

Evaluation of potato crop nitrogen status based on leaf chlorophyll and leaf flavonoids contents assessed by hand-held fluorimeters Dualox and Multiplex



Presentation

- 1 Basis to improve crop nitrogen management (Why?, How ?)**
- 2 Global objectives**
- 3 Experimental design and protocol (Optical measurements and plant sampling)**
- 4 Pertinence of using relative indices (Vs absolute indices)**
- 5 Comparison of the relative indices provided by the different optical devices**
- 6 Conclusion**

The evaluation of in-season crop nitrogen status improve crop nitrogen management and nitrogen fertilizer efficiency

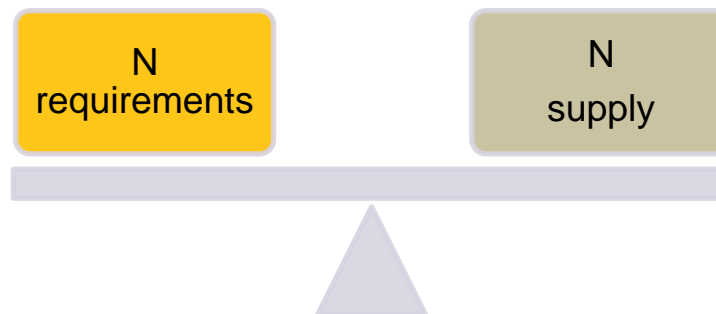
Why?

1. For all the known consequences of Nitrogen (N) fertilization on:
 - Tuber yield and quality (dry matter content, tuber size, ...)
 - Risks to the environment (N losses)
 - Economic loss for producers (fertilizer's prices)
2. The establishment of provisional field-specific N recommendation can never be accurate !
(influenced by several unpredictable factors)

Strategy to improve crop nitrogen management

1. Apply 70% of the N recommended rate

N balance sheet method



2. Monitor the crop for in-season N status assessment



3. Apply supplemental N if required according to monitoring

Evaluation of in-season crop N status

How?

Potato N monitoring implies the use of plant-based indicators:

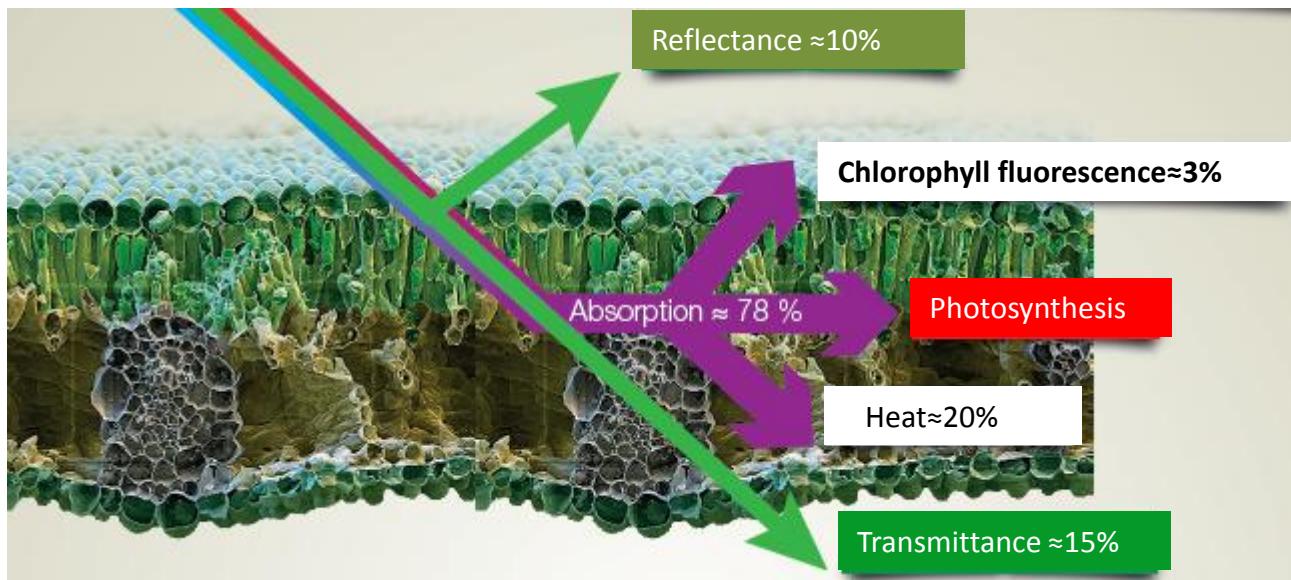
The Nitrogen Nutrition Index (NNI): likely the best indicator of crop N status

•requires destructive sampling !  But •long delay to get result !

