Project title:.	An on-line, low-cost, non-invasive MRI sensor of Basal Rot in Narcissus bulbs
Project number:	BOF 60
Project leader:	Dr. Brian Hills, Institute of Food Research
Report:	Final report, October 2011
Key staff:	Ben Piggott
Location of project:	Institute of Food Research, Norwich, NR4 7UA**.**
Industry Representative:	Gordon Flint, Winchester Growers
Date project commenced:	1st December 2010
Date project completed (or expected completion date):	31st October 2011

DISCLAIMER

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

We have shown that a low-cost, non-invasive, on-line MRI sensor should be able to detect basal rot in Narcissus bulbs with a high degree of confidence, although further work is needed to establish the commercial viability of the MRI sensor.

Background

Basal rot in Narcissus bulbs is a major problem for growers, but its automated, non-invasive detection with a high degree of confidence is not yet possible. At present, bulbs that have very extensive basal rot can be removed by hand sorting based on the softness of the bulb. This is a crude labour-intensive and expensive method that fails to detect basal rot in any but the most extreme cases of infection. This small scale project was therefore undertaken to investigate the feasibility of using a prototype MRI sensor for the automated detection of basal rot. This prototype had already been built and tested by the MRI research team at the IFR.

Summary

Basal rot continues to be the major disease of narcissus causing significant on farm losses of saleable bulbs, quality rejections by retailers and reduced yields of cut flowers.
The work reported here has shown that under laboratory conditions Basal rot can be detected in stationary Narcissus bulbs using an MRI sensor.

Further work is required to determine the practicality of using this technique on bulbs moving in a process flow situation.
In addition serious thought needs to be given to calculating the likely cost, to include all safety aspects, of an industrial sized sensor that could be used by the industry.
If the calculation indicates that the cost of such a machine is within the grasp of bulb growers/groups of bulbs grower (perhaps below £300k) then we should consider proposing to take the ‘project’ further.

50 intact Narcissus bulbs having varying degrees of basal rot were examined with the on-line MRI sensor. A 1-dimensional MRI relaxation spectrum was acquired for each of the bulbs

and analyzed for the existence of basal rot to give a straightforward “yes or no” result. Each bulb was then cut open and photographed and classified as to whether or not there was basal rot. The MRI prediction was then compared with the photographic evidence. All 50 bulbs were correctly classified by the MRI method, although two cases gave the correct result but could be described as “borderline”.

This success needs to be qualified by two points:

1) To test the validity of the method the MRI measurements were done under ideal circumstances, namely with stationary bulbs and with 16 accumulated acquisitions of the MRI data on each bulb to improve the signal/noise in the data. In a real on-line situation the bulbs would be travelling on a conveyor in single file through the MRI sensor at speeds of up to 1.3m/s and there would then only be time for a single acquisition of the MRI data on each bulb, so signal/noise would be worse and, potentially, the degree of confidence in the detection of basal rot correspondingly less. If the project is to be taken further then this would involve measurements on moving bulbs with single shot acquisition to test the reliability under realistic conditions.
2) The MRI sensor is still at the prototype stage and would require serious financial investment before it could be sold commercially, although this would not be necessary if the sensor were run as a “service” for sorting bulbs and selling basal-rot free bulbs.

Financial Benefits

There are two business scenarios to be considered:

- The first involves the replacement of the hand-sorters on a conveyor belt with a commercial version of the MRI sensor. This would require serious financial investment in the development of a factory-safe, and noise-insulated commercial version of the existing prototype MRI sensor. It is doubtful whether this could be commercially viable, but no attempt has been made a quantifying the cost/benefit ratio.

- The second business scenario is where just a few MRI sensors are located in an optimized, noise-free environment and used to grade bulbs and remove any bulb containing basal rot. The basal-rot-free bulbs could then be sold on to growers and to the general public with the guarantee of being basal rot free. Input from growers would be required before any cost/benefit analysis could be undertaken on this second business strategy.

Action Points

The next step, which would require additional funding, is to repeat the experiments under the more realistic conditions of moving, single-file bulbs with single-shot acquisition. This would require optimizing the design of the MRI sensor slightly. In particular, a new radiofrequency probe would be built around the conveyor to optimize the signal/noise ratio.

Subject to approval by the growers, similar studies could be undertaken to detect bruising in Narcissus bulbs and also a separate study could be undertaken for the quality of Onions.

SCIENCE SECTION

Introduction

Basal rot in Narcissus bulbs is a major problem for growers, but its automated, non-invasive, detection with a high degree of confidence is not yet possible. At present, bulbs that have very extensive basal rot can be removed by hand sorting based on the softness of the bulb. This is a crude labour-intensive and expensive method that fails to detect basal rot in any but the most extreme cases of infection. This small scale project was therefore undertaken to investigate the feasibility of using a prototype MRI sensor for the automated detection of basal rot. This prototype had already been built and tested by the MRI research team at the IFR.

