2004 BELTWIDE COTTON CONFERENCES, SAN ANTONIO, TX – JANUARY 5-9

MICROBIAL CENSUS AND COTTON BALE MOISTURE DURING A 6-MONTH STORAGE

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Abstract

As part of an ongoing investigation, a corroborative study was done to follow fiber quality, moisture content and microbial population changes for 1-, 2- and 6-month storage periods in cotton bales with high moisture contents. The target moisture contents were 6% (control/ambient moisture content), 8%, 10% and 12%. The high moisture content bales lost moisture after 6-months storage while the three other moisture content bales tended to retain the same moisture content. However, the distribution of moisture was not uniformly dispersed in the treatment bales with spottiness increasing with increased moisture. Microbial populations did not change significantly during 1 and 2 months storage, which were colder winter months. The greatest microbial changes associated with moisture content occurred after 6 months of storage, which took place during the warmer spring and summer months. Observations on fiber quality associated with moisture content indicate degraded color changes increased with increased added moisture.

Introduction

The practice of moisture restoration has value in reducing bale-packaging forces, increasing the bale weight to make up for excessively dry cotton resulting from ginning, and may even improve fiber quality and processing as shown by McAlister (1997). Anthony (2003) surveyed gins in Mississippi and Arkansas and found that the average moisture content prior to moisture restoration was 5.1 percent and 6.2 percent after moisture restoration, which are well below the 8 percent moisture level generally considered safe for bale storage. However, 8.6 percent of the bales surveyed exceeded the 8 percent level, which may subject the bales to quality degradation during extended storage (Chun and Anthony, 2002). This study was conducted as a companion study to the work done at the Stoneville Ginning Laboratory in response to industry concerns about high moisture during bale storage in adversely affecting fiber quality with the purpose of determining an acceptable cotton bale moisture range to avoid fiber quality degradation during long term bale storage. This part of the study looks at the bale moisture content and the microbial activity associated with it.

Materials and Methods

Cotton, Moisture Treatment

Cotton used was from the 2002 harvest year. The cotton was upland cotton, DP 565, grown by a local producer in New Mexico that had a reputation for producing good quality cotton and could harvest in good condition at least four modules from the same field during the same time frame. The harvested cotton was stored as properly shaped and tarped modules on sandy dry ground after harvest and before ginning at a commercial gin at its designed ginning capacity during the test. Ginning took place on December 12, 2003.

Water application was via a spray system where the spray level could be adjusted to apply an amount of water to meet specific preset levels for the processing rate used. Normally fiber moisture control was regulated by an infrared sensor located just after the battery condenser stripper rolls which would make sequential readings of lint moisture and running measurements of the seed cotton feed rate of each gin feeder and adjust the spray level accordingly with up to 5 active nozzles. For this study, the infrared sensor was bypassed and preset amounts of water, in pounds, was applied by the spray system to bring the bale moisture to predetermined levels of moisture based on an ambient bale moisture content of 6%, the moisture level of cotton harvested and moduled during the normally dry harvest conditions of the Southwest. To obtain the target moisture contents of 8%, 10% and 12% moisture, 4.54 kg, 9.07 kg and 13.61 kg (10 lb, 20 lb and 30 lb, respectively) of water per bale was added, respectively. The target bales weight was set for a 217.7 kg (480 pounds) bale. The bales were wrapped with a polyethylene bag and plastic strapping was used; the bale ends were secured by sewing the ends closed instead of heat seal-

ing. Nine bales were prepared for each of the moisture treatment, 6%, 8%, 10% and 12% moisture, with the weight of all 36-test bales averaging 216.5 kg. The finished bales were stored inside the gin building until they could be transported to the Cotton Quality Research Station where they were stored at the USDA Warehouse in the Agricultural Servicenter on Old Cherry Road in Clemson, South Carolina.

