Establishing the Nitrogen Dilution Curve for Potato Cultivar Bintje in Belgium

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Abstract The nitrogen nutrition index (NNI) is recognized as a reliable plant-based method for diagnosing the crop nitrogen status (CNS). The NNI is based on the concept of critical nitrogen (Nc) dilution curve describing the Nc concentration in the whole plant as a function of the total crop biomass (W). The objectives of this study were (i) to establish the specific potato Nc dilution curve for cv. Bintje under Belgian growing conditions; (ii) to assess if the established curve for cv. Bintje could be used for cv. Charlotte; (iii) to compare the established curve with the existing Nc potato curves; and (iv) to assess the possibility of using the obtained Nc curve to evaluate the potato CNS and to predict the final yield. Field experiments studying increasing nitrogen (N) rates were conducted at different locations in Belgium for several years. Biomass N concentration and W production were determined at different sampling dates, and the final tuber yield was determined at harvest. At a sampling date, the Nc was determined by selecting the data point from the treatment presenting the highest W. The NNI was calculated as the ratio between the measured N concentration in W and the predicted Nc according to the Nc dilution curve. The Nc curve (Nc (%) = 5.37 $W^{-0.45}$) was developed for potato for cv. Bintje under Belgian conditions matching also for cv. Charlotte. The Belgian Nc curve presented similar values as the curves developed previously in Scotland and the Netherlands. NNI was related to relative tuber yield (ratio between the tuber yield obtained for a given N rate and the highest tuber yield obtained among all N application rates). The Nc curve and the NNI adequately identified situations of limiting and non-limiting N nutrition and could be used to establish the potato CNS and to predict the final yield.

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Abbreviations
CNS   Crop nitrogen status
DM   Dry matter
Nc   Critical nitrogen
NNI   Nitrogen nutrition index
RY   Relative yield
W   Total biomass

Introduction

Plant-based diagnosis methods can be used to improve the efficiency of nitrogen (N) fertilizers use and reduce the risks of N losses to the environment. The nitrogen nutrition index (NNI) is recognized as a reliable, plant-based method for diagnosing the crop N status (CNS) (Justes et al. 1994; Bélanger et al. 2001; Ziadi et al. 2008; Lemaire and Gastal 2009; Li et al. 2012). The NNI was proposed to quantify the intensity of both N deficiency and luxury consumption of a given crop (Justes et al. 1994; Lemaire and Gastal 1997) and to establish its N requirements (Giletto and Echeverría 2012). The NNI is calculated as the ratio between the plant N concentration and the critical N (Nc) concentration. The Nc concentration is defined as the minimum plant N concentration allowing maximum shoot biomass (Ulrich 1952). The concept of an Nc curve based on the N concentration in whole plants was first developed by Lemaire and Salette (1984) for tall fescue (*Festuca arundinacea* Schreb.) and has been successfully applied to many crops including wheat (*Triticum aestivum* L.) (Justes et al. 1994; Ziadi et al. 2010), corn (*Zea mays* L.) (Plénet and Lemaire 2000; Ziadi et al. 2008), rapeseed (*Brassica napus* L.) (Colnenne et al. 1998), rice (*Oryza sativa* L.) (Sheehy et al. 1998) and potato (*Solanum tuberosum* L.) (Greenwood et al. 1990; Duchenne et al. 1997; Bélanger et al. 2001; Giletto and Echeverría 2012). The Nc is represented by an allometric function:

$$\text{Nc } (\%) = a\text{W}^{-b} \tag{1}$$

where W is the total biomass expressed in tons (t) of dry matter (DM) ha^{-1}, Nc is the total N concentration in the plant expressed in grams per 100 g of DM (%), and a and b are parameters to be estimated (Bélanger and Gastal 2000). The parameter a represents the N concentration at a total biomass of 1 t DM ha^{-1}, and b represents the coefficient of dilution which describes the relationship of decreasing N concentration with increasing W. In potato, due to the strong effect of N nutrition on the partitioning of DM between shoots and tubers, the values of a and b are estimated using the combined biomass of shoots and tubers and the corresponding N concentration of this combined biomass (Duchenne et al.1997; Bélanger et al. 2001; Giletto and Echeverría 2012).

For potato, parameters a and b were reported for different cultivars and under different pedo-climatic conditions. Greenwood et al. (1990) reported values of $a = 5.36$ and $b = 0.46$ for cultivar (cv.) Voran and cv. Eigenheimer (data from

Scotland and the Netherlands); Duchenne et al. (1997) reported values of $a = 5.21$ and $b = 0.56$ for cultivars Bintje, Kaptah Vandel, Russet Burbank and Saturna (data from France); Bélanger et al. (2001) reported values of $a = 5.04$ and $b = 0.42$ for cv. Shepody and values of $a = 4.57$ and $b = 0.42$ for cv. Russet Burbank (data from Canada); and Giletto and Echeverría (2012) reported values of $a = 5.30$ and $b = 0.42$ for cv. Innovator (data from Argentina). For biomass less than 1 t ha^{-1}, Nc takes a constant value due to its small decline with increasing W (Justes et al. 1994) and the lack of competition for light of isolated plants (Lemaire and Gastal 1997). For potato, Duchenne et al. (1997) reported a constant value for W less than 1.35 t ha^{-1} which coincides with the beginning of competition between plants for light and also with the time of tuber initiation. Greenwood et al. (1990), Duchenne et al. (1997) and Giletto and Echeverría (2012) based their estimation of the Nc curve on data with a range of W up to 12–15 t ha^{-1}. Bélanger et al. (2001) selected W ranging from 1 to 6 t DM ha^{-1} to avoid measurements with senescence towards the end of the growth cycle.

To our knowledge, no critical curve was established in Belgium for the potato crop. The objectives of this study were (i) to establish the specific potato Nc dilution curve for cv. Bintje under Belgian growing conditions; (ii) to verify if the established curve for Bintje could be used for cv. Charlotte; (iii) to compare the established curve with the existing Nc curves for potato established in other countries; and (iv) to assess the possibility of using the obtained Nc curve to evaluate the potato CNS and to predict the final yield.