The new MRI sensor used for this project works in the same way as the well-known MRI scanners in hospitals, except that it is not necessary to acquire a 2-dimensional image to answer the simple question as to whether a bulb has, or has not, got Basal Rot. This simple Yes/No question can be answered by analysing the signal from the whole bulb without any spatial/image resolution. This is possible because our preliminary experiments (see appendix) showed that a single MRI parameter (the water proton transverse relaxation time, called T2) for dissected pieces of basal rot was significantly different from the value for healthy tissue. In particular, healthy bulbs were associated with a single dominant T2 peak at ca. 100ms, with only a minor peak at ca 1-10ms. In contrast, the badly diseased basal rot segments were associated with a large peak at ca. 10ms, and no significant peak at 100ms. This suggested that acquisition of a T2 spectrum and taking the ratio of the amplitudes of the peaks at 100 and 10ms should be all that is required to identify the presence of basal rot. Our subsequent experiments have confirmed this idea.

Materials and methods

Narcissus bulbs were supplied by Winchester growers (Gordon Flint) and were placed in the MRI sensor located at the Institute of Food Research. This sensor had been successfully designed and developed by one of the researchers (Dr. Brian Hills and his colleague Kevin Wright) using LINK funding. The T2 characteristics of the bulb were measured using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence on a single stationary bulb. This gave a multiple-exponential decaying signal (the green figures in the results section) that was analyzed using WINDXP software (the grey figures) to give what is called a one-dimensional “T2-spectrum” (the yellow background figures in the Results section). A simple peak intensity ratio was then used to determine whether the bulb contained Basal Rot. A threshold value of the ratio was then chosen that gave the maximum discrimination between the healthy and basal rot bulbs. Finally, the bulb was cut in two and photographed to confirm the presence or absence of basal rot.

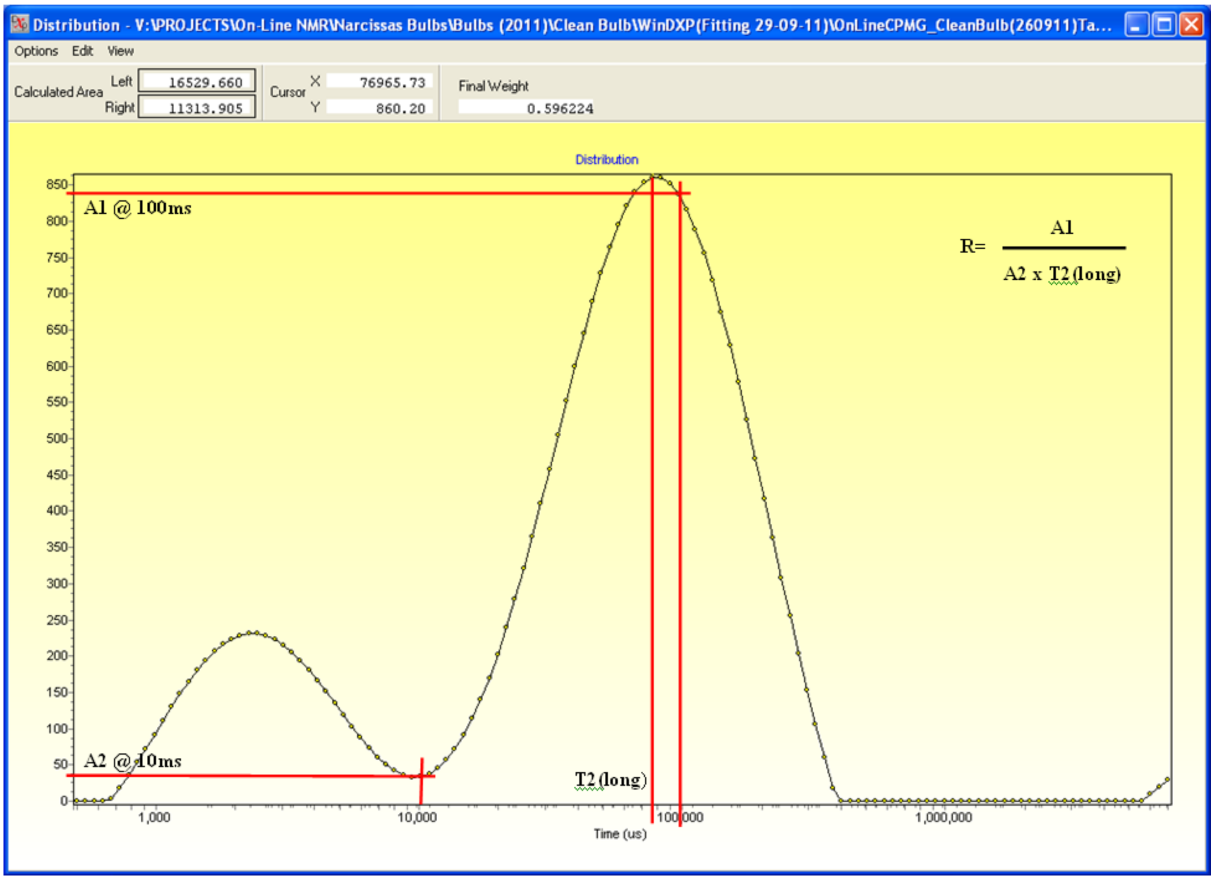

Figure 1. Classification formula successfully applied with appropriate 'test' threshold

The figure above shows a typical T2 spectrum for a healthy bulb. As discussed in the interim report, the criterion used to classify the bulbs was

$$R = A1/(A2 \; . \; T2(\text{long}))$$

where, as indicated in figure 1, A1 is the amplitude at a T2 of 100ms; A2 is the amplitude at 10ms; and T2(long) is the T2 of the major peak. A threshold value for R was then chosen that gave the best classification of all the bulbs.