Experimental Design and Statistical Analysis

The storage durations of approximately one-month, two-months and six-months, were used and sampling occurred on January 17, February 26 and July 8, 2003. For each storage period, 12 bales used, three bales from each moisture treatment were removed from storage and sampled. The sequence of processing each of the 12 bales was random. Each bale was placed on its side and before the plastic straps were removed, divided into 10 layers or zones parallel to the compression layers. After the straps were cut, a cardboard template that had 14 15.24-cm diameter holes, each hole representing a fixed location and equidistant from one another, was placed on the top surface of each layer. One location was randomly chosen from each layer and enough lint was removed from that location to compactly fill a 0.95 L wide-mouth canning bottle. The sampled layer was removed and the layer below it was exposed and sampled. This continued until each of the 10 layers were sampled. The samples were stored at room temperature in these tightly sealed canning bottles until the samples could be removed for fiber quality and microbial population measurements. The four moisture level treatments with three bale-replicates yielded 120 samples for each of the storage times. Each of the 120 samples was randomly given a new sample ID number and the samples were assayed sequentially. A $\log_{10}(cfu+1)$, where cfu = microbial population as colony forming units per gram lint (corrected for dry weight), transformation was used for the analysis dealing with the microbial data. Data were analyzed using release 8.00 or earlier releases of SAS (SAS, Statistical Analysis System; SAS system for Windows version 4.90.3000, SAS Institute Inc., Cary, NC, USA) for making mean comparisons. Otherwise, additional testing and data manipulation were done with Microsoft EXCEL 2000 or earlier releases of EXCEL (Microsoft Corporation, USA) or with SigmaPlot 2002 Version 8.0 (SPSS, Inc., USA).

Microbial Acitivity and Fiber Quality

Determining the extent of biological degradation in each moisture treatment consisted of measuring the viable microbial populations (total and Gram-negative bacteria, and total fungi). Changes to the physical properties of the fiber were measured by the Advanced Fiber Information System (AFIS) and by the High Volume Instrument (HVI) cotton classification analysis. The samples were tested on site at CQRS for the AFIS measurements, and the samples were sent to the USDA-AMS Cotton Division Classing Office in Memphis, TN, for HVI analysis. Only the color changes will be reported here. The microbial assays used 1-gram of lint from each sample for total bacterial and total Gram-negative bacterial populations using the method described by Chun and Perkins (1996); for fungal populations, the method described by Chun and McDonald (1987) was used. Changes made to the general protocol was that the dilution blanks used 0.05% agar to aid suspension; because of problems using DIFCO Bacto Potato Dextrose Broth, homemade potato dextrose agar (PDA) was used instead; and because of the size of the study, incubation was made at room temperature, 20°±2°C for 3 days for the total bacterial and Gram-negative bacterial assays, and for 7 days for the fungal population assay.

Results and Discussion

At the time of ginning, the actual ambient moisture content was found to be 5.8% by the oven drying method. This is not very far off from the assumed value of 6% and the difference from 6% shouldn't have much influence on the moisture values of the other treatments. However, the initial moisture content at the time of ginning was found to be 7.7%, 12.4% and 12.4% for the targeted 8.0%, 10.0% and 12.0% moisture treatments, respectively. The 12.4% moisture content for the 10% treatment stands out. Possibly the oven drying test gave higher values with the higher moisture applications or that some sampling plateau of the manual sampling method from the lint slide had been reached; but regardless, the manual sampling at the lint slide had difficulties providing accurate moisture measurements at the higher levels at this commercial gin.

The average moisture contents after 1-, 2- and 6-months of warehouse storage are shown in Table 1. As expected, very small changes were observed with the ambient or control bales. While the moisture content of the control bales were all below the initial 5.8% level at the gin and changed significantly during storage, it remained around 5.6%, about a 0.2% to 0.3% moisture loss. The 8% moisture treated bales, started at about 7.7% at the gin, but slowly lost moisture until it reached about 6.7% after 6-months in storage, about a percent loss from the initial ginning moisture or about a 1.3% loss from the target value at the gin. The moisture content of the10% treated bales, did not change significantly at all during storage and remained at about 8.3%, approximately 1.7% less than the initial target moisture content. The greatest change was observed with the 12% treated bales. After 2 months of storage, the moisture level had dropped about 1.7% from it's initial target moisture and by 6-months was 2.5% less than it's initial target moisture, or almost 3% less than the moisture content at the time of ginning. Clearly, with increased moisture application comes increased moisture loss during storage.

