

*6<sup>th</sup> Bergerac Tobacco Scientific Meeting, September 2005*

*Contribution of Near Infrared Spectrometry (NIRS)  
to tobacco breeding*



# Introduction : Principe

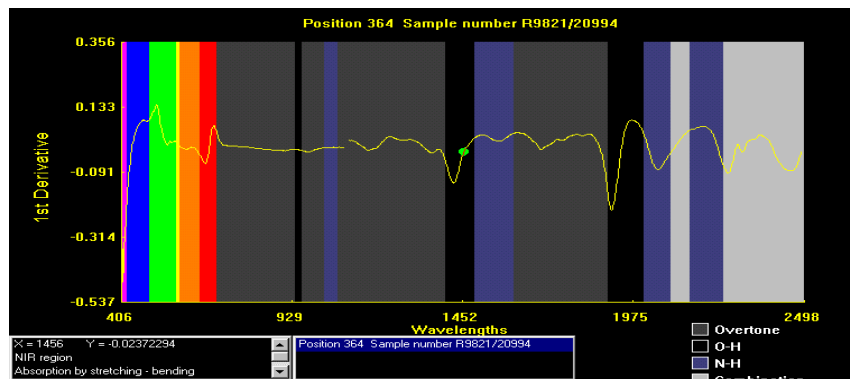
Tobacco powders : 500  $\mu\text{m}$



Spectra acquisition

Chemical analyses

$\log 1/R$



$\lambda$  : 400-2500 nm

variétés	famille	généa	Bloc ligne	N° éch	ALCA	CEN	Nt	MS
BAMM	S3	25-1-3	0101	02-23494	2,42	23,09	3,44	93,48
BAMM	S3	66-2-2	0102	02-23495	2,56	25,52	3,10	93,32
ITB 1000	temoin		0103	02-23496	3,08	20,54	3,64	93,20
BCCM	S3	14-2-2	0104	02-23497	0,70	25,60	3,07	92,89
BCCM	S3	14-2-3	0105	02-23498	1,83	23,28	4,08	94,41
BCCM	S3	20-1-1	0106	02-23499	1,48	23,40	4,14	93,61
BCCM	S3	20-1-6	0107	02-23500	1,57	22,47	4,35	93,68
ITB 1105	temoin		0108	02-23501	2,86	22,54	3,57	93,25
BGIC	S3	14-1-1	0109	02-23502	3,66	21,71	3,74	92,94
BGIC	S3	14-1-2	0110	02-23503	3,23	22,86	3,67	93,01
BGIC	S3	24-1-2	0111	02-23504	2,31	23,00	3,46	93,85
BGIC	S3	24-1-4	0112	02-23505	2,25	22,07	3,69	93,39

Data matrix

Setting up of the predictive models

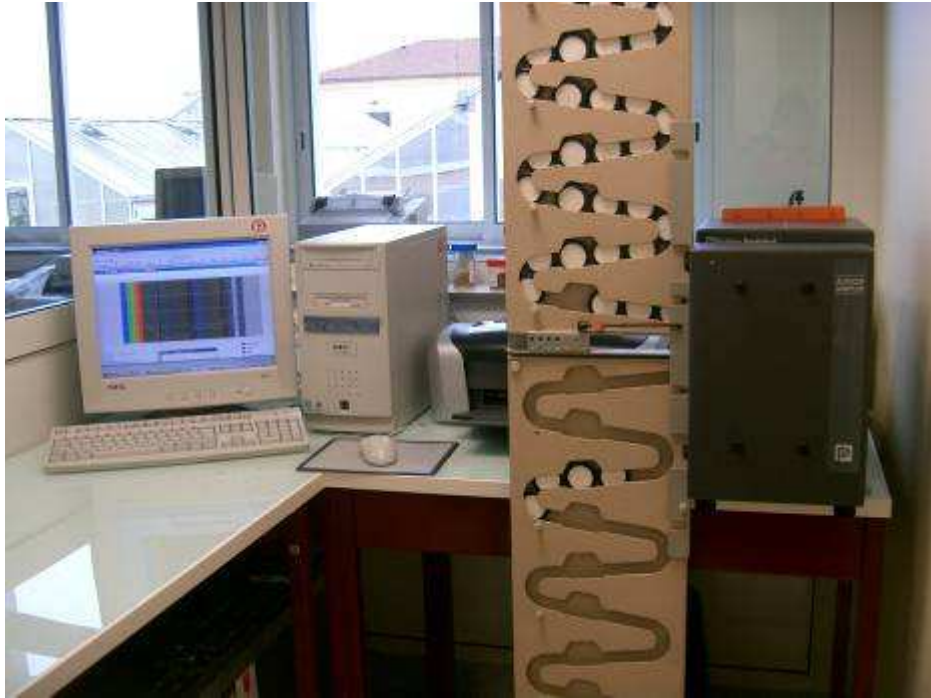
# Introduction

✚ Near Infrared Reflectance Spectrometry (NIRS) used for the characterization of tobaccos issued from the Bergerac Tobacco Institute (ITB) breeding programs.