The first set of experiments was undertaken on Karensa variety bulbs in October 2010 and was the subject of the brief mid-term report. Only 10 bulbs were examined (5 healthy and 5 with basal rot) but the results were so encouraging (100% correct classification with a threshold value of R equal to 0.06), that it was felt necessary to test the robustness of the method on a larger batch of bulbs at no extra cost.

This second set of experiments was completed in September 2011 on a larger number (40) of a different variety (Ellen) of bulbs. The success rate was again found to be 100%, but with a slightly different threshold value, R, of 0.049 for distinguishing good and bad bulbs. This slight difference in threshold value is expected because a different variety of bulbs was used and suggests that different bulb varieties will be associated with different threshold values.

Results

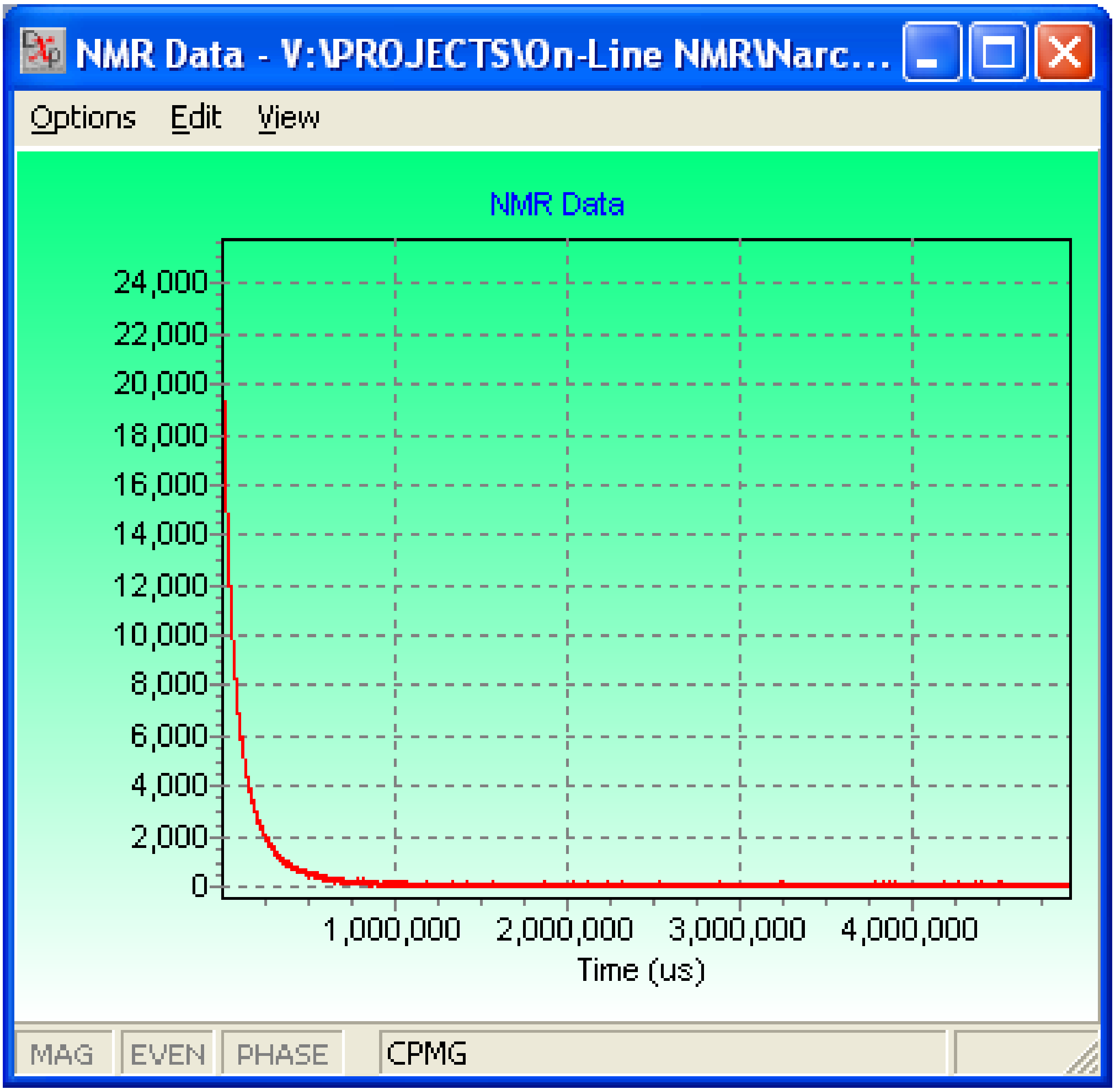

Figure 2. A typical raw data set showing the decay of the MRI signal with acquisition time