When the distribution of moisture in the bale is examined, all of the moisture restoration bales initially showed uneven distribution of moisture (Figure 1) with increasing 'spottiness' with increasing moisture. Even though the target 8% moisture treatment averaged about 6.7% moisture content during storage and only 7.7% at the gin, these bales had areas above 8%

moisture through at least 2 months of storage. Only after 6 months did we observe the 8% treated bales to have no areas above 8%. The moisture distribution of the 8%, 10% and 12% treated bales became more uniform as storage time increased. But the 10% and 12% moisture treated bales had greater variability with some of the locations having moisture contents more than 3% higher than the average bale moisture.

The picture with microbial effects is less clear. The storage conditions during the 1-month and 2-month storage periods were very different from the storage conditions of most of the storage period of the 6-month storage period. Since microbes are biological in nature, they were probably affected by the colder wintery months of the first two storage periods and then by the warmer spring and summer months of most of the 6-month storage period. This is reflected in the small differences in the viable microbial populations in the 1-month and 2-month storage period (Figure 2 and Table 2). There were no significant changes in the total and Gram-negative bacterial populations in the 1- and 2-month storage period for each of the moisture treatments. Only in the 6-months storage period do we see significant drops in population. Also, the lower populations seem to be associated with the higher moisture content. While higher moisture levels are generally associated with higher microbial levels, the lower populations probably represent a die off situation of the populations by the early moisture stimulation. The initial early higher moisture content probably broke the dormancy survival stage causing short-term high microbial activity. Later over time, such resources as available moisture or nutrients may become limiting or used up causing the microbes to be unable to sustain growth and caused die-off, leaving a lower number of viable bacteria to be assayed. The fungal populations seem to be breaking this trend. At the 6-month storage period, instead of dropping in population with added moisture, the fungal populations seem to have leveled off at the 8% and 10% target moisture treatment and at the 12% target moisture treatment actually increased to just above the control bale population of fungi (Figure 2). Since microbial activity is not expected at the control bale moisture level, there is good reason to believe that the viable fungal populations at the 12% moisture treatment may represent fungi that are exhibiting biologically active during the storage period at this high moisture level.

Individual fiber measurements were made of the samples from each storage period and for each of the moisture treatments. Studies have linked added moisture with decrease in grade because of color changes (Anthony, 2002; Chun et al., 2003; Chun and Anthony, 2002; Chun and Brushwood, 1998) and the results from associating each sample's moisture content with its reflectance and yellowness measurement as shown in Figure 3 strongly supports this supposition. Not only is moisture directly correlated with decrease in reflectance and increased yellowness, but also the effect of moisture appears to be influenced by the duration of exposure. For reflectance, RD (%), r = 0.68, 0.72 and 0.88, after 1-, 2- and 6-month storage, respectively. For yellowness, +b, r = 0.70, 0.76 and 0.91, after 1-, 2- and 6-month storage, respectively.

In summary, moisture restoration does add additional weight to the baled cottons. However, more weight loss is noticed with increased added moisture. Increased fungal activity was not as pronounced in this study, but very possibly bacterial and fungal activity may have occurred in the warmer months of storage and co-incidentally may possibly be associated with some of the color changes (Fischer, et al., 1980). Increased added moisture appears to be directly correlated with decreased reflectance and increased yellowness, but also seems to increase with the duration of storage. While probably not as great a concern to the ginner and producer, the poor distribution of moisture in the water-augmented bales may eventually cause serious production problems to ultimate bale purchaser. Many of these undesirable effects seem to be most noticeable with the 8% and higher moisture treatments (Figures 1 & 3). Further investigations to further refine the cotton bale moisture range where fiber quality degradation during long-term storage may be warranted.

Disclaimer

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Table 1. Moisture content after storage for 1-, 2- and 6-Months.