Materials and Methods

Field Experiments

Experiments in various regions of Belgium were used to estimate the Nc curve. These experiments consisted of several previous trials implemented in the framework of larger studies conducted at the Walloon Agriculture Research Centre (from 1997 to 2000, in 2004 and from 2010 to 2014). Trials included two cultivars: Bintje and Charlotte. Cultivar Bintje was chosen as it is the most important cv. in Belgian agriculture. In order to verify if the established curve could also be used for other cultivars with shorter growing season, cv. Charlotte was also used in this study as it has an early growing cycle compared to mid-early growing cycle for Bintje. Table 1 summarizes the site characteristics of the experiments. The experimental design for the period 1997–2010 was a randomized block scheme with increasing N-fertilizer rates. From 2011 to 2014, the design was a split-plot block scheme with cultivars Bintje and Charlotte as main plots and N rates as sub-plots. Ammonium nitrate fertilizer (solid granules, 27% N + 4% MgO) was broadcasted and incorporated just before planting. Phosphorus (P) and potassium (K) fertilizers were applied before planting at rates around 70–80 kg P_2O_5 ha^{-1} and 250–300 kg K_2O ha^{-1}. Trials were rain fed, and no period of significant drought stress was observed for those trials.

Analytical Methods

Plant samples were collected for all the sites for different sampling dates (except for the site Roisin 2000 for which there was only one sampling date) (Table 1). Eight plants per

Table 1 Soil and crop characteristics of the experimental sites in Belgium used for the calibration and the validation (*) of the critical N (Nc) curves

Year	Cultivar	Site	Soil organic matter (%)	Soil mineral N before plantation (kg ha⁻¹) (0–60 cm)	Soil type	Rainfall from April to August (mm)	Planting date	Crop emergence date	Applied N (kg ha⁻¹)	Plant sampling dates
1997	Bintje	Gembloux	2.2	29	Silt loam	339	8/4	23/5	0–75–113–150–188	12/6–24/6–3/7–16/7–30/7–12/8
	Bintje	Masnuy	2.3	72	Sandy loam	344	3/4	20/5	0–58–87–115–144	10/6–1/7–24/7
1998	Bintje	Gembloux	2.1	27	Silt loam	449	4/5	21/5	0–88–132–176–220	22/6–1/7–13/7–20/8
	Bintje	Gouy*	1.9	55	Sandy loam	429	23/4	25/5	0–88–132–176–220	24/6–7/7–15/7–25/8
1999	Bintje	Franière	2.6	26	Loam	322	4/5	25/5	0–119–170–221	28/6–27/8
	Bintje	Gembloux*	1.6	35	Silty clay loam	381	4/5	25/5	0–119–170–221	14/6–24/6–8/7–19/7–30/8
	Charlotte	Gembloux	1.6	35	Silty clay loam	381	4/5	26/5	0–84–120–156	6/7–22/7–5/8
	Bintje	Incourt	1.8	33	Loam	289	6/5	25/5	0–112–160–208	28/6–23/8
	Bintje	Marcq	1.8	7	Loam	328	4/5	25/5	0–105–150–195	1/7–18/8
	Bintje	Pontillas	1.5	13	Loam	374	2/5	25/5	0–88–125–163	28/6–16/8
2000	Bintje	Gembloux*	2.0	33	Silty clay loam	431	11/4	15/5	0–102–145–189	15/6–29/6–13/7–16/8
	Bintje	Longpré	2.2	27	Loam	458	14/4	14/5	0–98–140–182	09/8
	Bintje	Roisin	2.2	27	Loam	445	13/4	6/6	0–102–145–189	19/7
2004	Bintje	Gembloux	2.2	26	Silt loam	332	16/4	21/5	0–100–140–180–240	14/6–21/6–28/6–5/7–12/7–19/7–27/7–9/8
	Bintje	Gembloux	1.6	36	Silt loam	375	26/4	26/5	0–115–165–215–248	14/6–28/6–5/7–12/7–26/7
2010	Charlotte	Gembloux	1.6	36	Silt loam	375	26/4	3/6	0–100–140–180–210	17/6–1/7–8/7–15/7–19/7
2011	Bintje	Gembloux	1.7	39	Silt loam	303	19/4	19/5	0–98–140–180–210	14/6–6/7–20/7–25/7
	Charlotte	Gembloux	1.7	39	Silt loam	303	19/4	19/5	0–84–120–156–180	20/6–4/7–11/7–18/7
2012	Bintje	Gembloux	2.2	46	Silt loam	342	13/5	2/6	0–50–100–150–200–250	27/6–4/7–11/7–25/7

Table 1 (continued)

Year	Cultivar	Site	Soil organic matter (%)	Soil mineral N before plantation (kg ha^{-1}) (0–60 cm)	Soil type	Rainfall from April to August (mm)	Planting date	Crop emergence date	Applied N (kg ha^{-1})	Plant sampling dates
	Charlotte	Gembloux	2.2	46	Silt loam	342	13/5	1/6	0–50–100–150–200–250	27/6–2/7–9/7–16/7
2013	Bintje	Gembloux*	1.3	36	Silt loam	298	25/4	7/6	0–50–100–150–200–250	19/6–26/6–11/7–23/7
	Charlotte	Gembloux*	1.3	36	Silt loam	298	25/4	5/6	0–50–100–150–200–250	17/6–24/6–1/7–15/7
2014	Bintje	Gembloux	2.1	72	Silt loam	428	15/4	21/5	0–100–200–250	18/6–3/7–30/7
	Charlotte	Gembloux1	2.1	72	Silt loam	428	15/4	18/5	0–100–200–250	16/6–26/6–28/7
	Charlotte	Gembloux2*	2.4	39	Silt loam	428	15/4	19/5	0–100–250	24/6–8/7–15/7–23/7