These data were processed to give a T2 relaxation time spectrum, such as that illustrated below:

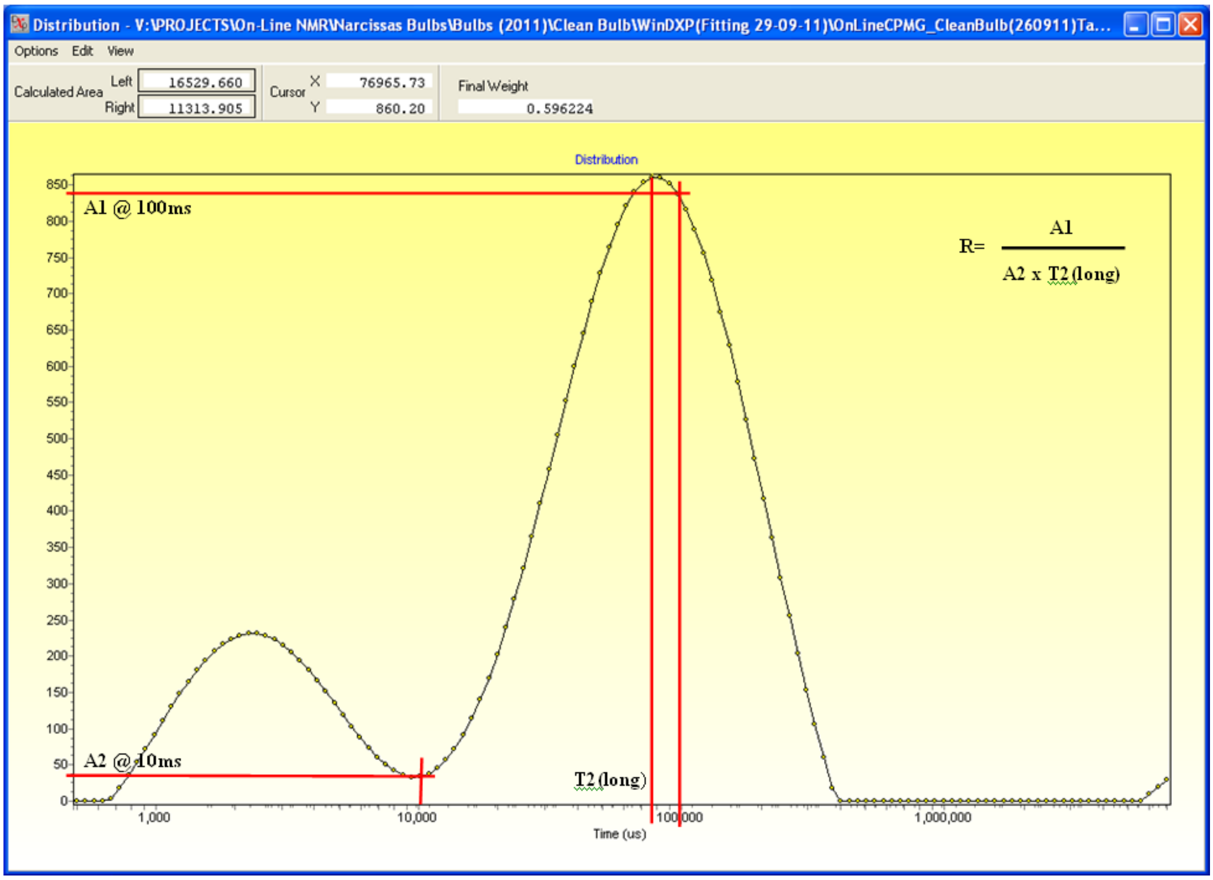

Figure 3. Illustration of classification formula successfully applied with appropriate 'test' threshold.

Figure 4. A photo of the sliced bulb was then taken to test for the presence of Basal Rot

The results for all 50 bulbs are listed below. It can be seen that the bulbs have been correctly classified with a 100% success rate.

Table 1. Classification of Basal Rot Bulbs of Clean 'Karensa' bulbs and Basal Rot 'Malvern City' bulbs. Results for the first batch of bulbs delivered in October 2010

		Clean and Basal Rot Bulb (Oct-2010)						
							Healthy Threshold	
Sample	*Actual Classification*	A1@T2(100ms)	A2@T2(10ms)	A1/A2	T2(Long)	R=A1/(A2*T2(long))	>0.06=TRUE()	Test Flag
C1	*TRUE*	416	66	6.303	66608	0.095	TRUE	1
C2	*TRUE*	575	81	7.099	100000	0.071	TRUE	1
C3	*TRUE*	530	80	6.625	91366	0.073	TRUE	1
C4	*TRUE*	570	78	7.308	76270	0.096	TRUE	1
C5	*TRUE*	621	71	8.746	87333	0.100	TRUE	1
BR1	*FALSE*	227	71	3.197	125325	0.026	FALSE	1
BR2	*TRUE*	299	42	7.119	91366	0.078	TRUE	1
BR3	*FALSE*	516	73	7.068	119793	0.059	FALSE	1
BR4	*FALSE*	303	63	4.810	114505	0.042	FALSE	1
BR5	*FALSE*	340	47	7.234	131113	0.055	FALSE	1

Table 2. Results for the 2nd batch of bulbs delivered in September 2011.

