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Flue-cured tobacco and Cl rates: Implications on yield, quality, and nutrient concentration

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Abstract

The increase in flue-cured tobacco (*Nicotiana tabacum* L.) yields in recent decades due to genetic improvements of new cultivars and management technologies may increase the plant demand for Cl, and the increased dry mass may dilute Cl concentration, thereby reducing negative effects. This study evaluated the effect of increasing doses of Cl on tobacco production, quality, and chemical composition of leaves, in four growing environments located at research stations where flue-cured tobacco is produced in North Carolina. The treatments consisted of 11 rates of Cl (0, 11, 22, 34, 45, 56, 67, 78, 90, 101, and 112 kg ha^{-1}) in each growing environment, with four replications in a randomized complete block design. The yield and visual quality, total alkaloids, and reducing sugars concentrations of cured leaf were determined. In addition, the concentration of selected nutrients (N, P, K, Ca, Mg, S, and Cl) and nitrate (NO_3^-) in tobacco leaves was measured in five different periods. Rates of Cl up to 112 kg ha^{-1} did not reduce the productivity or quality of flue-cured tobacco in any environment. The Cl rate required to reach the threshold of 1.0% Cl content in cured leaf was site-specific, being surpassed even in the control treatment at one location, or with Cl rates higher than 34 and 90 kg ha^{-1} in two environments. In one environment, the Cl rates increased tobacco yield, probably due the direct effect of Cl as a nutrient. Although the increasing Cl rates increased the reducing sugars concentration, visual quality was not attenuated.

1 | INTRODUCTION

Chlorine, known as a plant nutrient since the mid-20th century because of its low requirement, is classified as an essential micronutrient (Broyer et al., 1954). When sufficient, the element enhances fresh and dry biomass, leaf expansion, elongation of leaf and root cells, water relations, mesophyll diffusion to CO_2, and water- and nitrogen-use efficiency (Colmenero-Flores et al., 2019). In non-halophyte crops, Cl concentration in shoots is largely variable (1–20 g kg^{-1} or 0.1%–2.0% of dry matter; Marschner, 2012). However, under certain environmental conditions, a concentration as high as 50 g kg^{-1} or 5.0% of dry matter for tobacco is reported (Franco-Navarro et al., 2016) and, in tobacco grown in a

Abbreviation: 2WAL, 2 weeks after layby; 2WAT, 2 weeks after Cl application; layby, last cultivation; LCPRS, Lower Coastal Plain Research Station in Kingston, NC; OTRS, Oxford Tobacco Research Station in Oxford, NC; UCPRS, Upper Coastal Plain Research Station near Rocky Mount, NC.

greenhouse, McCants and Woltz (1967) found concentrations up to 10% of dry weight in leaves. This concentration is similar to those for macronutrients like N and K. High concentrations of Cl in tobacco leaves could be beneficial to plants by regulating leaf osmotic potential and turgor, thus improving leaf water balance. Due to these findings, it is suggested that Cl be classified as both an essential micronutrient and a beneficial macronutrient (Franco-Navarro et al., 2016).

Research involving tobacco's response to Cl application dates back to the beginning of last century (Garner et al., 1930). Since then, researchers continued to investigate how Cl affects tobacco and have discovered controversial results. Studies demonstrate positive effects on tobacco yields at low rates of Cl ranging from 22 to 45 kg ha^{-1} (McCants & Woltz, 1967; Warren et al., 1990), whereas others determine that high Cl rates can decrease the quality of tobacco (Chen et al., 2010; Myhre et al., 1956). A general recommendation for tobacco growers is difficult to establish due to several factors. One of them is that the element can enter the system through several paths, for example, via the atmosphere or via irrigation water, as contaminants in fertilizers, or directly via phytosanitary products (Xu et al., 2000). For example, tobacco growers applying chloropicrin (CCl_3NO_2) in the southeast United States may apply 28–84 kg ha^{-1} of active ingredient (Becker et al.,2005), which is approximately 18–54 kg ha^{-1} of Cl. This amount alone can often meet the demand for tobacco plants.