✚ Chemical components routine-predicted since 1999 :

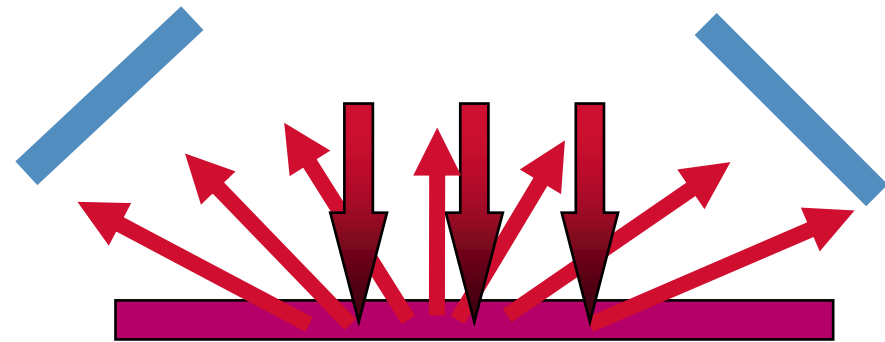
- Total alkaloids (A),
- Ashes (Ash),
- Total Nitrogen (N),
- Total Reducing Sugars (TRS).

# The spectrophotometer



✚ FOSS NIRSystem 6500 : from 400 to 2500 nm (1050 measure points, 2 nm steps)

✚ WinISI II spectral acquisition and calibration software



# Spectral and chemical data

- ✚ Tobaccos cultivated in ITB from 1995 to 2000
- ✚ Lines and varieties from breeding programs
- ✚ 3 tobacco types :