		Clean and Basal Rot Bulb Classification (Sept-2011)								
						Healthy Threshold			Healthy Threshold	
Sample	*Actual Classification*	A1@T2(100ms)	A2@T2(10ms)	A1/A2	T2(Long)	Ratio A1/A2 Test (>5)	Test Flag	(A1/A2) x 1/T2(long)	>0.049=TRUE()	Test Flag
C1	*TRUE*	838	34	24.65	82974	TRUE	1	0.2970	TRUE	1
C2	*TRUE*	816	76	10.74	120831	TRUE	1	0.0889	TRUE	1
C3	*TRUE*	761	96	7.93	103964	TRUE	1	0.0762	TRUE	1
C4	*TRUE*	717	66	10.86	96436	TRUE	1	0.1127	TRUE	1
C5	*TRUE*	530	79	6.71	112081	TRUE	1	0.0599	TRUE	1
C6	*TRUE*	378	51	7.41	96436	TRUE	1	0.0769	TRUE	1
C7	*TRUE*	520	66	7.88	66222	TRUE	1	0.1190	TRUE	1
C8	*TRUE*	421	52	8.10	89452	TRUE	1	0.0905	TRUE	1
C9	*TRUE*	536	36	14.89	103964	TRUE	1	0.1432	TRUE	1
C10	*TRUE*	434	68	6.38	89452	TRUE	1	0.0713	TRUE	1
BR1	*FALSE*	77	538	0.14	12670	FALSE	1	0.0113	FALSE	1
BR2	*FALSE*	161	390	0.41	24923	FALSE	1	0.0166	FALSE	1
BR3	*FALSE*	479	270	1.77	89452	FALSE	1	0.0198	FALSE	1
BR4	*FALSE*	99	441	0.22	21444	FALSE	1	0.0105	FALSE	1
BR5	*FALSE*	278	402	0.69	24923	FALSE	1	0.0277	FALSE	1
BR6	*FALSE*	237	406	0.58	24933	FALSE	1	0.0234	FALSE	1
BR7	*FALSE*	127	377	0.34	175959	FALSE	1	0.0019	FALSE	1
BR8	*FALSE*	429	305	1.41	112081	FALSE	1	0.0125	FALSE	1
BR9	*FALSE*	42	317	0.13	175959	FALSE	1	0.0008	FALSE	1
BR10	*FALSE*	495	325	1.52	103964	FALSE	1	0.0147	FALSE	1
U1	*TRUE*	605	45	13.44	100000	TRUE	1	0.1344	TRUE	1
U2	*TRUE*	420	57	7.37	83477	TRUE	1	0.0883	TRUE	1
U3	***FALSE***	**478**	**98**	**4.88**	**100000**	**FALSE**	**1**	**0.0488**	**FALSE**	**1**
U4	*TRUE*	509	48	10.60	95586	TRUE	1	0.1109	TRUE	1
U5	*TRUE*	375	74	5.07	79792	TRUE	1	0.0635	TRUE	1
U6	*FALSE*	279	186	1.50	104618	FALSE	1	0.0143	FALSE	1
U7	*FALSE*	286	125	2.29	87333	FALSE	1	0.0262	FALSE	1
U8	*FALSE*	292	196	1.49	44367	FALSE	1	0.0336	FALSE	1
U9	*FALSE*	223	158	1.41	100000	FALSE	1	0.0141	FALSE	1
U10	*FALSE*	197	165	1.19	44367	FALSE	1	0.0269	FALSE	1
U11	*FALSE*	261	151	1.73	66608	FALSE	1	0.0259	FALSE	1
U12	*TRUE*	449	61	7.36	76270	TRUE	1	0.0965	TRUE	1
U13	***TRUE***	**384**	**128**	**3.00**	**60857**	**FALSE**	**0**	**0.0493**	**TRUE**	**1**
U14	*TRUE*	278	55	5.05	87333	TRUE	1	0.0579	TRUE	1
U15	*TRUE*	308	51	6.04	114505	TRUE	1	0.0527	TRUE	1
U16	*FALSE*	337	143	2.36	83477	FALSE	1	0.0282	FALSE	1
U17	*TRUE*	299	51	5.86	83477	TRUE	1	0.0702	TRUE	1
U18	*FALSE*	178	190	0.94	30917	FALSE	1	0.0303	FALSE	1
U19	*FALSE*	246	184	1.34	50802	FALSE	1	0.0263	FALSE	1
U20	*FALSE*	323	141	2.29	72903	FALSE	1	0.0314	FALSE	1

Discussion and Conclusions

The above results confirm that the on-line MRI sensor, in its ideal configuration, is capable of giving a 100% successful detection of Basal Rot in Narcissus bulbs. However, this “ideal” configuration involved using stationary bulbs and 16 accumulations of the MRI signal on each bulb. This robustness of this method now needs to be tested on samples moving at about 1m/s with only a single acquisition.