Harvesting dates for Bintje were from the end of August to the beginning of October and for Charlotte from the mid of August to the end of September

plots were collected and taken to the laboratory. The harvested plots were spaced out to avoid the influence of the previous sample collection. The plants were washed, air dried and weighed in order to determine separately the shoots (leaves + stems) and tubers' fresh weights. Sub-samples of each plant part were then dried at 80 °C up to constant dry weight. The DM concentration of each sample was then calculated. W was calculated by adding the shoot biomass and tuber DM. The dried samples of shoots and tubers were finely crushed with a Cyclotec 1093 sample mill (FOSS, Tecator). The N concentration in shoots and tubers was determined with near-infrared (NIR) spectroscopy using a FOSS-NIR Systems 6500 scanning instrument (NIR-Systems, Silver Springs, MD) and calibrated using the Dumas combustion method (LECO, St. Joseph, MI, USA) and the statistical method developed by Shenk and Westerhaus (1993). The N concentration (% N) in W was calculated as

$$\%N = \frac{\left(\%N_{shoots} \times W_{shoots}\right) + \left(\%N_{tubers} \times W_{tubers}\right)}{W} \quad (2)$$

where $\%\,N_{shoots}$ and $\%\,N_{tubers}$ are the N concentrations in shoots and tubers respectively, expressed in grammes per 100 g^{-1} of DM. W is the total biomass in t DM ha^{-1}, W_{shoots} is the shoot biomass in t DM ha^{-1} and W_{tubers} is the tuber biomass in t DM ha^{-1}.

Data Processing

The collected data set for cv. Bintje was divided in a calibration and a validation data set (Table 1).

The calibration of the Nc curve requires the identification of data points for which N does not limit crop growth. Therefore, a procedure was used similar to Greenwood et al. (1990) as Duchenne et al. (1997), Bélanger et al. (2001), Ziadi et al. (2008), Giletto and Echeverría (2012) and Li et al. (2012) did. At each sampling date, for cv. Bintje, W and total biomass N concentration were analysed by analysis of variance (F test) and the LSD test (SAS 9.4). Sampling dates were not used for the determination of the Nc curve when the F value indicated no significant difference ($p \leq 0.05$) of W among the N application rates. For the remaining sampling dates, the significantly highest W obtained with any level of fertilizer and the corresponding N concentration (%) were identified using the LSD test ($p \leq 0.05$). These data points were used for the establishment of the Nc curve. Where the highest W was obtained with more than one N-fertilizer level, the data obtained with the lower N level was selected for the establishment of Nc curve. Only data with W higher than 1 t DM ha^{-1} were used for the calibration of Nc curve. The set of data was limited to the period of June and July (period between 20 and 70 days after emergence (DAE)) to avoid including measurements in the senescence period corresponding to the end of the growth cycle. The parameters of Nc curve in Eq. (1) were obtained using the SAS non-linear regression procedure (PROC NLIN). Pseudo-R^2 was used to assess model fit with the SAS non-linear procedure (Brook et al. 2008).

The validation data for cv. Bintje were also classified using the LSD test ($p \leq 0.05$). For each sampling date, a treatment with significantly lower W than the W of the treatment having received higher N rate was considered to be a limiting N condition. A

treatment with significantly higher W than the W of the treatment having received lower N rate was considered to be a non-limiting N condition. Treatments were not selected if their W was classified in more than one group.

For cv. Charlotte, due to the limited number of data points meeting the afore mentioned criteria (only four points), the Nc curve was not established. This set of data was used to verify if the established curve for cv. Bintje could also be used for cv. Charlotte by identifying the limiting N conditions and the non-limiting N conditions (LSD test at $p \leq 0.05$).

The NNI for Bintje was calculated at each sampling date as the ratio between the measured % N concentration in W (as in Eq. 2) and the predicted % Nc. The relative yield (RY) was calculated as the ratio of the tuber yield obtained for a given N rate with the highest tuber yield obtained among all N application rates.

The relationship between NNI and RY was defined for three periods of the crop growth season. The three functions were estimated using the SAS quadratic plateau procedure (PROC NLIN). The first quadratic function took into account the average of the NNI values across all sampling dates (Ziadi et al. 2010; Li et al. 2012). The second function took into account the average of the NNI values across the period between 20 and 55 days after emergence (DAE). This period corresponds to the optimal period for assessment of CNS as previously stated by Olivier et al. (2006). The third function took into account the NNI calculated for the last sampling dates for each site (dates between 44 and 94 DAE). The last sampling date corresponded to the available one closest to the harvesting date. This third period is similar to that used by Chambenoit et al. (2004) (NNI measured just before haulm destruction) and Giletto and Echeverría (2012) (NNI measured at tuber bulking) for establishing the relationship between RY and NNI.

Results and Discussion

Total Crop Biomass Production and Nitrogen Concentration

The W production during the growing season ranged from 0.33 to 12.71 t DM ha^{-1} for Bintje and from 0.46 to 12.83 t DM ha^{-1} for Charlotte depending on N applications rates, sampling dates, site and year (Table 2). Except for some situations (particularly at the beginning of the growing cycle), W generally increased with increasing N rates. This effect, not always statistically significant, was observed especially by comparing the non-fertilized treatments to N fertilized treatments.