1255 Flue-cured

507 Dark air-cured

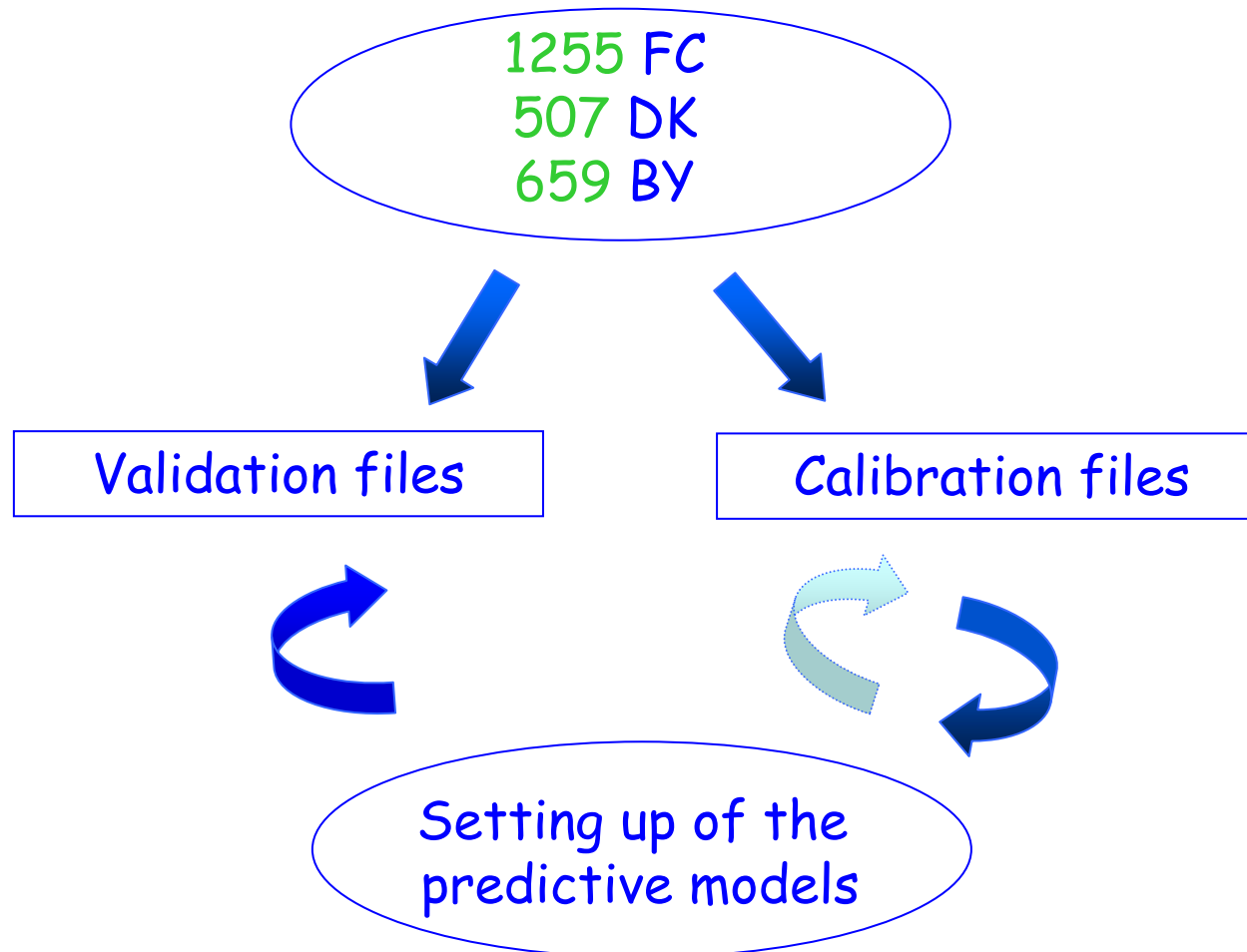
659 Burley

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2421 Samples

- spectral variability
  - important chemical component level ranges
  - year effects, varieties, stalk positions ... integrated
- ✚ Achieved on the 3 tobacco types separately

# Setting up of the predictive models



Modified PLS predictive models (*Modified Partial Least Squares*)

$$Y = b_0 + b_1x_1 + b_2x_2 + \dots + b_kx_k$$

# Setting up of the predictive models

	Files	Number of samples
FC	Calibration	720
	Validation	535
DK	Calibration	375
	Validation	132
BY	Calibration	515
	Validation	144

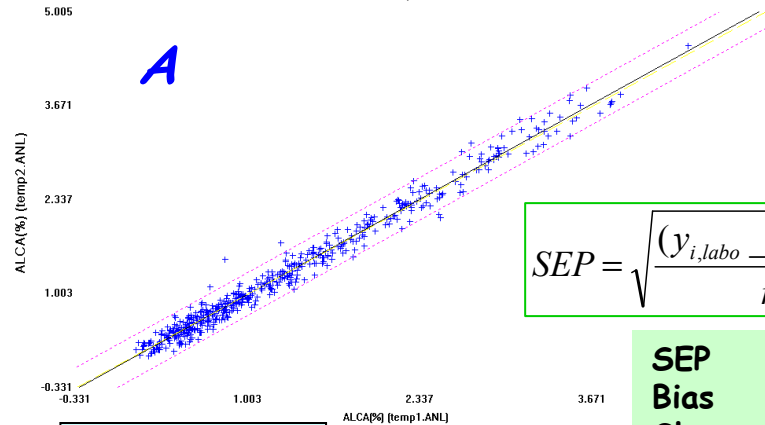
Classical chemical analyses performed on a sample set from each new year field trials (2001 ... 2004) :

- ✚ Confirmation of the performance of the models.
- ✚ No enrichment with new samples because of :
  - satisfying predictions,
  - spectra integrated in the initial spectral population,
  - component levels integrated in the range of values observed in the calibration files.