One could expect the response to the element would diminish over time in fields where tobacco has been grown for several years. However, due to the low retention of the chloride ion (Cl^-) by the binding sites present in clays and soil organic matter particles, the buildup of soil Cl over time is nearly impossible (Liu et al., 2021). Field measurements and prediction in two Canadian soils indicated that over 70% of the applied Cl can be lost to deep drainage (below 80-cm depth) during the winter months, November to April (Saso et al., 2012). Therefore, Cl leaching below from the root uptake zone is very rapid, particularly in sandy soils after high rainfall volumes (Moore et al., 2011).

Contrasting with the increase in tobacco yield, high Cl rates can compromise the quality of tobacco. According to Zehler et al. (1981), the cured tobacco leaves can be classified by Cl content as excellent (<1%), good to satisfactory (1%–2%), unsatisfactory (2%–3%), and poor (>3%). High Cl reduces flammability and increases the hygroscopicity of the leaf, creating problems during leaf drying (Ishizaki & Akiya, 1978). During curing, excess Cl creates muddy, dingy, and uneven colors (McCants & Woltz, 1967). In addition, the increase in Cl content decreases total alkaloids and increases reducing sugars (Sierra, 1966). Moreover, greenhouse experiments found that concentrations of 3.6%–5.6% Cl in leaves can be toxic to tobacco plants and reduce yields (Honda et al., 1963). Results also indicated that Cl inhibited the absorption of the other anions such as sulfate (SO_4^{-2}) and NO_3^- by burley tobacco plants but did not influence the composition of cations (Fuqua et al., 1974). Finally, Cl concentrations higher than 3% may not maintain combustion (Akehurst, 1981).

Core Ideas

- Rates of Cl up to 112 kg ha^{-1} did not reduce the productivity or quality of flue-cured tobacco in any environment.
- The Cl rate required to reach the quality threshold of 1.0% Cl content in cured leaf was site-specific.
- In one site, the Cl rates increased tobacco yield, probably due the direct effect of Cl as a nutrient.
- Although the increasing Cl rates increased the reducing sugars concentration, visual quality was not attenuated.

Because of the possible negative effects of excess Cl in tobacco leaves, the nutrient is handled cautiously and is a constant concern among tobacco agronomists and farmers in the southern US. As a result, preference has been given to the application of Cl-free potassium fertilizers or the use of sources of N containing NO_3^- to reduce the absorption of Cl (Pace et al., 2020; Rosales et al., 2020; Warren, 1990); these strategies often increase production costs. However, the increase in tobacco yields in recent decades due to genetic improvements of new cultivars and management technologies may increase the plant demand for Cl, and the increased dry mass may dilute Cl concentration reducing its negative effects. Therefore, the aim of the present study was to evaluate the effect of increasing doses of Cl on tobacco production, quality, leaf chemistry, and nutrient composition, in four growing environments in North Carolina.

2 | MATERIALS AND METHODS

2.1 | Growing environments and experimental design

Field experiments in NC were conducted at the Lower Coastal Plain Research Station (LCPRS) near Kinston in 2016 and 2017 and in 2017 at the Oxford Tobacco Research Station (OTRS) in Oxford and the Upper Coastal Plain Research Station (UCPRS) near Rocky Mount. Soils were classified as a Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) at LCPRS and UCPRS which are typical of flue-cured production in eastern NC and an Appling sandy loam (fine, kaolinitic, thermic Typic Kandiudults) at OTRS, which is representative of the flue-cured tobacco-producing area of the piedmont or central part of NC.