The change of crop N concentration with time for Bintje in 1998 at Gembloux is shown as an example (Fig. 1). Crop N concentrations varied from a maximum of 5.23% of DM (observed for the 176 N rate at 22/6) to a minimum of 0.88% of DM (observed for the 0 N rate at 20/8). The N concentration decreased as W increased during the growing season. This decline of N concentration with time reported by many studies (Justes et al. 1994; Duchenne et al. 1997; Bélanger et al. 2001; Li et al. 2012; Giletto and Echeverría 2012) corresponds to the dilution phenomenon which is mainly attributed to two processes. The first process corresponds to self-shading of leaves that induces a non-uniform leaf N concentration from the top of the canopy layers with high N concentration to the shaded layers with low N concentration (Lemaire et al. 1991; Pons and Pearcy 1994). The second process is the increase in the proportion of plant

Table 2 Total biomass production for different potato trials conducted in Belgium with increasing N-fertilizer rates from 1997 to 2014 of cultivar Bintje or Charlotte

Cultivar	Sites	Year	Date	DAE[a]	Total biomass (t DM ha^{-1})					F test[b]	LSD[c]
Bintje	Applied N (kg N ha^{-1})				0	75	113	150	188		
	Gembloux	1997	12/6	21	0.79	1.15	1.14	1.2	1.25	*	0.27
			24/6	33	2.17	3.08	3.38	3.33	3.31	**	0.68
			3/7	42	3.42	5.32	4.87	5.19	5.03	*	1.09
			16/7	55	6.44	8.82	8.17	8.25	8.84	**	1.08
			30/7	69	9.37	12.36	10.85	12.34	12.71	*	2.03
	Applied N (kg N ha^{-1})				0	58	87	115	144		
	Masnuy	1997	10/6	22	1.08	0.89	1.06	1.08	0.84	ns	
			1/7	43	4.05	5.63	4.51	4.67	4.95	ns	
			24/7	66	10.42	11.76	11.24	9.43	10.58	ns	
	Applied N (kg N ha^{-1})				0	88	132	176	220		
	Gembloux	1998	22/6	33	0.93	1.78	1.66	1.82	1.66	**	0.46
			1/7	42	2.44	3.59	3.41	3.66	3.66	**	0.60
			13/7	54	5.16	8.23	6.48	6.58	7.08	**	1.31
	Gouy	1998	24/6	31	1.07	1.73	1.89	1.90	1.51	ns	
			7/7	44	**3.47**	4.62	4.33	5.50	**4.06**	*	1.27
			15/7	52	**4.40**	6.75	6.61	7.97	**5.59**	*	1.97
	Applied N (kg N ha^{-1})				0	119	170	221			
	Franière	1999	28/7	35	1.87	2.00	1.75	1.97		ns	
	Gembloux	1999	14/7	21	0.53	0.50	0.40	0.44		ns	
			24/7	31	1.66	2.12	1.72	2.00		ns	
			8/7	45	**4.44**	**5.39**	**5.50**	**5.38**		*	0.73
			19/7	56	7.82	8.96	7.29	8.24		ns	
	Applied N (kg N ha^{-1})				0	112	160	208			
	Incourt	1999	28/6	35	2.64	2.53	2.48	2.34		ns	
	Applied N (kg N ha^{-1})				0	105	150	195			
	Marcq	1999	1/7	38	1.96	2.06	2.24	2.27		ns	
	Applied N (kg N ha^{-1})				0	88	125	163			
	Pontillas	1999	28/6	35	2.64	3.31	3.31	3.36		*	0.55
	Applied N (kg N ha^{-1})				0	102	145	189			
	Gembloux	2000	15/6	32	**1.75**	**2.78**	2.38	**2.71**		*	0.68
			29/6	46	4.69	5.83	5.15	5.62		ns	
			13/7	66	8.06	8.92	8.32	8.49		ns	
	Applied N (kg N ha^{-1})				0	102	145	189			

Table 2 (continued)

Cultivar	Sites	Year	Date	DAE[a]	Total biomass (t DM ha^{-1})						F test[b]	LSD[c]
	Roisin	2000	19/6	44	2.43	2.88	2.68	2.60			ns	
	Applied N (kg N ha^{-1})				0	100	140	180	240			
	Gembloux	2004	14/6	25	0.62	0.94	1.01	0.96	0.92		ns	
			21/6	32	1.88	2.49	2.52	2.45	2.37		ns	
			28/6	39	2.94	4.10	3.73	3.85	3.99		*	0.60
			5/7	46	4.19	5.53	5.78	5.46	6.08		**	0.71
			12/7	53	5.67	7.29	6.82	6.98	7.47		***	0.48
			19/7	60	6.70	9.01	8.73	8.39	9.35		**	1.32
			27/7	68	8.16	10.81	10.24	11.06	10.98		*	2.04
	Applied N (kg N ha^{-1})				0	115	165	215	248			
	Gembloux	2010	14/6	25	0.65	0.83	0.71	0.77	0.68		ns	
			28/6	34	3.10	3.19	3.42	3.22	3.42		ns	
			5/7	41	4.20	4.33	4.34	4.06	4.41		ns	
			12/7	48	5.12	5.50	5.24	5.43	5.27		ns	
			26/7	62	7.19	7.72	8.15	7.07	7.80		ns	
	Applied N (kg N ha^{-1})				0	98	140	182	210			
	Gembloux	2011	14/6	27	1.44	1.46	1.27	1.55	1.36		ns	
			6/7	49	5.55	5.15	5.41	5.37	5.53		ns	
			20/7	63	8.26	8.25	8.04	8.88	8.53		ns	
			25/7	68	8.77	10.22	8.86	9.42	9.30		ns	
	Applied N (kg N ha^{-1})				0	50	100	150	200	250		
	Gembloux	2012	27/6	26	1.02	1.10	1.21	1.37	1.28	1.32	ns	
			4/7	33	1.91	2.83	2.83	2.75	3.25	2.97	**	0.55
			11/7	40	3.25	3.41	4.00	4.28	4.29	4.66	***	0.41
			25/7	54	5.82	7.31	6.06	7.54	5.40	6.21	ns	
	Gembloux	2013	19/6	13	0.33	0.44	0.41	0.44	0.52	0.43	ns	
			26/6	20	1.00	1.41	1.16	1.45	1.23	1.29	ns	
			11/7	35	3.79	4.33	4.35	4.64	5.04	4.50	ns	
			23/7	47	**5.19**	**6.67**	**7.14**	**7.21**	**7.13**	**6.76**	*	1.36
	Gembloux	2014	18/6	29	2.51		2.91		2.85	2.67	ns	
			3/7	44	5.99		5.99		6.75	6.76	ns	
			30/7	71	10.23		11.70		11.10	11.77	ns	
Charlotte	Applied N (kg N ha^{-1})				0	84	120	156				
	Gembloux	1999	6/7	42	3.07	3.43	4.22	4.32			ns	
			22/7	58	5.79	8.47	7.70	8.29			ns	
	Applied N (kg N ha^{-1})				0	100	140	180	210			
	Gembloux	2010	17/6	15	0.88	1.04	1.03	0.83	0.96		ns	