# Application of the models on validation files

😊 Flue-cured tobacco

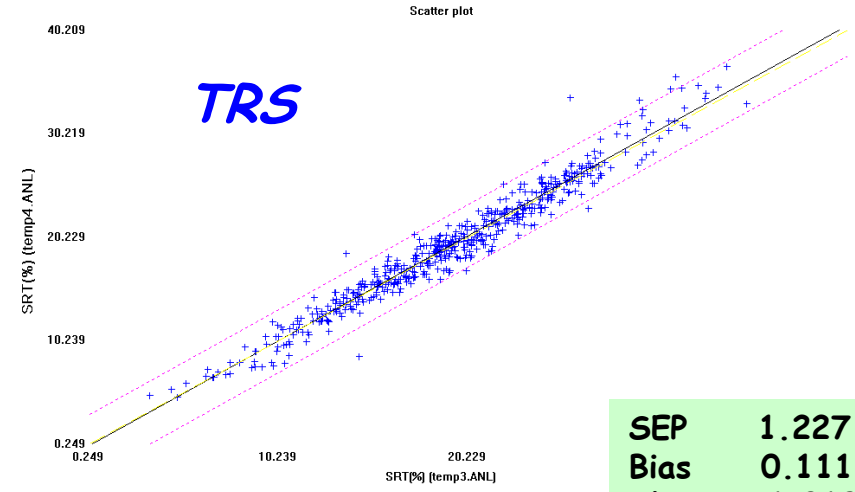
Laboratory



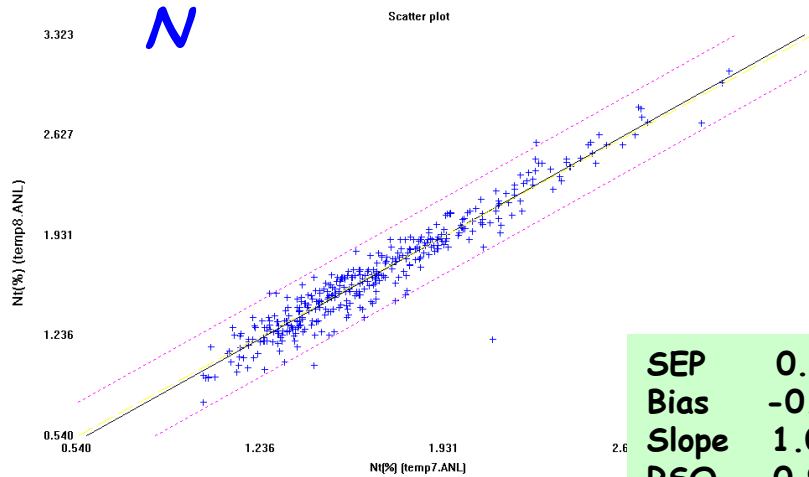
Predicted

$$SEP = \sqrt{\frac{(y_{i,labo} - y_{i,SPiR})^2}{n}}$$

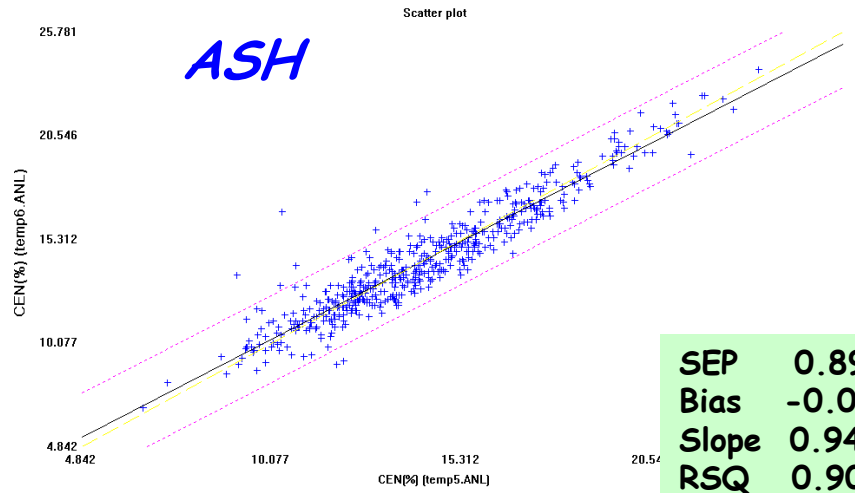
SEP	0.123
Bias	0.002
Slope	1.017
RSQ	0.982



SEP	1.227
Bias	0.111
Slope	1.019
RSQ	0.957



SEP	0.108
Bias	-0.007
Slope	1.027
RSQ	0.927



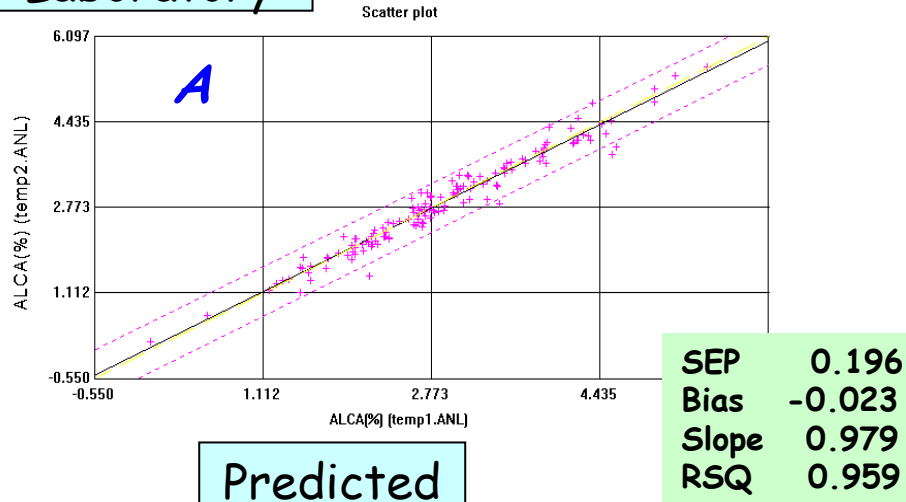
SEP	0.899
Bias	-0.020
Slope	0.947
RSQ	0.901