Can be used as a reference to calibrate other
easier, quicker and non-destructive optical methods

Evaluation of in-season crop N status

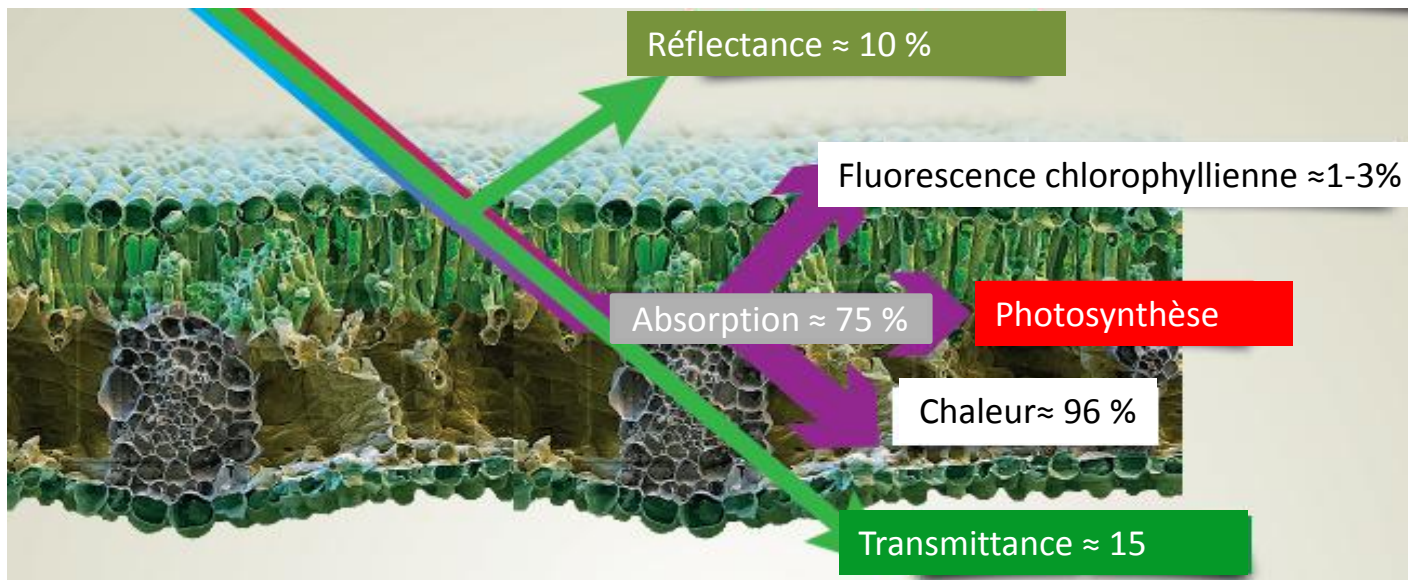
Optical methods are currently based on spectral properties of the leaf



The % reflectance, transmittance, fluorescence and heat dissipation are indicative values

Evaluation of in-season crop N status

Optical methods are currently based on spectral properties of the leaf



The % reflectance, transmittance, fluorescence and heat dissipation are indicative values

Evaluation of in-season crop N status

How ?

The optical methods allow the estimation of **N related indicators**

1. **Chlorophyll content**: highly correlated to N status

- Leaf transmittance: hand held chlorophyll-meters

But: Chlorophyll-meters can detect only deficiency situations for N !