Table 2 (continued)

Cultivar	Sites	Year	Date	DAE[a]	Total biomass (t DM ha^{-1})						F test[b]	LSD[c]
			1/7	29	3.34	4.01	3.71	3.30	3.35		ns	
			8/7	36	4.72	5.13	4.38	5.07	5.22		ns	
			15/7	43	5.83	5.91	6.11	5.19	5.93		ns	
			19/7	47	5.36	6.08	6.05	5.95	6.16		ns	
Applied N (kg N ha^{-1})					0	84	120	156	180			
Gembloux		2011	20/6	33	2.36	2.64	2.32	2.77	2.95		ns	
			4/7	47	5.17	5.59	5.32	6.03	5.19		ns	
			11/7	54	6.45	7.60	6.37	7.57	6.47		ns	
			18/7	61	8.51	9.64	9.40	9.68	10.13		ns	
Applied N (kg N ha^{-1})					0	50	100	150	200	250		
Gembloux		2012	27/6	27	1.27	1.35	1.46	1.49	1.50	1.46	ns	
			2/7	32	2.09	2.38	2.74	2.64	2.69	2.63	ns	
			9/7	39	4.09	4.54	4.10	4.54	4.57	4.30	ns	
			16/7	48	5.01	4.80	5.50	5.47	5.96	5.49	ns	
Gembloux		2013	17/6	13	0.46	0.58	0.63	0.53	0.61	0.54	**	0.09
			24/6	20	**1.03**	1.37	1.51	1.43	**1.70**	1.36	**	0.31
			1/7	27	**2.19**	2.53	2.91	2.94	**3.27**	2.98	**	0.51
			15/7	41	**5.34**	6.70	6.91	**7.41**	6.96	6.94	***	0.68
Gembloux1		2014	16/6	30	2.42		2.41		2.69	2.53	ns	
			26/6	44	5.43		5.73		5.74	5.87	ns	
			28/7	72	9.45		11.16		11.67	12.83	ns	
Gembloux2		2014	24/6	36	3.31		3.87			3.53	ns	
			8/7	51	**6.02**		**7.22**			**7.24**	*	0.89
			15/7	58	7.22		7.88			8.91	ns	
			23/7	66	8.92		10.89			10.03	ns	

Bold numbers represents total biomass used for the validation

DAE days after emergence; *ns* indicates no significance ($p > 0.05$); *LSD* least significant difference calculated if the *F* test indicated significant difference among total biomass (t of dry matter ha^{-1})

*F test indicating statistical significance at $p \leq 0.05$; **F test indicating statistical significance at $p \leq 0.01$; ***F test indicating statistical significance at $p \leq 0.001$

structural and storage tissues with a lower nitrogen concentration (Caloin and Yu 1984). Crop N concentration varied significantly with N rates; higher N rates mostly resulted in higher N concentrations (Fig. 1).

Determination of the Critical Nitrogen Dilution Curve for Cultivar Bintje

Among the sites and years used to establish the critical dilution curve, 16 sampling dates (between 21 and 69 DAE) for Bintje met the previously defined statistical criteria. Each of these sampling dates provided a point of Nc concentration for a given W (Table 3).
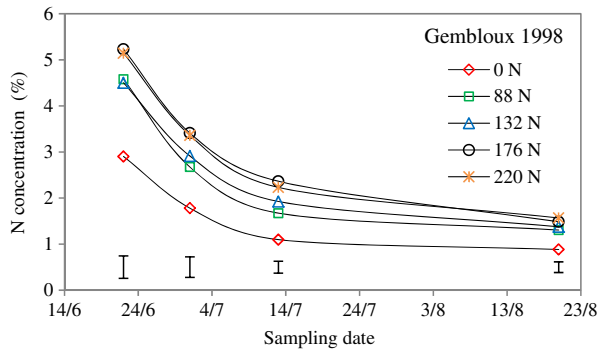
Fig. 1 Changes of nitrogen concentration in total biomass (W, expressed in % of dry matter) with time for potato cultivar Bintje under different N rates (kg N ha^{-1}) at the site Gembloux in 1998. The *vertical bars* represent LSD values ($p \le 0.05$) at each plant sampling date

As illustrated in Fig. 2, the allometric equation obtained for cv. Bintje was

$$Nc\ (\%)\ =\ 5.37\ W^{-0.45} \qquad (3)$$

The model accounted for 86% of the total variance. The 95% confidence interval of the mean was 1.30 N (in % of DM) for a W of 1.15 t DM ha^{-1} and 0.54 N (in % of DM) for a total biomass of 10.85 t DM ha^{-1}.

Table 3 Critical nitrogen concentration data points selected for the calibration of the Belgian critical nitrogen dilution curve for cultivar Bintje

Sites	Sampling dates	DAE	Applied N	Total biomass	Nc concentration
Gembloux	12 June 1997	21	75	1.15	4.45
Gembloux	24 June 1997	33	75	3.08	3.53
Gembloux	3 July 1997	42	75	5.32	2.79
Gembloux	16 July 1997	55	75	8.82	1.86
Gembloux	30 July 1997	69	113	10.85	1.55
Gembloux	22 June 1998	33	88	1.78	4.58
Gembloux	1 July 1998	42	88	3.59	2.67
Gembloux	13 July 1998	54	88	8.23	1.67
Pontillas	28 June 1999	35	88	3.31	3.29
Gembloux	28 June 2004	39	100	4.10	2.96
Gembloux	5 July 2004	46	100	5.53	2.22
Gembloux	12 July 2004	53	240	7.47	2.48
Gembloux	19 July 2004	60	100	9.01	1.57
Gembloux	27 July 2004	68	100	10.81	1.47
Gembloux	4 July 2012	33	50	2.83	3.47
Gembloux	11 July 2012	40	250	4.66	3.40