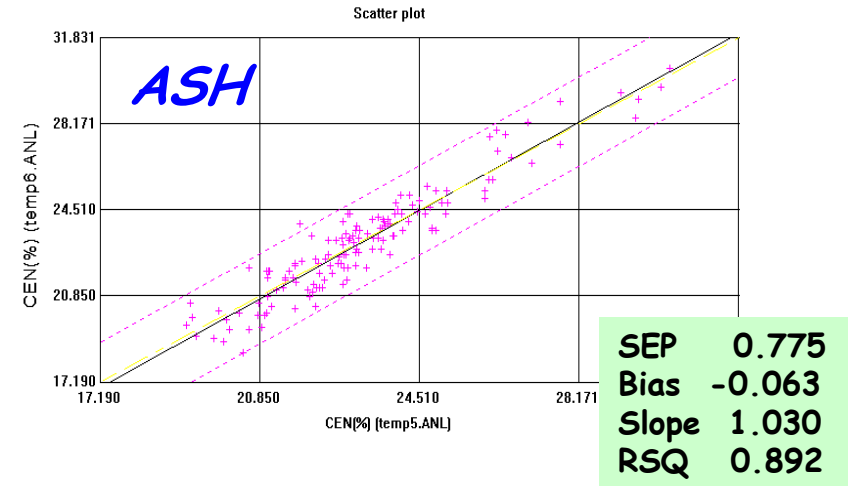
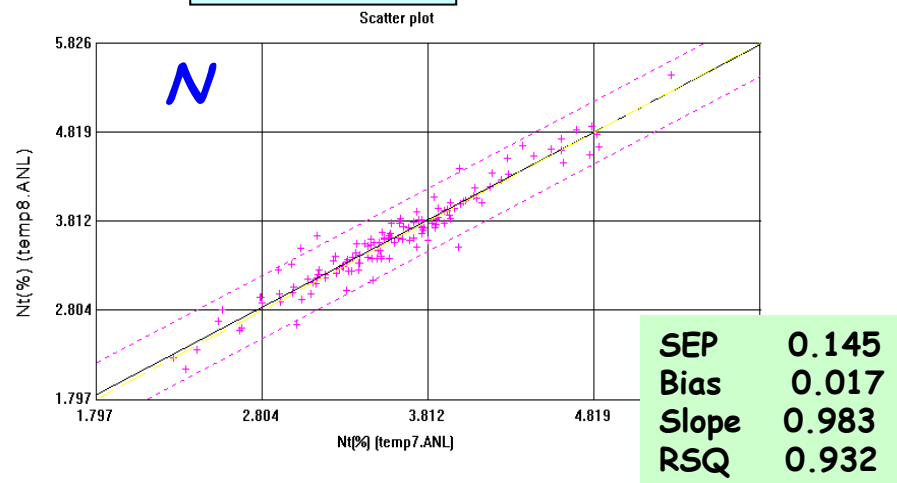
# Application of the models on validation files