TABLE 1 Soil chemical properties prior to nutrient application within each growing environment

Growing environment	Soil depth	pH	CEC	BS	Humic matter	Cl	P	K	Mg	Ca	S
	cm		$cmol_c\ dm^{-3}$	%		kg ha^{-1}					
LCPRS-16[a]	0–15	6.3	2.8	85.5	0.60	8.24	182	109	150	555	29
	15–30	5.7	5.8	58.8	0.25	18.24	223	156	225	773	63
LCPRS-17[b]	0–15	6.0	2.4	67.1	0.29	0.02	329	106	78	397	19
	15–30	6.0	2.5	68.4	0.44	0.01	396	121	84	416	20
OTRS-17[c]	0–15	5.8	3.8	78.8	0.28	81.97	194	203	192	657	24
	15–30	5.6	3.1	74.3	0.28	0.02	166	125	145	525	29
UCPRS-17[d]	0–15	6.0	2.4	74.5	0.21	27.57	103	133	59	478	16
	15–30	5.8	2.3	74.4	0.17	12.34	96	125	64	448	35

Abbreviations: BS, base saturation; CEC, cation exchange capacity.
[a]LCPRS-16: Lower Coastal Plain Research Station in Kinston, NC, in 2016.
[b]LCPRS-17: Lower Coastal Plain Research Station in Kinston, NC, in 2017.
[c]OTRS-17: Oxford Tobacco Research Station in Oxford, NC, in 2017.
[d]UCPRS-17: Upper Coastal Plain Research Station near Rocky Mount, NC, in 2017.

Treatments consisted of 11 rates of Cl (0, 11, 22, 34, 45, 56, 67, 78, 90, 101, and 112 kg ha^{-1}) in each growing environment, with four replications in a randomized complete block design. The Cl rates were obtained by using a dry blend containing KCl (50% K and 45% Cl), K_2SO_4 (42% K and 18% S), and $CaSO_4$ (22% Ca and 18% S) to ensure equal application rates of K and S within each Cl application rate. Therefore, the amount of Ca was uneven among treatments and increased with Cl rate, ranging from 0 to 59 kg ha^{-1}. Each experimental unit (plot) contained four rows of flue-cured tobacco. Row spacing varied among environments (1.12 m at LCPRS and 1.22 m at OTRS and UCPRS); however, row length and planting density were consistent (15.2 m and 14,820 plants ha^{-1}). The variety NC 196 (Gold Leaf Seed Company) was planted in each growing environment.

2.2 | Field operations

Prior to transplanting, research areas at the LCPRS and OTRS were fumigated with Telone C-17 (78.3% 1,3-dichloropropene at 98.2 L ha^{-1}) and PicPlus (85.5% chloropicrin at 37.4 L ha^{-1}), respectively. Soil fumigation was not used at the UCPRS. Before fertilizer treatment application, soil cores were collected from each field site at a depth of 0–15 and 15–30 cm for quantification of pH, humic matter, base saturation, CEC, and nutrient concentration by Waters Laboratory in Warsaw, NC (California State Transportation Agency, 2014) (Table 1; Mehlich, 1984). The monthly rainfall in each month is shown in Table 2. Fertilizer treatments were applied 10 days after transplanting in each environment. Nutrient placement was in a single furrow adjacent to each planted row, approximately 12 cm away from the ridge and 12 cm deep. Nutrient application timing and methodology were consistent with Pace et al. (2020). Total N rates used in the growing environments were 80, 85, and 92 kg ha^{-1} of N in the OTRS, LCPRS, and UCPRS, respectively, split in two applications, at 10 days after transplanting and at layby (when plant height was approximately 38 cm), using liquid 28% urea–ammonium–nitrate as an N source.

2.3 | Nutrient concentration assessments in tobacco leaf

The concentration of selected nutrients (N, P, K, Ca, Mg, S, and Cl) and NO_3^- in tobacco leaves was measured at 2 weeks after Cl application (2WAT), last cultivation (layby), 2 weeks after layby (2WAL), flowering (topping), and after curing leaf (cured leaf). For this purpose, in the three first sampling intervals (2WAT, layby, and 2WAL), the fourth leaf below the apical meristem was collected from five randomly chosen plants of each plot. At flowering, five random samples were taken from upper most leaves in each plot. In the first four, green leaf sampling periods, the leaf dimensions were approximately 10-cm wide by 15-cm long. Following traditional harvest and curing, five leaves were randomly sampled from the uppermost stalk position.