Note that this threshold criterion for the ratio R was chosen to give the correct identification of healthy and basal rot bulbs. Inevitably, one or two bulbs were then very close to the threshold value and could then be described as “borderline”. But in a commercial application the threshold criterion would need to be agreed with the end user to determine the relative costs of discarding healthy bulbs against the risks and associated costs of retaining bulbs close to the threshold level.

So far we have only studied Basal Rot in Narcissus Bulbs; but other quality factors that may be amenable to MRI sensor detection include a) Bruising b) Onion quality and c) the size (weight) of the bulbs/onions. In particular we expect the weight of the bulb to be proportional to the amplitude of the MRI signal, so could be determined in the same measurement used to detect Basal Rot.

Knowledge and Technology Transfer

There is, at present, no IPR protection either on the on-line MRI sensor hardware and software; or on the specific methodology used to detect basal rot in Narcissus bulbs. The risk of any other research group being able to build the MRI sensor in a reasonable time is, however, at this time, minimal.

References specific to the On-line MRI sensor

B.P.Hills and K.M.Wright, Motional Relativity and Industrial MRI Sensors, Journal of Magnetic Resonance, 2006, 178, 47-59.

APPENDIX: Preliminary experiments

Our earlier report showed the results of preliminary experiments undertaken on a commercial NMR spectrometer operating at 23.4MHz. The aim of these first

Initial experiments were designed see whether tissue affected by basal rot could be distinguished from healthy tissue using NMR relaxometry. To do this tissue affected by basal rot was dissected out of the bulb and examined separately from healthy tissue. This was necessary because the commercial NMR equipment could only hold samples less than 1cm in diameter. A one-dimensional relaxation time T2-spectrum was acquired on each sample. It was noted that the basal rot tissue gave a single peak at about 20ms; whereas the healthy tissue gave a single peak at ca. 110ms, so discrimination was 100%. (see figure 5).

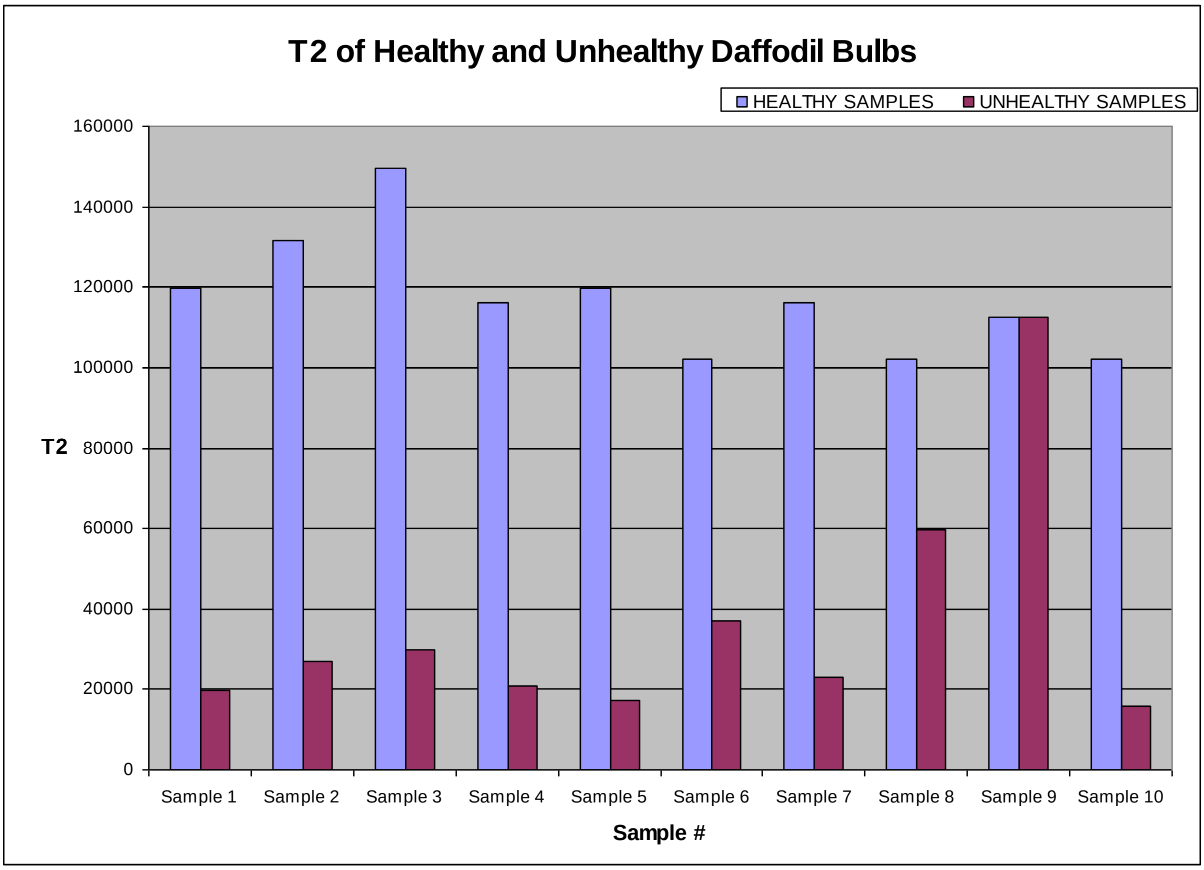

Figure 5. The T2 (ms) of the peak for pure basal rot tissue (red peaks) compared to the T2 of pure healthy tissue (blue peaks). Data acquired at 23.4 MHz on a commercial spectrometer.