😊 Burley tobacco

Laboratory



Predicted



➦ Good performances :  
robustness and accuracy  
for each tobacco type

# Conclusion

✦ Potential of the NIRS technology for the prediction,

with an error close to the laboratory error,

of

total alkaloids,

ashes,

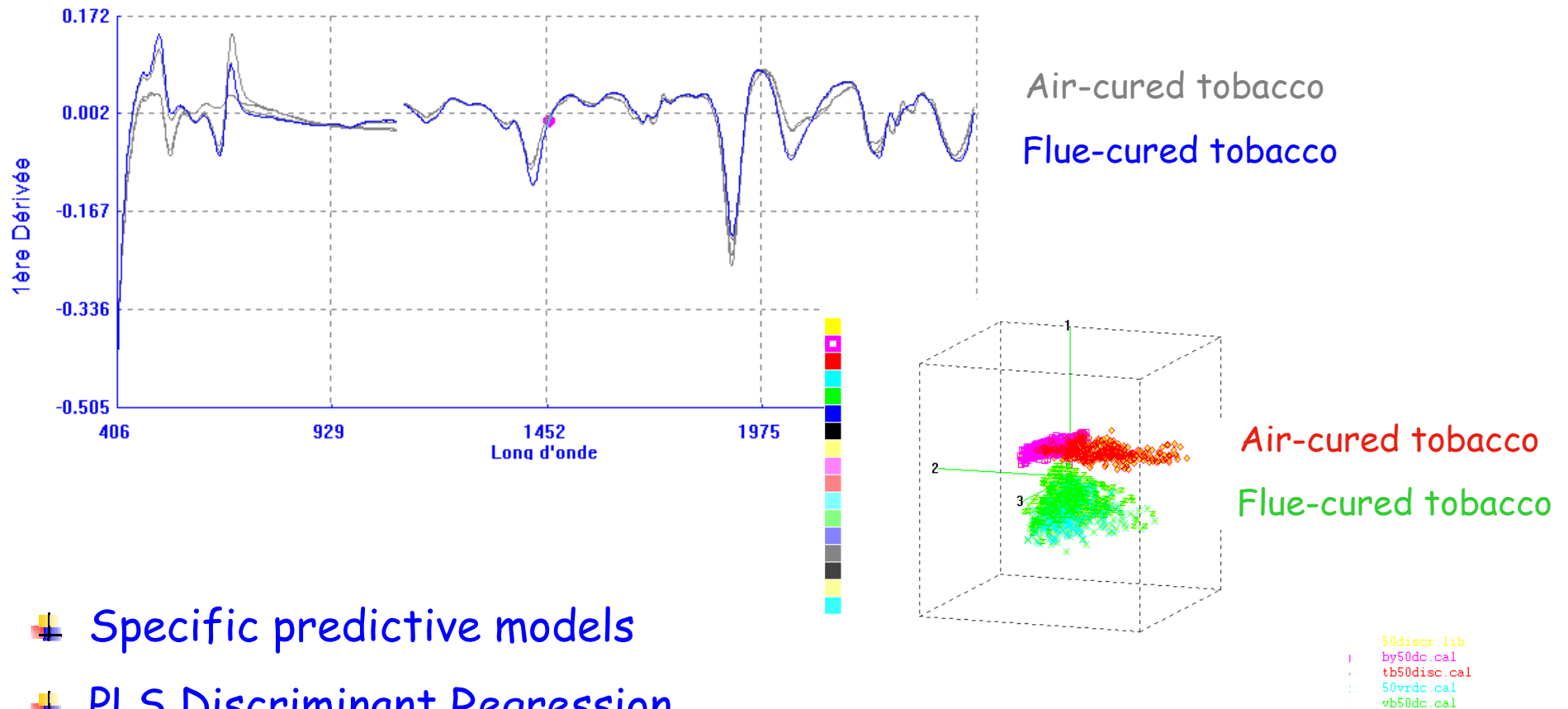
total nitrogen

and total reducing sugar levels

in tobacco.

# Setting up of a discriminant equation

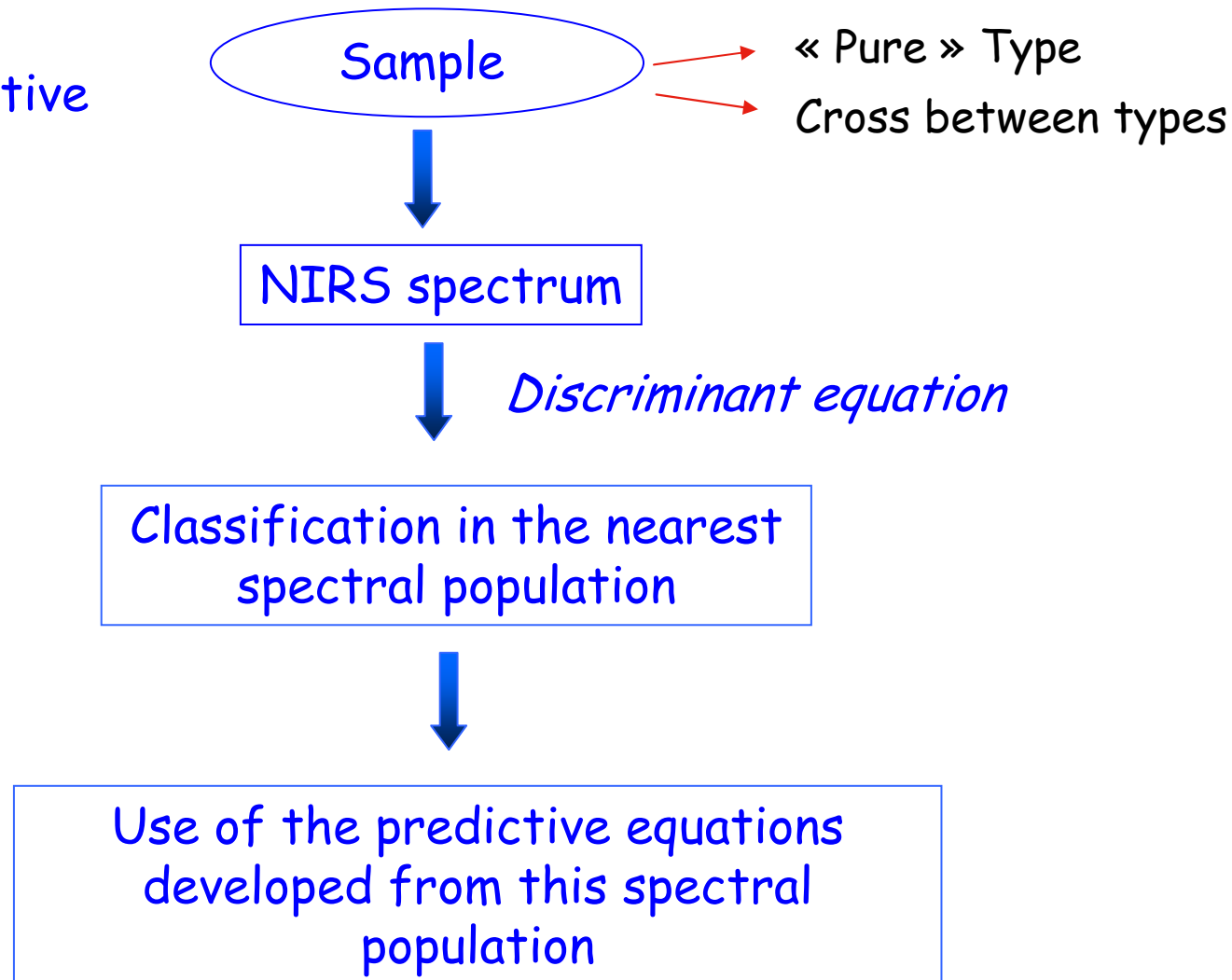
- Spectral profiles different according to tobacco types