- Crop light reflectance: Ground-based radiometers

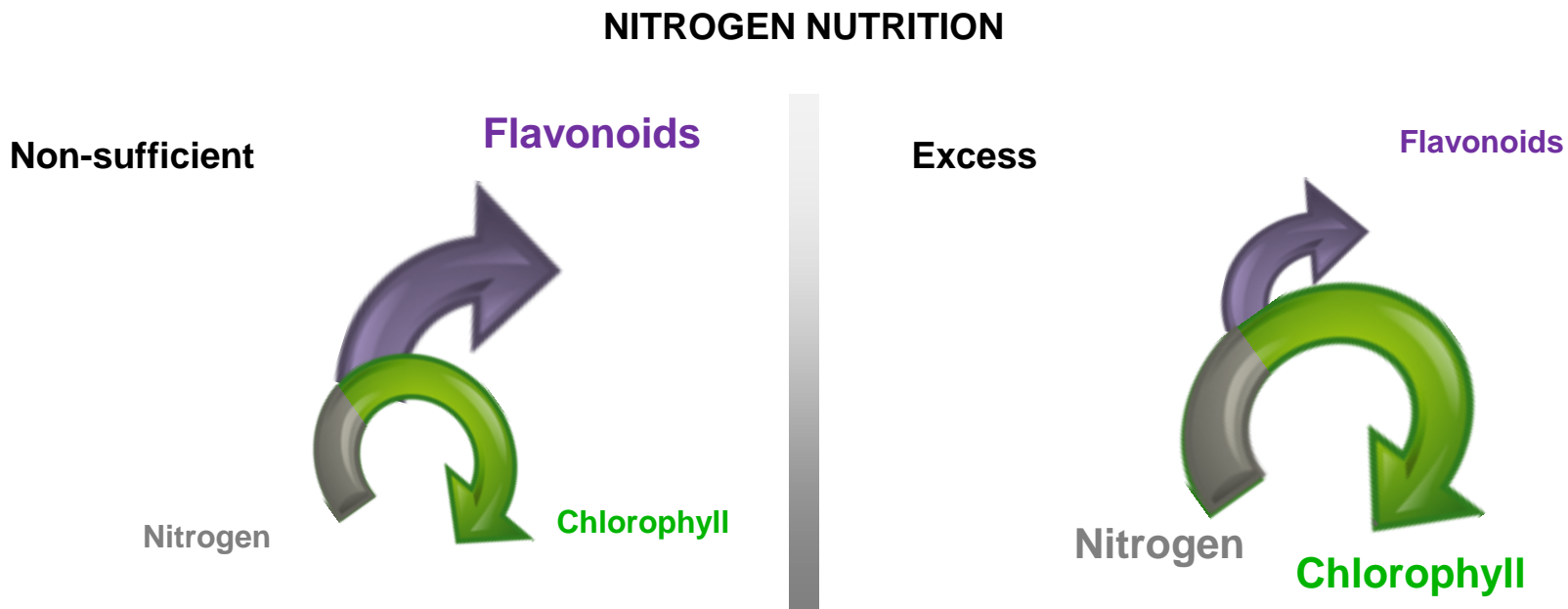
But: Interferences such as soil reflectance should be taken into account !

2. **Flavonoids content**: potential indicators of N status

- Leaf fluorescence: fluorimeters

How?

3. Chlorophyll and Flavonoids content



The combined ratios should improve the discrimination between N treatments due to the opposite dependance of **chlorophyll** and **flavonoids** with N status

Global objective

The objective of the current study was to evaluate the potentialities of leaf flavonoids content considered alone or combined to leaf chlorophyll content for the evaluation of potato crop N status.

3 criteria were evaluated:

- ① The sensitivity of the studied index,
- ② The earliness of the diagnosis,
- ③ The accuracy (precision and repeatability) of the index.

Experimental design

2 trials on 2012 et 2013

- 2 potato cultivars: Bintje and Charlotte
- 6 increasing N rates
0, 50, 100, 150, 200, 250 kg N/ha
- SPLIT PLOT bloc (4 replications)
- Weekly observations dates with optical devices and plant sampling



Optical data collection

Transmittance & Chlorophyll fluorescence



Dualex fluorimeter
(Force-A, Orsay, Paris, France)

CHL index
Chlorophyll

FLV index
flavonoids

NBI index
Chlorophyll
flavonoids

Chlorophyll fluorescence

Multiplex fluorimeter
(Force-A, Orsay, Paris, France)



SFR-G index
chlorophyll

SFR-R index
chlorophyll

FLAV index
flavonoids

NBI-R index
Chlorophyll
flavonoids

Transmittance



HNTChlorophyll-meter
(Yara, Oslo, Norway)