Months in Storage	Target Moisture, %			
	6%, Control[1]	8%[1]	10%[1]	12%[1]
1	5.53^{B}	6.99^{A}	8.33^{A}	10.11^{A}
2	5.41^{C}	6.62^{B}	8.16^{A}	10.45^{A}
6	5.72^{A}	6.68^{B}	8.40^{A}	9.50^{B}

[1]Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

Table 2. Microbial population after storage for 1-, 2- and 6-Months.

	Months in Storage	Target Moisture, %			
		6%, Control	8%	10%	12%
Total Bacteria	1	6.29^{A}	6.31^{A}	6.08^{A}	5.99^{A}
	2	6.25^{A}	6.22^{A}	6.27^{A}	6.26^{A}
	6	5.95^{B}	5.99^{B}	5.12^{B}	3.52^{B}
G(-) Bacteria	1	6.29^{A}	6.26^{A}	6.06^{A}	5.87^{A}
	2	6.25^{A}	6.18^{A}	6.28^{A}	6.25^{A}
	6	5.95^{B}	5.91^{B}	4.66^{B}	0.85^{B}
Fungi	1	4.92^{B}	4.89^{A}	4.51^{A}	4.75^{B}
	2	4.54^{C}	4.35^{C}	3.97^{B}	4.35^{C}
	6	5.28^{A}	4.64^{B}	4.74^{A}	5.42^{A}

[1]Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

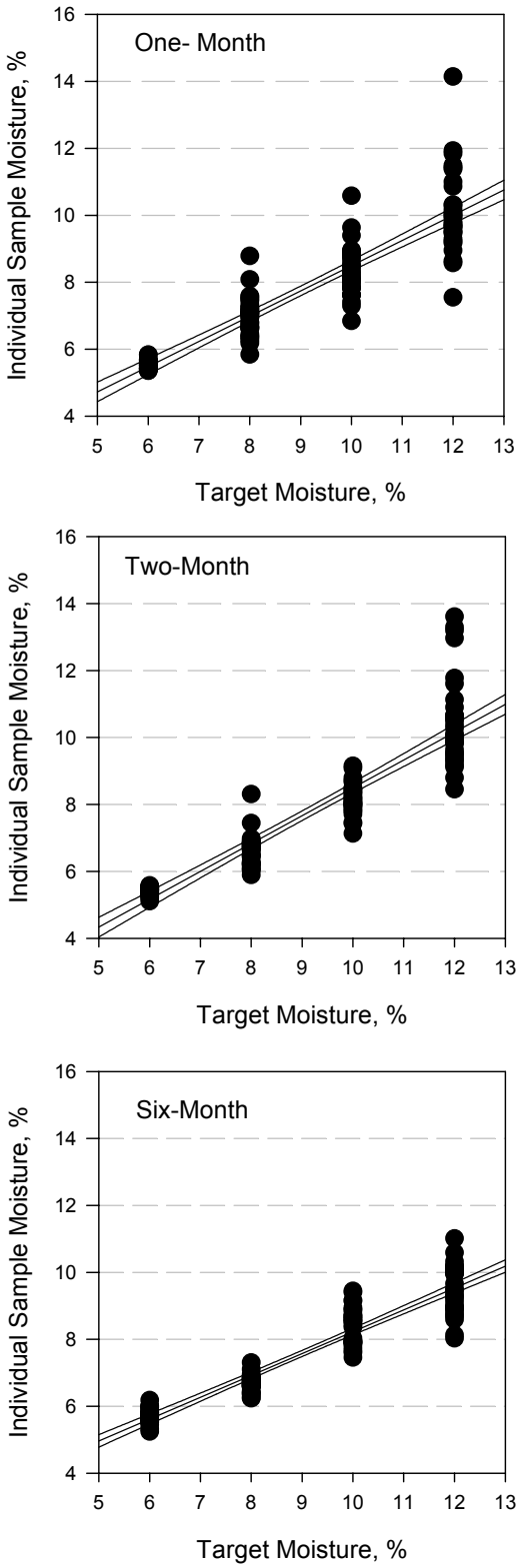

Figure 1. Moisture content, %, of all the samples after 1-, 2- and 6-month storage. The regression line represents the individual sample moistures plotted against the target moistures (6%, 8%, 10% and 12%), the outer lines depict the 95% confidence interval.