- Specific predictive models
- PLS Discriminant Regression from 1756 spectra (Calibration files)

# Setting up of a discriminant equation

± Objective



# Other results : chemical content

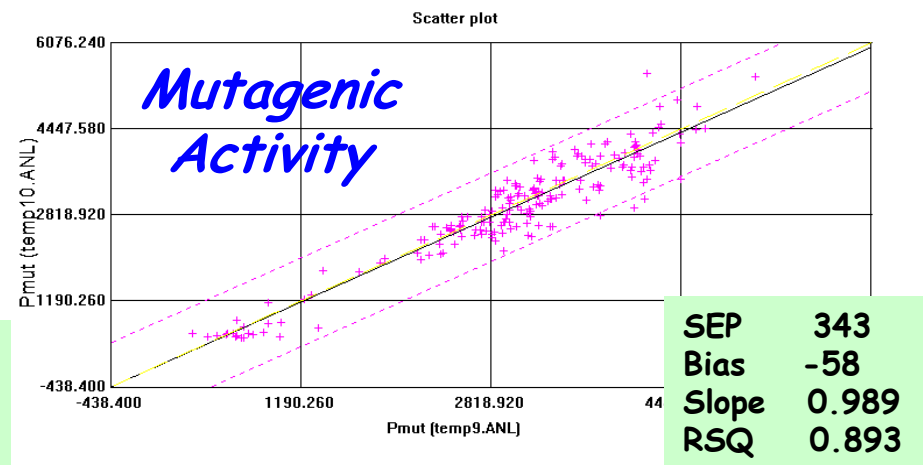
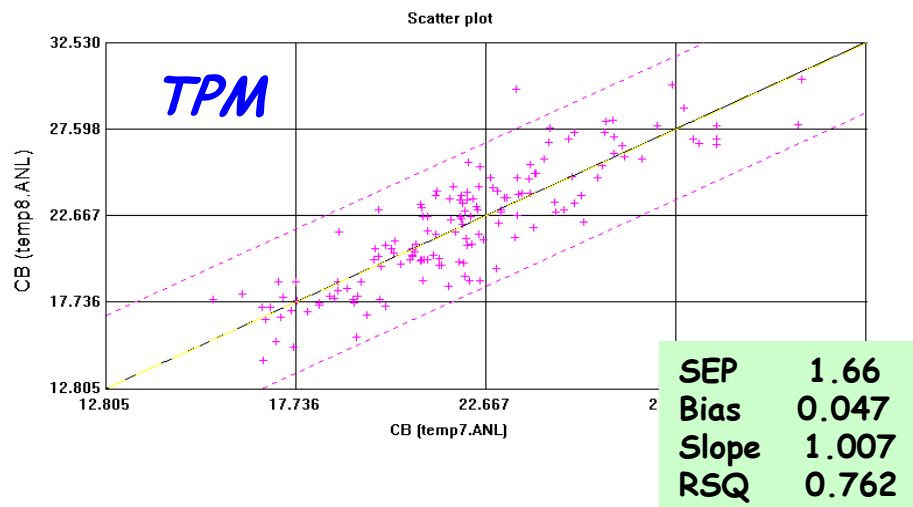
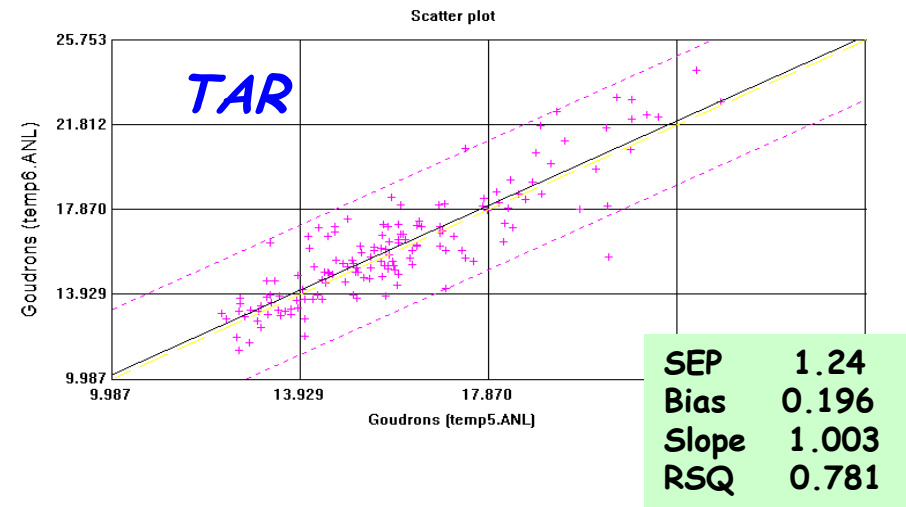
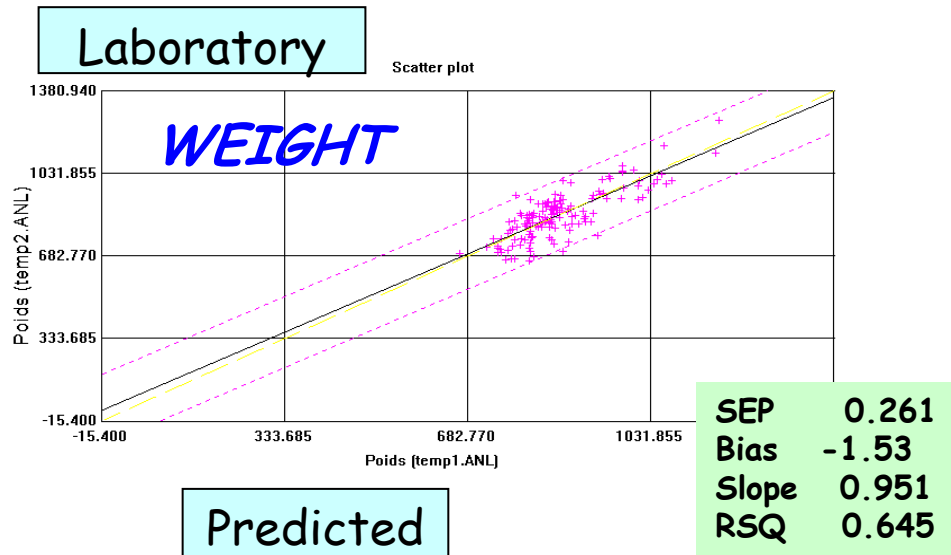
	Nicotine	NN/A	NH <sub>3</sub>	ASN	AT	CIT	EP	CI	MAL	PP	PRO
FC				x	x		x		x	x	x
DK			x		x	x	x		x		
BY	x	x	x	x	x	x	x	x			