HNT index
chlorophyll

Reflectance

Cropscan radiometer
(Cropscan Inc, Rochester, USA)



G index
chlorophyll

R index
chlorophyll

NIR index
chlorophyll

VI's
chlorophyll



HNT& Dualex
or HNT& Multiplex

HNT/FLV index
Chlorophyll
flavonoids

HNT/FLAV index
Chlorophyll
flavonoids

Optical measurements were carried weekly during the potato growth (mid of June to end of July). Measurements dates are expressed as Day After Emergence: DAE

Samples collection and analysis



Plant samples were collected periodically during the growing cycle allowing the determination of:

- Total biomass
- Tuber yield

Plant tissue sub-samples were subjected to laboratory analyses allowing the determination of:

- Total N for different parts of the plant
- Total N-uptake
- NNI

Absolute indices Vs. Relative indices

Nitrogen effect (N) – Cultivar effect (Cv): combining 2012 and 2013 trials

Optical devices	Indices	Absolute Indices		250N Relative Indices (trt x / trt 250N)		0N Relative Indices (trt x /trt 0N)	
		N	V	N	V	N	V
Dualex	CHL	***	NS	***	NS	***	NS
	FLV	***	NS	***	NS	***	NS
	NBI	***	NS	***	NS	***	NS
Multiplex	SFR-G	NS	NS	NS	NS	NS	NS
	SFR-R	***	***	***	NS	NS	NS
	FLAV	***	**	***	NS	***	NS
	NBI-G	***	**	***	NS	NS	NS
	NBI-R	***	***	***	NS	***	NS
Hydro N-tester	HNT	***	***	***	NS	***	NS
Cropscan	G	**	NS	***	NS	NS	NS
	R	***	**	***	NS	***	NS
	NIR	***	NS	***	NS	***	NS
	NormG	***	*	***	NS	***	NS
	NormR	***	**	***	NS	***	NS
	NormNIR	***	***	***	NS	***	NS
	DVI-GDVI	***	NS	***	NS	***	NS
	RVI	***	*	***	NS	***	NS
	GRVI	***	**	***	NS	***	NS
	NDVI-GNDVI	***	***	***	NS	***	NS
	SAVI-OSAVI	***	***	***	NS	**	NS
	GSAVI-GOSAVI	***	**	***	NS	***	NS
Hydro N-tester et Dualex	SPAD/FLV	***	**	***	NS	***	NS
Hydro N-tester et Multiplex	SPAD/FLAV	***	*	***	NS	***	NS

*, **, and *** : statistical significance respectively at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$. NS : no significance at $P > 0.05$

0N Relatives indices Vs. 250 N Relative indices

Nitrogen effect (N) – Year effect (Yr): combining 2012 and 2013 trials

Optical devices	Indices	0N Relative Indices (trt x / trt 0N)				250N Relative Indices (trt x / trt 250N)			
		N		Yr		N		Year	
		Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F
Dualetx	CHL	***	106.4	**	124.86	***	96.59	**	81.61
	FLV	***	444.05	**	102.15	***	324.01	**	35.29
	NBI	***	196.59	***	174.55	***	253.45	**	77.39
Multiplex	SFR-G	NS	0.9	NS	2.2	NS	0.66	NS	0.12
	SFR-R	NS	1.37	NS	0.83	***	61.26	NS	3.13
	FLAV	***	164.29	**	66.99	***	164.94	**	89
	NBI-G	NS	2.52	NS	1.58	***	86.42	*	12.35
	NBI-R	***	10.75	**	12.77	***	246.34	**	88.14
Hydro N-tester	HNT	***	97.9	**	128.7	***	157.5	*	12.79
Cropscan	G	NS	1.21	***	2771.66	***	21.44	NS	0.81
	R	***	11.54	**	168.22	***	65.91	NS	0.38
	NIR	***	14.36	**	56.03	***	53.09	NS	0.67
	NormG	***	20.81	**	79.31	***	139.41	*	10.19
	NormR	***	17.67	**	125.64	***	79.76	NS	0.01
	NormNIR	***	14.3	**	95.9	***	105.72	NS	0.68
	DVI	***	14.46	**	68.69	***	57.3	NS	0.24
	GDVI	***	16.68	**	103.4	***	63.2	NS	1.38
	RVI	***	15.41	**	53.41	***	51.84	NS	0.29
	GRVI	***	34.35	**	87.42	***	122.27	NS	5.9
	NDVI	**	5.89	*	21.91	***	61.31	NS	0.1
	GNDVI	***	14.01	**	130.65	***	114.21	NS	4.25
	SAVI	**	6.03	*	22.5	***	61.42	NS	0.09
	OSAVI	**	5.93	*	22.1	***	61.35	NS	0.1
	GSAVI	***	14.25	**	132.2	***	113.19	NS	4.31
	GOSAVI	***	14.09	**	131.15	***	113.88	NS	4.27
Hydro N-tester et Dualetx	HNT/FLV	***	238.48	**	166.22	***	291.32	**	35.19
Hydro N-tester et Multiplex	HNT/FLAV	***	237.73	***	626.8	***	235.24	**	55.23