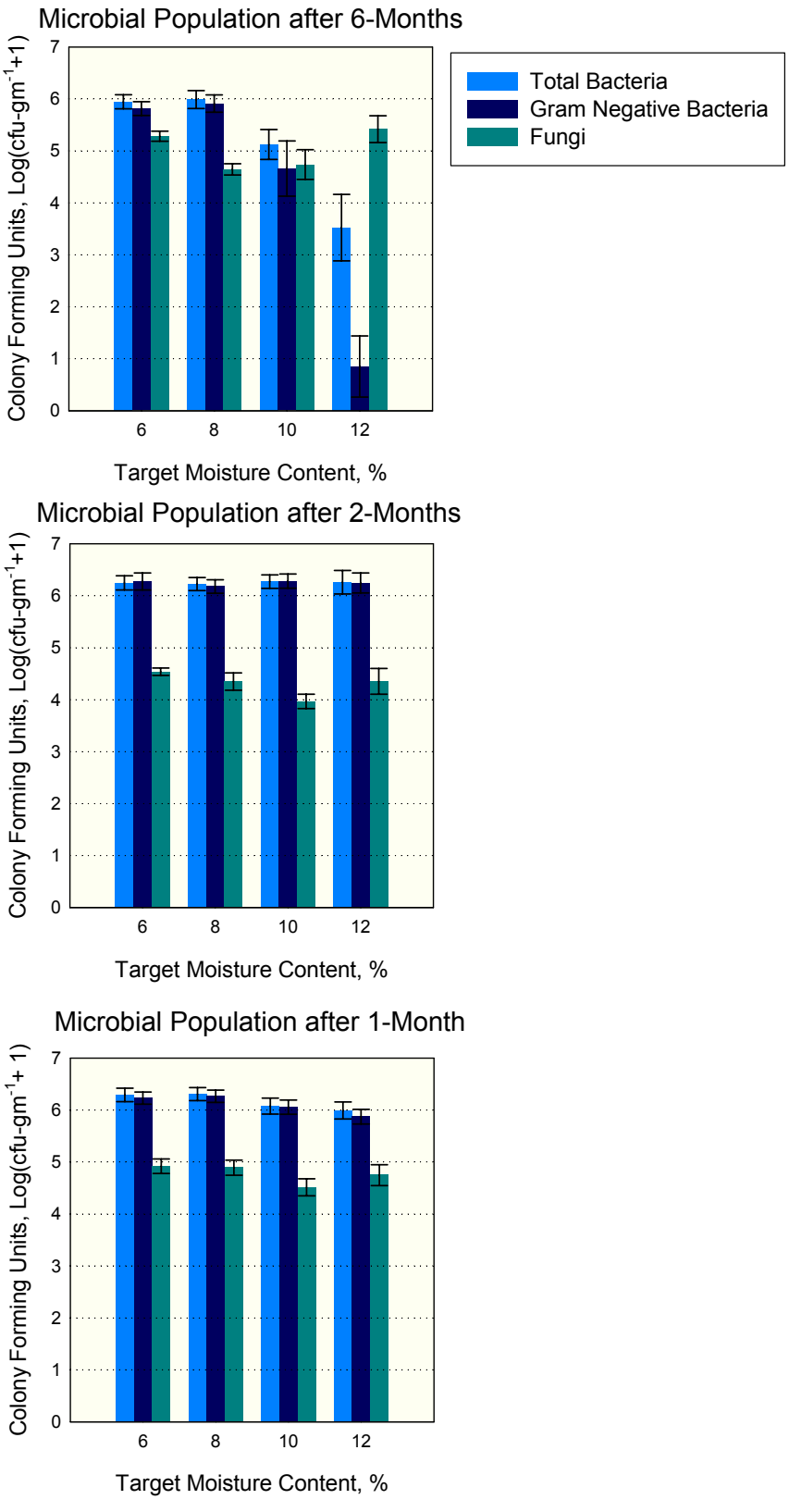

Figure 2. Microbial Populations at 1-, 2- and 6-month storage for the 6%, 8%, 10% and 10% target moisture levels. Each half bar represents 2 s.e.