Applied nitrogen (N) in kg ha^{-1}, total biomass expressed in t of dry matter ha^{-1} and critical nitrogen (Nc) concentration expressed in percentage of dry matter

DAE day after emergence of the crop
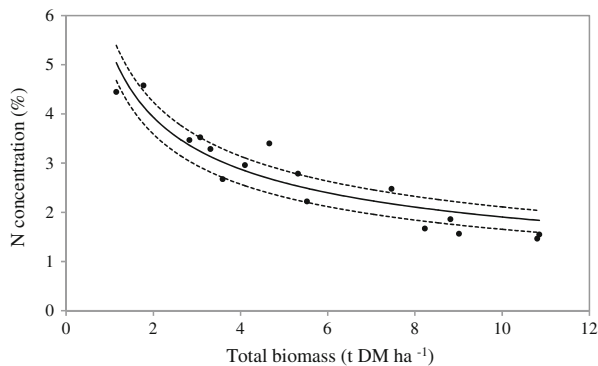
Fig. 2 Critical nitrogen (Nc) concentration data points used to define the critical N dilution curve of potato for cultivar Bintje in Belgium. The *solid line* represents the critical N dilution curve with the relation Nc (%) = 5.37 W$^{-0.45}$ (R^2 = 0.86). The *dotted lines* represent the confidence band (p = 0.95) for mean prediction

Comparison with Available Critical Nitrogen Dilution Curves for Potato Crop

Figure 3 shows that the Nc curve defined in this study was very similar to the reference curve of Greenwood et al. (1990) and clearly above the reference curve established by Duchenne et al. (1997). Taking into account the curves of Bélanger et al. (2001), the Belgian curve is relatively similar to the one established for cv. Shepody (particularly from medium W productions) and above the one established for cv. Russet Burbank. Comparing the curve obtained by Giletto and Echeverría (2012), the Belgian curve is slightly lower, particularly for the medium and the higher W productions.

In this study, the coefficient *a* (5.37) for the dilution curve equation was very similar to the ones of Greenwood et al. (1990) (5.36) and Giletto and Echeverría (2012) (5.30). Similarly, the coefficient of dilution *b* (0.45) was close to the coefficient obtained by
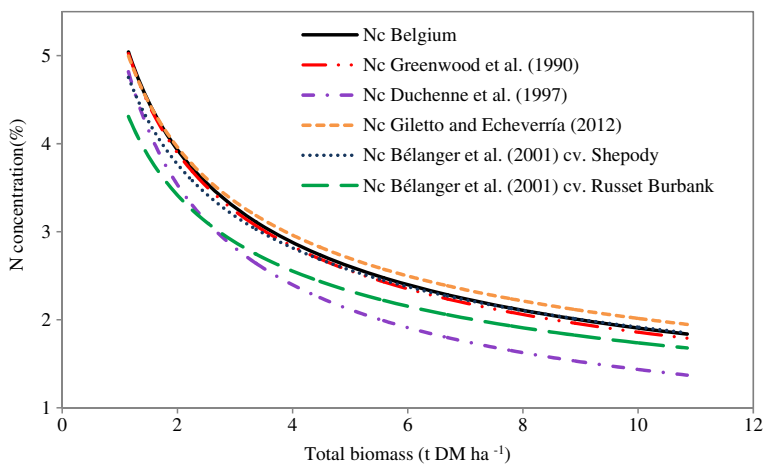
Fig. 3 Comparison of different critical nitrogen curves for potato. The *full black line* represents the Belgian critical N curve (this study) (Nc (%) = 5.37 W$^{-0.45}$ for cv. Bintje). The other curves represent the proposed reference by Duchenne et al. (1997) (Nc (%) = 5.21 W$^{-0.56}$), by Greenwood et al. (1990) (Nc (%) = 5.36 W$^{-0.46}$), by Giletto and Echeverría (2012) (Nc (%) = 5.30 W$^{-0.42}$) and by Bélanger et al. (2001) (for cv. Shepody Nc (%) = 5.04 W$^{-0.42}$ and for cv. Russet Burbank Nc (%) = 4.57 W$^{-0.42}$)

Greenwood et al. (1990) (0.46) and Giletto and Echeverría (2012) and Bélanger et al. (2001) (0.42).

The reasons for the difference observed between the coefficients of the Belgian curve and the reference curves of Duchenne et al. (1997) and Bélanger et al. (2001) for cv. Russet Burbank could be related to various factors including differences in cultivars, climatic conditions and field management. Duchenne et al. (1997) established the Nc curve on the basis of three experimental years on two cultivars Bintje and Kaptah Vandel. The curve was then validated for Saturna, El Kana and Russet Burbank in loamy soils and for Bintje in calcareous clay and calcareous soils (Chambenoit et al. 2004). The critical points obtained for each cultivar were close to the established Nc curve for Bintje and Kaptah Vandel. Chambenoit et al. (2004) stated that varietal differences may exist but were not detectable given the precision level of the used experimental design. The cultivar differences seem mainly related to the earliness of each cultivar that determines the maximum production of dry matter and therefore the dilution of the nitrogen in the plant biomass (Chambenoit et al. 2004). Vos (1997) reported that the relationship between N uptake and biomass accumulation should be different for genotypes of different earliness. Giletto and Echeverría (2015) found that cultivars with shorter growing season (cultivars Innovator and Gem Russet) had lower plant N concentration for a same level of total biomass than cultivars with longer growing seasons (cultivars Umatilla Russet, Bannock Russet and Markies Russet). Contrary to Giletto and Echeverría (2015), Bélanger et al. (2001) found greater N concentration for the cultivar Shepody with shorter season than for Russet Burbank. Giletto and Echeverría (2015) reported different statistical value in the coefficient b between the tested cultivars but showed no statistical differences in coefficient a. However, Bélanger et al. (2001), reported different values of coefficients a and similar values of coefficient b for Shepody and Russet Burbank. According to Bélanger et al. (2001), the observed difference in the coefficient a was caused by differences in plant density. The cultivar with greater plant density had smaller plants with greater N concentration than the cultivars with low plant density. Similar b coefficients indicated that both cultivars had similar N dilution (Bélanger et al. 2001). In our study, the plant density was different according to the seed size used.