*, **, and *** : statistical significance respectively at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$. NS : no significance at $P > 0.05$

Illustration of the Sensitivity, the Earliness and the Accuracy of the flavonoids indicators combined or not to the chlorophyll indicators



Studied criteria

1 Sensitivity

2 Earliness of the diagnosis

3 Accuracy

Precision

Repeatability

Statistical analysis

***** N effect combining 2012 and 2013 trials**

Good discrimination on 2012 and 2013 between all experimented N rates

** or *** N effect per DAE and per cultivar





** or *** N effect at the first DAE (15 DAE on 2012 and 9 DAE on 2013)

High Pearson correlation coefficient (***) between the index and NNI

Good repeatability estimated by the coefficient of variation between the 4 replications

1 The sensitivity of the studied index

Responses of relative indices to the increasing nitrogen including all measurement dates and the two cultivars (combining 2012 and 2013 trials)

Optical devices		Relative indices (trt x/trt 250 N)	N	
			P	F value
Dual ex		CHL	***	96.59
		FLV	***	324.01
		NBI	***	253.45
Multi plex		SFR-G	NS	0.66
		SFR-R	***	61.26
		FLAV	***	164..94
		NBI-G	***	86.42
		NBI-R	***	246.34
Hydro N-tester		HNT	***	157.5
		G	***	21.44
Crop scan		R	***	65.91
		NIR	***	53.09
		NormG	***	139.41
		NormR	***	79.76
		NormNIR	***	105.72
		DVI	***	57.3
		GDVI	***	63.2
		RVI	***	51.84
		GRVI	***	122.27
		NDVI	***	61.31
		GNDVI	***	114.21
		SAVI	***	61.42
		OSAVI	***	61.35
		GSAVI	***	113.19
		GOSAVI	***	113.88
Hydro N-tester&Dual ex		HNT/FLV	***	291.32
Hydro N-tester & Multi plex		HNT/FLAV	***	235.24

FLV >
 HNT/FLV >
 NBI >
 NBI-R >
 HNT/FLAV >
 FLAV >

HNT>

NormG >
 GRVI >
 GNDVI >
 GOSAVI >
 GSAVI >
 NormNIR >

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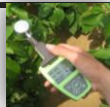



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1 The sensitivity of the studied index

Responses of relative indices to the increasing nitrogen on all measurement dates and for the two cultivars (combining 2012 and 2013 trials)

Optical devices		Relative indices (trt x/trt 250 N)	N		Tested N rates (kg N/ha)				
			P	F value	0	50	100	150	200
Duallex		CHL	***	96.59	d	c	b	a	a
		FLV	***	324.01	a	b	c	d	e
		NBI	***	253.45	e	d	c	b	a
Multiplex		SFR-G	NS	0.66	a	a	a	a	a
		SFR-R	***	61.26	d	c	b	a	a
		FLAV	***	164..94	a	b	c	d	d
		NBI-G	***	86.42	d	c	b	a	a
		NBI-R	***	246.34	e	d	c	b	a
Hydro N-tester		HNT	***	157.5	e	d	c	b	a
Cropscan		G	***	21.44	a	b	b	b	b
		R	***	65.91	a	b	c	c	c
		NIR	***	53.09	c	b	a	a	a
		NormG	***	139.41	a	b	c	dc	d
		NormR	***	79.76	a	b	c	c	c
		NormNIR	***	105.72	c	b	a	a	a
		DVI	***	57.3	c	b	a	a	a
		GDVI	***	63.2	c	b	a	a	a
		RVI	***	51.84	c	b	a	a	a
		GRVI	***	122.27	d	c	b	ba	a
		NDVI	***	61.31	c	b	a	a	a
		GNDVI	***	114.21	c	b	a	a	a
		SAVI	***	61.42	c	b	a	a	a
		OSAVI	***	61.35	c	b	a	a	a
		GSAVI	***	113.19	c	b	a	a	a
		GOSAVI	***	113.88	c	b	a	a	a
Hydro N-tester&Duallex		HNT/FLV	***	291.32	a	b	c	d	e
Hydro N-tester & Multiplex		HNT/FLAV	***	235.24	a	b	c	d	e

