24.5 - 25.2	5.3 - 7.4	3.5 - 4.8	0.3 - 0.5
QS10-7123	40.0 - 41.8	21.7 - 23.1	8.5 - 8.8	5.0 - 6.1	0.2 - 0.5
QS10-8770	40.6 - 43.0	24.0 - 26.0	5.8 - 7.4	3.6 - 4.4	0.1 - 0.4
QSl 0-8770	36.8 - 36.8	16.4 - 16.4	10.7 - 10.7	9.6 - 9.6	0.2 - 0.4
QSl0-7123	37.4 - 39.4	16.4 - 18.4	11.1 - 11.1	9.7 - 9.7	0.2 - 0.4
QSl0-8770	37.2 - 37.2	17.6 - 17.6	10.4 - 10.4	9.1 - 9.1	0.3 - 0.5
SRA3	39.3 - 41.2	20.4 - 21.3	8.9 - 9.4	6.6 - 7.7	0.2 - 0.5
WSRA24	37.1 - 39.9	24.0 - 26.5	6.6 - 6.8	3.3 - 3.5	0.2 - 0.5

[1]Trehalose, mannose, galactose, arabinose, sorbitol, rhamnose and xylose

0.05% of total dry mass and could potentially be recovered from the tissue (Table **??**). An assessment of the potential commercial value of these metabolites has not been done yet.

References

[1] Hervé Abdi and Lynne J. Williams. Principal component analysis. 2(4):433–459.

[2] E. O. Ajala, J. O. Ighalo, M. A. Ajala, A. G. Adeniyi, and A. M. Ayanshola. Sugarcane bagasse: A biomass sufficiently applied for improving global energy, environment and economic sustainability. *Bioresources and Bioprocessing*, 8(1):87, September 2021.

[3] A. G. Alexander. *The Energy Cane Alternative*. Sugar Sciences. Elsevier.

[4] A. G. Alexander. Sugarcane as a source of biomass. In R. Sansoucy, G. Aarts, and T. R. Preston, editors, *FAO Expert Consultation on Sugarcane as Feed*. FAO.

[5] N. Berding and D. H. Marston. Operational validation of the efficacy of spectracanetm, a high-speed analytical system for sugarcane quality components. pages 445–459.

[6] Osmar V. Carvalho-Netto, Jose A. Bressiani, Hugo L. Soriano, Celso S. Fiori, Joao M. Santos, Geraldo VS Barbosa, Mauro A. Xavier, Marcos GA Landell, and Goncalo AG Pereira. The potential of the energy cane as the main biomass crop for the cellulosic industry. 1(1):20.

[7] Geraldo Veríssimo de Souza Barbosa, João Messias dos Santos, Carlos Assis Diniz, Danilo Eduardo Cursi, and Hermann Paulo Hoffmann. Chapter 6 - Energy cane breeding. In Fernando Santos, Sarita Cândida Rabelo, Mario De Matos, and Paulo Eichler, editors, *Sugarcane Biorefinery, Technology and Perspectives*, pages 103–116. Academic Press.

[8] Roni Maryana, Dian Ma'rifatun, A.I. Wheni, K.W. Satriyo, and W. Angga Rizal. Alkaline Pretreatment on Sugarcane Bagasse for Bioethanol Production. *Conference and Exhibition Indonesia Renewable Energy & Energy Conservation [Indonesia EBTKE-CONEX 2013]*, 47:250–254, January 2014.

[9] R. Sanjuán, J. Anzaldo, J. Vargas, J. Turrado, and R. Patt. Morphological and Chemical Composition of Pith and Fibers from Mexican Sugarcane Bagasse. *Holz als Roh- und Werkstoff*, 59(6):447–450, December 2001.

[10] T.L. Tew and R.M. Cobill. Genetic improvement of sugarcane (Saccharum spp.) as an energy crop. pages 249–272.

Frikkie Botha, Crop Science, Queensland Alliance for Agriculture & Food Innovation The University of Queensland, St Lucia QLD 4072
(+61) 048 840 0074 f.botha@uq.edu.au

Table 3: *The most abundant polar metabolites in the culm of 15 sugarcane genotypes grown in the Mossman mill production area.Values represent the mean levels present in the culm tissue.)*