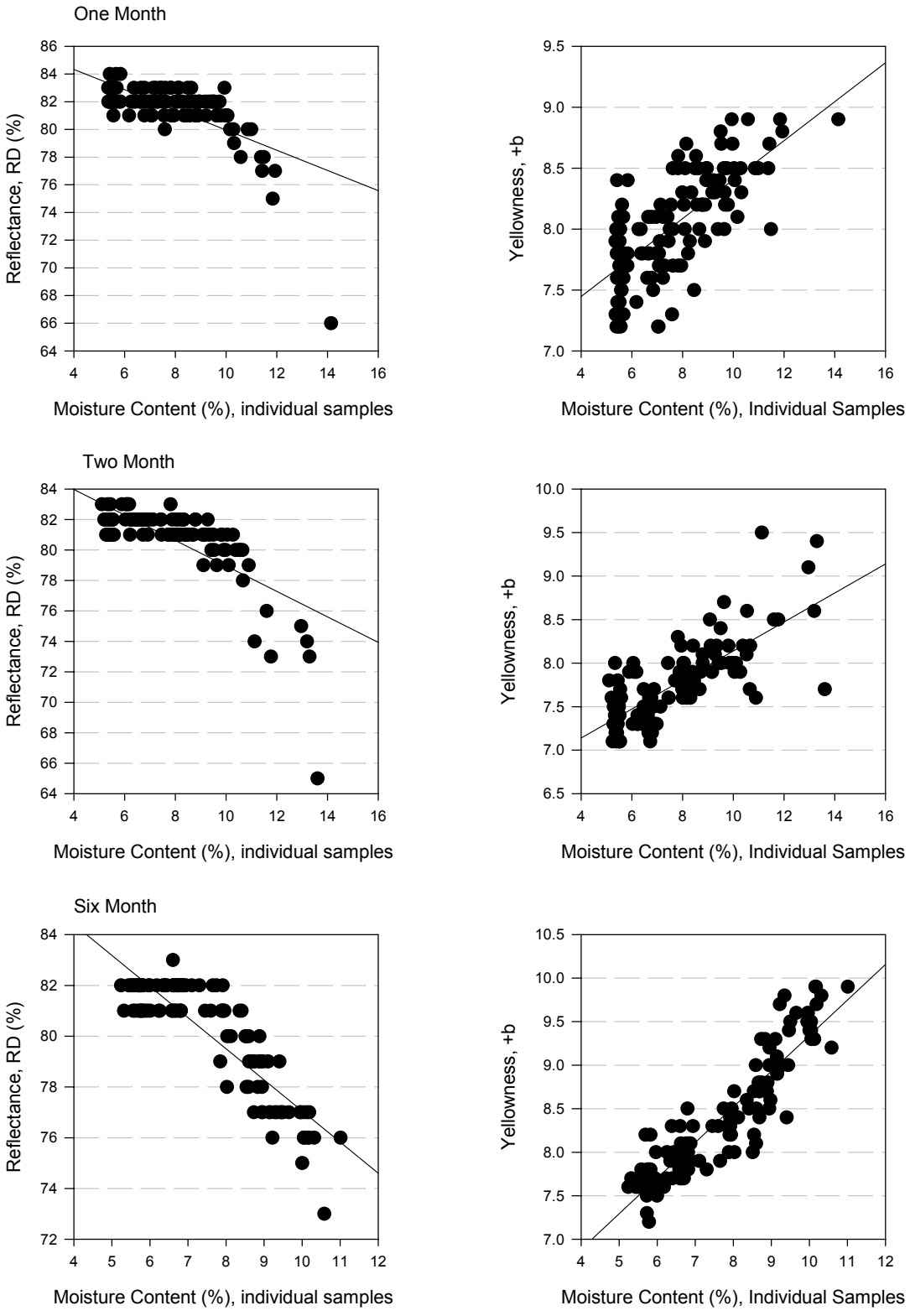

Figure 3. Reflectance and Yellowness at 1-, 2- and 6-month storage associated with the moisture content of the individual samples.