On-line sensor results

This initial result encouraged us to proceed to the next step which was to examine whole bulbs on the on-line NMR sensor operating at the lower frequency of 2.8MHz. A second batch of bulbs was acquired, which had been pre-sorted by the grower into healthy bulbs and basal-rot bulbs. The bulbs were placed in the middle of the sensor in a stationary position and a one-dimensional T2-spectrum was acquired. A typical spectrum is seen in figure 6.

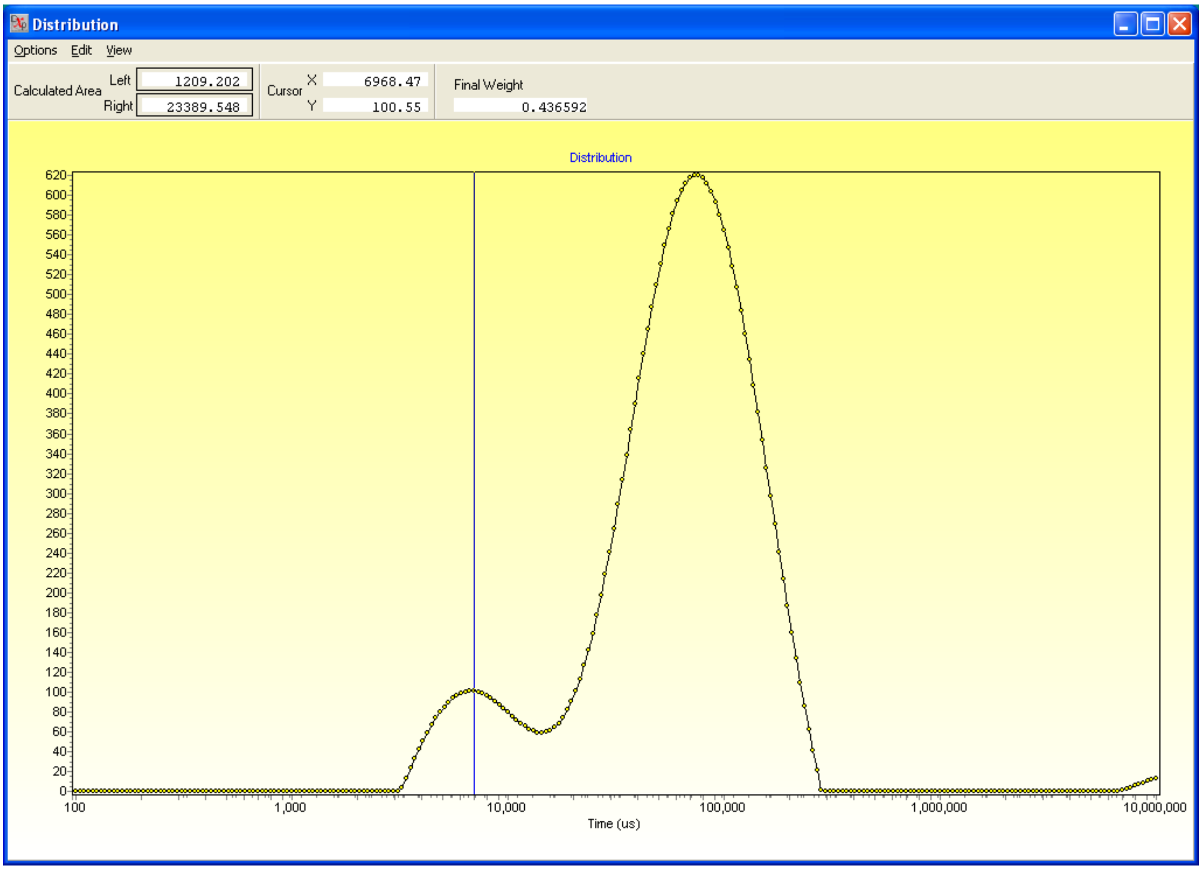

Figure 6. A representative T2 spectrum showing two peaks. The one on the left has a T2 about 10ms and contains contributions from basal rot tissue. The larger peak on the right at a T2 of ca. 100ms arises from healthy tissue. Data acquired with the prototype on-line sensor. The vertical line simply is a marker of the peak maximum

Because we are now looking at the whole bulb we see two peaks. The smaller one at a T2 of ca. 8ms contains the contribution from basal rot tissue, along with tissue components from healthy tissue. The major peak at a T2 of about 100ms arises exclusively (we believe) from healthy tissue. To discriminate between the basal rot and healthy bulbs it is therefore necessary to take a ratio. A suitable ratio for distinguishing the healthy from basal rot is given by the formula:

$$R = \frac{A1}{A2 \times T2(long)}.$$

Where:

A1 = Amplitude of the peak maximum of the long T2 peak

A2 = Amplitude of the peak maximum of the short T2 peak

T2(long) = Transverse relaxation time (T2) of the long T2 peak in milliseconds.