Genotype	Aconitate	Cellobiose	Citrate	Fructose	Galactose	Gentibiose	Glucose	Glycerate	Isocitrate	Lactate	Malate	Maltose	Mannose	Myo-inositol	Quinate	Raffinose	Ribitol	Sedoheptulose	Sucrose	Tartrate	Threitol	Trehalose	Turanose	Xylose
	gram kilogram^{-1}																							
KQ228	2.3	6.6	0.0	146.2	151.0	4.3	55.2	0.0	0.0	0.6	1.0	24.4	107.9	0.7	1.6	5.2	0.4	96.3	52.3	0.0	0.0	2.2	5.0	2.4
Q200	1.3	6.2	0.0	128.9	138.2	3.9	48.2	0.0	0.0	0.6	1.8	22.7	94.3	0.6	1.4	4.9	0.0	92.7	45.8	0.0	0.0	2.1	4.6	2.2
Q208	2.3	6.9	0.0	146.6	148.8	4.4	53.9	0.0	0.0	0.7	0.5	23.6	105.5	0.7	1.6	5.4	0.5	96.7	44.8	0.0	0.1	2.4	5.2	2.5
Q240	2.3	6.9	0.0	145.0	143.6	4.4	47.8	0.0	0.0	0.6	0.5	25.6	93.4	0.7	1.6	5.5	0.5	102.8	43.6	0.2	0.1	2.3	5.2	2.4
QS07-9185	1.5	6.8	0.0	153.6	153.3	4.3	55.9	0.0	0.0	0.7	0.0	22.9	109.5	0.7	1.6	5.5	0.4	99.9	52.2	0.0	0.0	2.3	5.1	2.4
QN12-512	2.5	10.2	341.6	167.1	158.5	4.9	50.3	0.0	0.5	11.9	0.0	173.0	95.9	0.6	7.7	4.6	0.2	85.4	56.4	0.0	0.0	4.1	4.4	15.8
QN13-173	5.0	6.7	0.7	188.3	178.7	4.3	57.5	0.5	0.0	0.7	2.3	118.6	112.5	0.7	2.8	5.2	0.2	133.3	26.1	0.0	0.0	2.3	5.0	2.4
QN13-609	2.6	7.5	0.0	211.7	235.0	4.6	73.2	0.5	1.4	0.9	2.1	41.0	144.3	0.8	1.9	5.4	0.2	173.4	37.0	0.0	0.2	2.8	5.5	2.6
QN132-520	2.2	8.3	0.0	258.1	214.8	4.9	66.6	0.1	0.9	1.1	2.1	25.7	133.1	0.9	1.9	6.0	2.1	136.2	54.6	0.0	0.7	3.4	6.1	3.3
QS07-9185	3.0	8.3	0.0	220.4	204.2	5.1	64.7	0.3	2.1	1.0	2.3	33.3	129.4	0.9	1.9	5.9	0.7	125.0	54.7	0.0	0.2	3.2	6.1	3.3
QS08-7370	1.9	8.2	0.0	213.2	168.6	4.9	60.5	0.0	1.0	1.1	1.1	18.1	121.0	0.9	1.9	6.1	2.6	88.3	64.5	0.5	0.7	3.5	5.5	3.3
QS08-8662	8.9	8.2	1.7	205.9	233.8	4.9	70.9	0.7	0.0	1.2	2.6	49.4	141.8	0.9	3.7	5.8	0.1	151.1	46.4	0.0	0.0	3.2	6.0	3.2
QS09-8348	2.7	8.5	0.0	290.9	213.7	5.0	73.5	0.0	0.0	1.1	1.8	25.8	146.9	0.9	1.9	6.3	5.5	116.9	67.8	0.7	0.9	4.0	6.2	3.6
QS09-8404	2.7	8.4	0.0	214.2	195.2	5.0	65.8	0.0	1.4	1.0	1.1	31.7	131.6	0.9	1.9	6.2	1.2	111.5	59.8	0.0	0.3	3.3	6.2	3.3
QS10-7123	2.2	7.9	0.0	245.7	194.6	4.7	66.4	0.0	0.3	1.1	1.4	23.0	132.8	0.9	1.8	5.9	2.4	109.3	56.4	0.3	0.5	3.5	5.8	3.2
QS10-8770	2.7	8.2	0.0	219.1	188.9	5.0	60.8	0.0	0.0	1.1	0.6	23.3	121.7	0.9	1.9	5.8	2.0	113.8	45.2	0.5	0.4	3.3	6.0	3.3
QSO9-8348	2.7	8.0	0.0	204.4	164.1	4.8	56.0	0.0	0.0	1.0	2.2	34.1	112.0	0.8	1.8	5.7	1.1	91.4	50.2	0.4	0.5	3.6	5.8	3.1
QSO9-8406	2.6	8.0	0.0	181.6	174.6	4.8	59.7	0.0	0.0	1.1	0.0	25.3	119.5	0.9	1.9	5.9	0.6	97.8	64.8	0.0	0.0	3.2	5.9	3.1
SRA3	5.1	8.5	1.0	221.3	162.3	5.0	57.3	0.5	0.0	1.2	2.7	52.7	114.5	1.0	3.3	6.4	5.3	87.5	57.4	0.5	0.8	3.9	6.2	3.3
WSRA24	2.7	9.5	0.0	259.7	235.9	5.7	70.5	0.2	0.0	1.1	0.0	33.2	140.9	1.0	2.2	7.1	1.2	155.3	65.3	0.0	0.3	3.7	7.0	3.8