Bélanger et al. (2001) reported also different parameters a and b of the Nc curve for both studied cultivars according to the water regime. The N concentration at a given level of total biomass, primarily for Russet Burbank, was lower without than with irrigation. The water deficiency decreased N uptake and therefore indirectly created conditions of N deficiency (Bélanger et al. 2001). The curve of Duchenne et al. (1997) was established under growing conditions for which water was not limiting growth. In this study, the Nc curve for Bintje was established for Belgian climate and soil conditions for which temporary water deprivation may occur. The climate in Belgium is a temperate maritime climate with cool summers and moderate winters which is similar to the climate of the north of France, Scotland and the Netherlands. According to Lemaire and Gastal (1997), the relationship between Nc concentration and crop mass does not vary considerably with major environmental factors others than those affecting soil N supply. Ziadi et al. (2010) stated that climatic conditions could explain part of the difference observed between the Canadian Nc curve of spring wheat and the French curve of winter wheat developed by Justes et al. (1994).

The observed difference with the Nc curve of Duchenne et al. (1997) could also be due to their applied statistical method which corresponds to the classification of the W values using the Newman and Keuls test for $p = 0.10$. The range of plant weights used for the calibration of the Nc curves could also have influenced the relationship between Nc and W. Contrary to Bélanger et al. (2001), who limited the data set to W less than 6 t DM ha^{-1}, the W used in this study for the establishment of the curve ranged from 1.15 to 10.85 t DM ha^{-1} corresponding to the period from June to July. Greenwood et al. (1990), Duchenne et al. (1997) and Giletto and Echeverría (2012) based their estimation on data with a range of W up to 12–15 t DM ha^{-1}.

Validation of the Critical Nitrogen Dilution Curve for Cultivars Bintje and Charlotte

The crop is considered in non-limiting N conditions if the actual N concentration is higher than the Nc concentration required for maximum growth rate. The crop is in limiting growth condition if the actual N concentration is below the Nc curve. In the validation data set for cv. Bintje (see Table 1), 5 points were in limiting N conditions and 12 points in non-limiting N conditions (Fig. 4). All data points identified as limiting N conditions were under the Nc curve and all the data points identified as non-limiting N conditions (except 1) were above the curve (Fig. 4). These observations tend to confirm that the Nc curve can be used for the discrimination between the limiting and non-limiting N conditions and the determination of CNS for the cv. Bintje in Belgian conditions.

Following the same approach, 4 limiting N conditions and 5 non-limiting N conditions were identified for cv. Charlotte. As for cv. Bintje, all the limiting N conditions were under the Nc curve and all the non-limiting N conditions (except 1) were above the curve. According to the limited number of points for validation, the Nc curve could also be used for the discrimination between limiting and non-limiting N conditions for cv. Charlotte. But since Charlotte is an earlier cultivar than Bintje and, as mentioned before, the earliness of the genotype is a factor of intra-specific variability in potato for the relationship between N concentration and biomass, thus when establishing the Nc curve, the cultivar factor should be taken into account. In this study, due to the limited number of data points, a cultivar difference was not detectable. A study
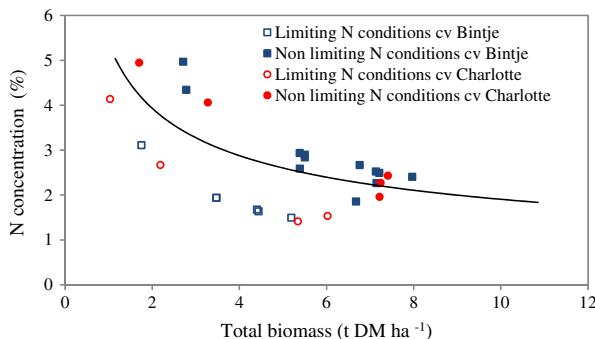
Fig. 4 Validation of the Belgian critical N curve using data from potato cultivars Bintje and Charlotte under limiting and non limiting N growth conditions. The *solid line* represents the predicted critical N dilution curve with the relation Nc (%) = 5.37 W$^{-0.45}$

based on a larger data set for cv. Charlotte is therefore needed in order to be able to draw more definitive conclusions.

Nitrogen Nutrition Index and Its Relation to Relative Yield

The NNI was calculated for each plant sampling date. The NNI in this study ranged from 0.39 to 1.53 (data not shown). As an example, the NNI values over three plant sampling dates at Gembloux in 2014 are given in Fig. 5. Values of NNI ≥1 indicate that the crop is non-limiting for N, whereas values of NNI < 1 indicate N deficiency (Duchenne et al. 1997). Contrary to the maximum N treatment (250 N), the data points for the non-fertilized treatment were clearly under the reference of 1 indicating N deficiency, whatever the sampling date. The NNI values for the 100 and 200 N treatments were over this reference at the first sampling date but dropped below this threshold at the second sampling date for the 100 N treatment and at the last sampling date for the 200 N treatment.

The relationship between NNI and RY expressed by a quadratic plateau function accounted for 79, 75 and 70% of the variation considering the three different periods of plant sampling for the calculation of NNI, i.e. respectively, the mean of all the sampling dates, the mean of sampling between 20 and 55 DAE and the last sampling date closest to harvest (Fig. 6). Based on this relationship, the potato RY reached a plateau for a determined NNI value. This value corresponds to the joint point of the quadratic phase and the plateau phase for each of the three curves and was equal to 1.1 (Fig. 6a), 1.16 (Fig. 6b) and 0.97 (Fig. 6c) depending on the considered plant-sampling period. With decreasing NNI value below this joint point, the RY decreased (Fig. 6). This confirms the link between N uptake and yield of potato and thus the relevancy of the Nc model and the resulting NNI for forecasting actual yield with respect to potential yield (Chambenoit et al. 2004).