*, **, and *** : statistical significance respectively at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$. NS : no significance at $P > 0.05$.

Indices with the same letter are not significantly different

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[illegible]

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3 The accuracy of the index: Precision

**Pearson correlation coefficient between NNI and the studied index
per year and per cultivar N = 80**

		Dualex			Multiplex			Cropscan										HNT	HNT & Dualex	HNT & Multiplex	
		Chl	Flv	NBI	SFR_G	SFR_R	FLAV	NBI_G	NBI_R	R	NIR	NorrmG	NormR	NormNIR	RVI	GRVI	GNDVI	GOSAVI	HNT	HNT/FLV	HNT/FLAV
NNI	Bintje 2012	0.6 ***	-0.6 ***	0.7 ***	0.2 NS	0.3 *	-0.6 ***	0.5 ***	0.7 ***	-0.5 ***	0.4 ***	-0.7 ***	-0.6 ***	0.6 ***	0.6 ***	0.6 ***	0.6 ***	0.6 ***	0.7 ***	0.7 ***	0.8 ***
	Charlotte 2012	0.6 ***	-0.7 ***	0.7 ***	0.1 NS	0.1 NS	-0.5 ***	0.5 ***	0.6 ***	-0.3 **	0.4 **	-0.1 NS	-0.5 ***	0.2 *	0.5 ***	0.0 NS	0.0 NS	0.0 NS	0.6 ***	0.7 ***	0.6 ***
	Bintje 2013	0.7 ***	-0.7 ***	0.7 ***	0.3 *	0.6 ***	-0.7 ***	0.7 ***	0.8 ***	-0.6 ***	0.6 ***	-0.7 ***	-0.7 ***	0.6 ***	0.7 ***	0.7 ***	0.5 ***	0.6 ***	0.8 ***	0.8 ***	0.8 ***
	Charlotte 2013	0.6 ***	-0.8 ***	0.7 ***	0.0 NS	0.3 **	-0.7 ***	0.6 ***	0.7 ***	-0.6 ***	0.7 ***	-0.6 ***	-0.7 ***	0.5 ***	0.6 ***	0.6 ***	0.5 ***	0.5 ***	0.7 ***	0.8 ***	0.8 ***

r = -1

r = 0

r = 1

r = -1 r = 0 r = 1

*, **, and ***: statistical significance respectively at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$. NS : no significance at $P > 0.05$.

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3 The accuracy of the index: Repeatability

Evolution of the coefficient of variation of the studied indices between the 4 replications (combining all the N levels)