# Other results : cigarette

## ✚ Successfull calibrations :

- tar  
(mg / cigarette)
- tobacco smoke condensate  
(mg / cigarette)
- tobacco weight  
(mg /cigarette)
- mutagenic activity  
(number of revertants / mg tobacco smoke  
condensate)

# Application of the models on validation files



# In progress

- ✚ Stem percent
- ✚ Tobacco filling power
- ✚ Burning capacity
- ✚ Seeds ...



# Advantages of the technique (1)

- ✦ Fast (2 mn / scan)
- ✦ Rapid results from a sample set
- ✦ Allows the screening of a large number of samples
- ✦ Multiplicity of predictions from one sample scan  
(spectra can later be used for predictions when new models are developed)
- ✦ Systematic prediction from each inbred line and variety



## Advantages of the technique (2)

- ✚ Simple to perform (sample preparation)
- ✚ Non-destructive
- ✚ Safe (no extraction)
- ✚ Inexpensive
- ✚ Possible standardization for calibration transfer



# Disadvantages of the technique

- ✚ Cost of the NIRSystem
- ✚ Calibration step
- ✚ Annual enrichment to include maximum variability among samples used for calibration
- ✚ Annual controls to be continued
- ✚ Unsuccessful results

# Conclusion

- ✚ Perfectly well-adapted to tobacco breeding in order to improve tobacco leaf quality.

# Prospects : Working with fresh or dried leaves

## Portable NIRSystem :

- ± 7.5 kg
- ± Wheeled carrier for transport
- ± Internal batteries
- ± High intensity contact probe with flexible cable



LabSpec Pro  
(FONDIS Electronic,  
Analytical Spectral  
Devices Inc. (USA))



- ± 0,1 s scan time

# Prospects : Working with fresh or dried leaves





✚ Thank you for your attention !