Figure 7 shows a plot of **R** for all the bulbs examined in the on-line sensor and shows that the healthy bulbs have **R** ≥ 0.065; whereas the bulbs containing basal rot have **R** < 0.065. This then gives 100% discrimination on this limited data set.

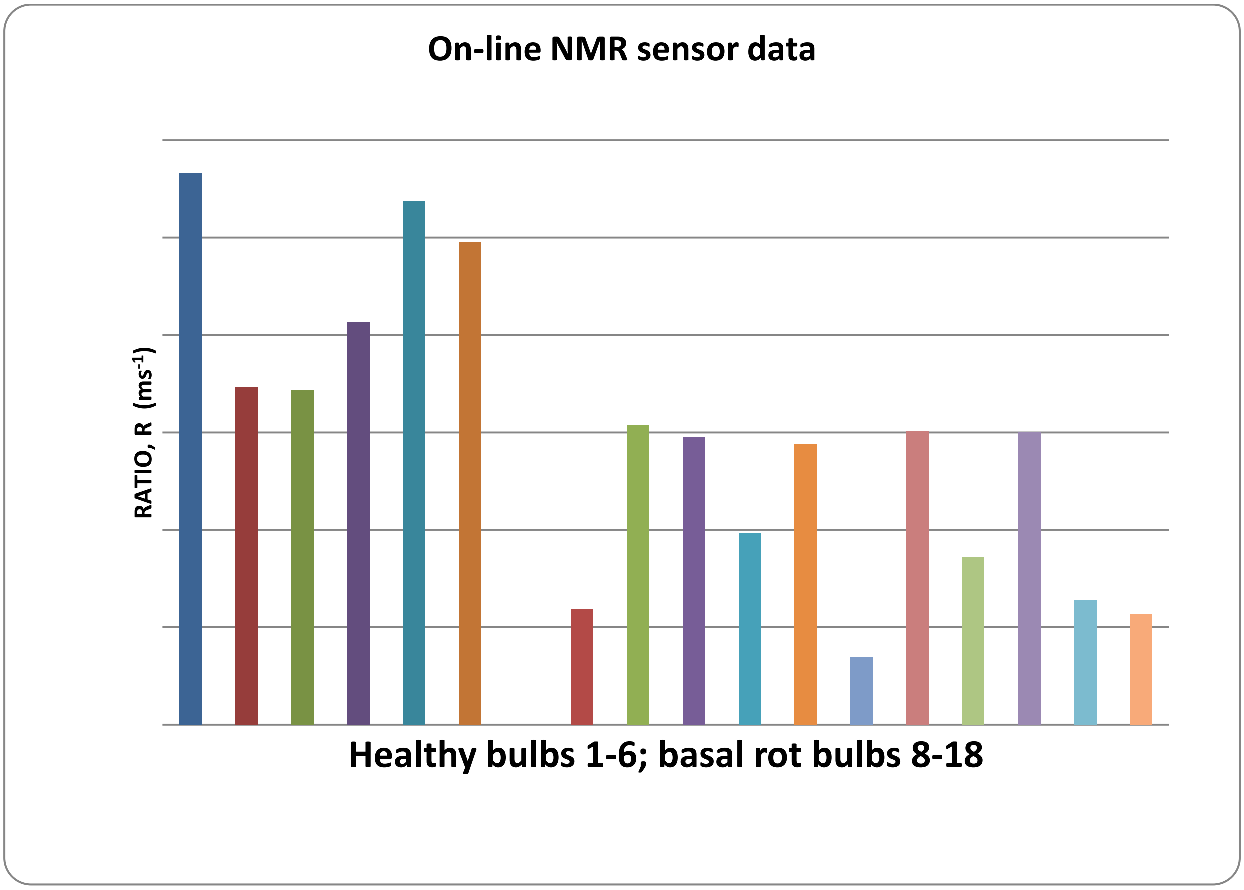

Figure 7. Results from the latest on-line NMR measurements on the intact bulbs showing a plot of the ratio R against bulb number. Bulbs numbered 1 to 6 were healthy; those labelled 8 to 18 contained basal rot.

Recommendations for next Steps

a) After the NMR acquisition the bulbs were cut open and photographed with a digital camera. To our unskilled eye it was hard to identify the basal rot or judge to what extent the rot had progressed. The labelling in figure 7 was that on the batches sent to us. We therefore recommend that a third batch of bulbs is sent to us and we are shown by an expert grower how to assess the degree of basal rot in the bulb.

b) Clearly we wish to strengthen the correlation seen in figure 3 by examining a lot more bulbs. This will be at no extra cost.

c) The above results were acquired on a stationary bulb in the centre of the on-line sensor. The last and final challenge is to be able to repeat these results for bulbs that are continuously moving through the sensor at a typical conveyor speed of say 1m/s. This can be done using our on-line prototype sensor and will be the main aim of the next batch of work.

Brian Hills, 11/01/2011