	Dates	Chl	Flv	NBI	SFR	GSFR	R	FLAV	NBI	GNBI	R	G	R	NIR	HNT	HNT/FLV	HNT/FLAV
cv (%)	Bintje	20/6/12	3.9	6.4	6.8	1.7	1.3	5.5	7.9	7.4	5.1	5.9	4.5	2.0	4.1	4.1	
		26/6/12	3.9	5.3	2.1	4.4	2.8	5.5	2.6	5.2	8.1	10.9	3.0	1.4	4.3	4.5	
		3/7/12	3.5	3.6	4.4	2.9	0.6	1.7	1.8	3.3	7.2	3.9	3.1	0.8	2.6	0.9	
		10/7/12	2.2	2.6	4.3	1.1	0.9	0.6	2.1	0.9	16.8	4.8	6.4	1.1	2.1	0.8	
		17/7/12	3.4	2.1	3.6	4.4	2.8	3.3	11.7	9.5	3.4	8.1	5.0	1.0	1.6	2.7	
		24/7/12	5.0	2.6	7.2	34.5	3.1	8.6	35.7	6.4	5.2	6.7	6.4	1.3	3.6	9.4	
	Charlotte	14/6/12	8.3	2.8	7.1	1.9	1.8	5.9	5.7	6.4	7.4	2.8	4.8	1.9	1.6	4.9	
		19/6/12	5.5	1.4	5.6	1.7	1.2	5.5	7.6	7.7	1.8	2.1	1.8	2.4	2.4	3.1	
		25/6/12	3.5	3.6	2.2	1.1	0.9	6.8	8.8	9.9	6.2	3.1	2.0	2.3	2.6	4.6	
		2/7/12	4.2	5.1	8.8	4.0	1.2	4.2	4.4	8.2	1.9	4.3	3.3	1.1	4.3	3.7	
		9/7/12	6.8	5.0	11.5	6.1	2.7	4.7	6.6	8.7	10.0	8.1	7.3	0.2	5.1	4.8	
		16/7/12	5.4	4.1	9.5	3.4	1.2	2.1	2.7	3.6	37.9	9.6	7.3	0.7	3.4	1.9	
23/7/12	4.8	6.6	10.5	21.9	2.4	10.6	19.4	8.2	4.2	4.3	5.9	1.8	7.6	10.8			
cv (%)	Bintje	14/6/13	2.6	3.0	5.1	3.2	1.2	2.5	1.9	2.6	4.1	4.8	1.8	1.4	1.9	1.6	
		20/6/13	9.4	0.4	9.8	6.7	3.1	7.0	10.2	6.8	4.2	4.1	2.2	2.3	2.8	5.5	
		26/6/13	3.4	3.4	6.4	4.5	2.7	3.3	4.2	2.9	5.2	5.2	3.0	1.5	2.5	2.1	
		4/7/13	3.8	2.5	5.7	0.8	0.4	4.4	5.6	7.0	2.1	3.4	2.1	0.9	2.7	4.4	
		10/7/13	5.8	3.2	8.5	3.3	2.3	2.5	6.7	6.2	2.7	3.4	3.6	1.0	2.4	2.5	
		18/7/13	3.8	2.4	5.8	4.2	2.6	2.5	9.5	6.4	1.5	3.0	3.9	1.1	3.1	3.7	
	24/7/13	3.3	4.5	7.6	6.6	3.4	2.8	3.0	6.3	11.1	4.1	5.2	1.5	4.3	3.0		
	Charlotte	11/6/13	6.3	2.8	9.0	8.3	3.8	1.0	8.8	4.7	2.3	6.0	3.1	3.8	3.6	3.7	
		19/6/13	3.4	0.6	4.0	2.3	0.8	2.1	2.7	4.7	1.5	3.3	2.8	1.5	2.0	2.3	
		25/6/13	2.9	0.9	2.3	2.8	1.5	1.2	4.0	3.4	16.4	4.6	4.2	1.9	2.3	3.5	
		1/7/13	4.1	1.5	4.4	5.3	1.6	1.3	4.7	2.0	1.8	1.7	2.3	1.4	0.5	1.5	
		8/7/13	7.0	2.3	7.2	10.0	5.3	1.8	10.1	4.9	10.7	11.2	11.0	1.8	2.0	2.4	
15/7/13		5.6	0.9	3.9	8.7	1.3	0.9	12.7	4.8	4.9	2.1	2.8	1.0	1.6	2.1		
22/7/13	5.9	2.0	7.8	14.1	5.2	0.7	13.1	7.8	7.9	5.4	5.2	1.4	2.8	3.2			

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Precision

Repeatability

FLV(Dualex) , HNT/FLV (Hydro N-tester & Dualex), HNT/FLAV (Hydro N-tester & Multiplex) in 2012 and FLV, NBI (Dualex), HNT/FLAV indices in 2013 were able to meet all the requirements successfully and were selected as provisionally promising indices from this study.

Conclusion

The indices based on leaf flavonoids content assessed by fluorimetry, and combined or not with the assessed leaf chlorophyll content as more relevant to assess potato CNS.

The pertinence of using the 250N relative indices.

Following steps:

Study further criteria (specificity)

Establishment of critical values of chlorophyll and flavonoids indicators for decision on supplemental N need.