The relationship between NNI and RY, however, varied with the periods of plant sampling for NNI determination (Fig. 6). The maximum RY obtained was the same for the three functions (0.97), but the equation of RY corresponding to NNI below the joint point was different. This joint point is important for determining the threshold value under which the crop nitrogen status is considered as deficient with respect to
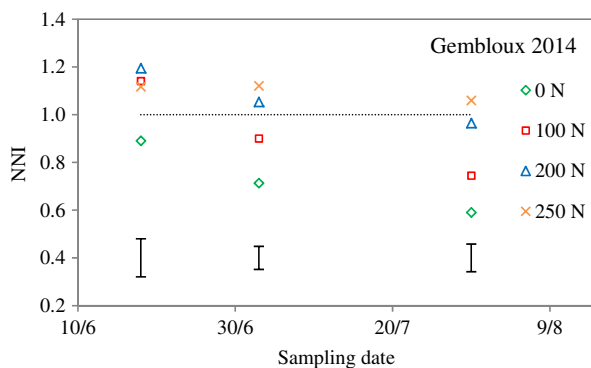

Fig. 5 Nitrogen Nutrition Index (NNI) of three sampling dates for increasing N rates (kg N ha^{-1}) at the site of Gembloux in 2014 for cultivar Bintje. The *dotted horizontal line* represents an NNI of 1.0. The *vertical bars* represent LSD values ($p \leq 0.05$) at each plant sampling date
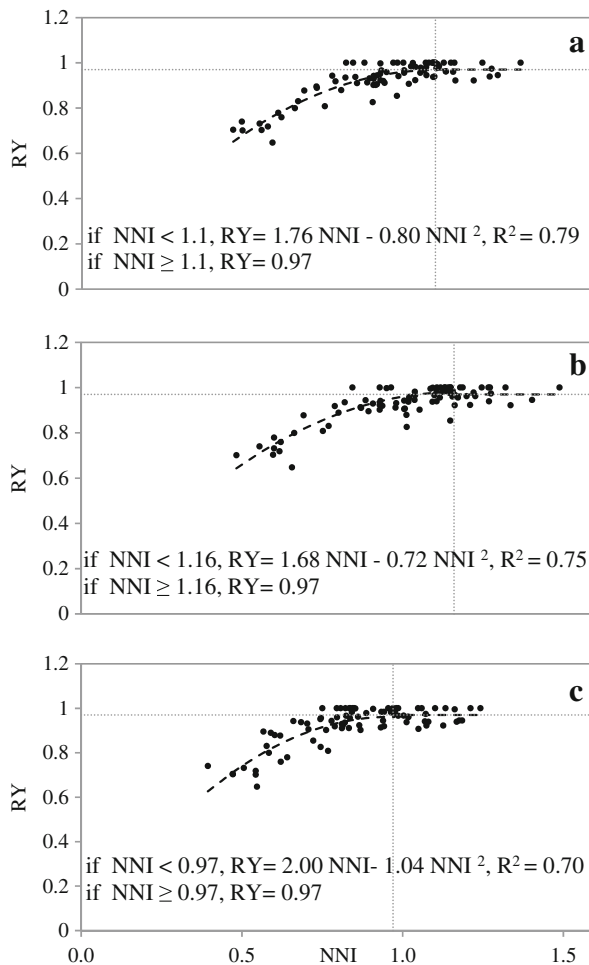
Fig. 6 Relationship between relative yield (RY) measured at harvest and nitrogen nutrition index (NNI) for cultivar Bintje measured across all the sampling dates (**a**), across the period of 20 and 55 DAE (**b**) and for the last sampling date (**c**). The *vertical bars* indicate the joint point for each curve, and the *horizontal bars* indicate the plateau of the curve

maximum potential yield. The NNI reference value of 1 given by Duchenne et al. (1997) corresponds to the optimum CNS required to achieve the maximum growth rate. Chambenoit et al. (2004) reported values of NNI between 1.1 and 1.2 to maximize the tuber yield. In this study, the reference value of NNI was slightly higher, as in Chambenoit et al. (2004), for Fig. 6a, b with values of 1.1 and 1.16 (when considering all the plant sampling dates or the period between 20 and 55 DAE) and similar for Fig. 6c with value of 0.97 (when considering the latest sampling date) compared to the reference value of 1. The period between 20 and 55 DAE has been demonstrated as the optimal period for the assessment of the CNS, according to a previous study by Olivier et al. (2006). Consequently, in this study, we consider the reference NNI value of 1.16 (Fig. 6b), under which the CNS is considered as deficient during the optimal period for CNS assessment.

Conclusion

A Nc dilution curve (Nc (%) = 5.37 $W^{-0.45}$) was developed for potato for cv. Bintje under the Belgian conditions. The Belgian Nc dilution curve was different from the one developed for potato in France but similar to the one developed in Scotland and the Netherlands. The usefulness of the Belgian Nc dilution curve for cv. Charlotte was not fully verified since the validation considered only a limited data set and cultivar difference is reported as a factor of variability of the Nc curve. The Belgian Nc curve and the resulting NNI adequately identified situations of limiting and non-limiting N nutrition and could be used to establish the potato CNS and to predict the final yield. A reference NNI value of 1.16 was established as the threshold value to be achieved during the optimal period for CNS assessment, under which threshold the CNS is considered as deficient.

However, establishing the NNI at field level requires destructive and chemical analysis and is not appropriate for a quick assessment of CNS. The NNI can be used as a reference for calibrating other non-invasive methods for a quick and easy in-season monitoring of CNS (Goffart et al. 2008).

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