After drying at 65°C for 72 h, the leaves were ground to pass a 1-mm sieve and stored until further analysis. The concentration of N, P, K, Ca, Mg, S, Cl, and NO_3^- was estimated using the methodology proposed by Plank (1992). The macronutrient concentration obtained was then compared with the sufficiency range proposed by Campbell (2013) (Table 3). In addition, the total alkaloids and reducing sugars concentrations of each experimental unit were determined using 50-g composite cured leaf samples collected from each unit following the method developed by Davis (1976).

TABLE 2 Monthly and total amount of rainfall (mm) during tobacco growing in each of the growing environments

Month	LCPRS-16	LCPRS-17	OTRS-17	UCPRS-17
April	72	237	195	165
May	142	114	139	125
June	110	109	139	122
July	161	90	42	151
August	104	82	89	176
September	307	112	60	76
October	272	74	95	87
Total	1168	818	758	902

Abbreviations: LCPRS-16, Lower Coastal Plain Research Station in Kinston, NC, in 2016; LCPRS-17, Lower Coastal Plain Research Station in Kinston, NC, in 2017; OTRS-17, Oxford Tobacco Research Station in Oxford, NC, in 2017; UCPRS-17, Upper Coastal Plain Research Station near Rocky Mount, NC, in 2017.

TABLE 3 Sufficiency range of macronutrients in the most recent mature or fully expanded leaf of tobacco used as the indicator of nutritional status

Growth stage	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Early growth	4.0–5.0	0.2–0.5	2.5–3.5	0.75–1.5	0.2–0.6	0.15–0.6
Flowering	3.5–4.5	0.2–0.5	2.5–3.5	0.75–1.5	0.2–0.6	0.15–0.6

Source: Campbell (2013).

2.4 | Yield and quality assessment

In each growing environment, the center-two rows of each plot were hand-harvested four times. Harvested leaves were cured in bulk curing barns, and the yield was quantified by weighing each harvest after curing. Leaf maturity and ripeness were described by a grade index ranging from 1 to 100 according to the USDA government grade (Bowman et al., 1988).

2.5 | Data analysis

For the analysis of variance (ANOVA) of yield, quality, price, value, total alkaloids, reducing sugars, and reducing sugars:total alkaloids ratio comparing the four growing environments, the following model was used: $Y_{ijk} = \mu + R_i + E_j + error\ a(i, j)$; where μ = overall average; R = replications ($i = 1, 2, 3, 4$); E = growing environment ($j = 1, 2, 3, 4$); and *error* = experimental error. When growing environment effects were significant at $p < 0.05$ probability of error by F test, the differences between means of treatments were compared by Tukey test ($p < 0.05$).

In each growing environment, for the ANOVA of nutrient concentration in tobacco leaf, the following model was used: $Y_{ijk} = \mu + R_i + T_j + error\ a(i, j) + S_k + error\ b(i, k) + TS_{jk} + error\ c(i, j, k)$; where μ = overall experimental average; R = replications ($i = 1, 2, 3, 4$); T = treatment ($j = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11$); S = sampling interval ($k = 1, 2, 3, 4, 5$); and *error* = experimental error. When treatment effects were significant at $p < 0.05$ probability of error by F test, regression equations were adjusted for Cl rates, and the differences between sampling intervals were compared by the Tukey test at $p < 0.05$.

3 | RESULTS AND DISCUSSION

3.1 | Yield, quality, value, and leaf chemistry of tobacco in different growing environments

The four growing environments presented different tobacco yields and qualities (Figure 1a). At the LCPRS, lowest (2.73 Mg ha^{-1}) and highest (3.71 Mg ha^{-1}) yields occurred in 2016 and 2017, respectively, whereas in 2017, similar yield (3.14 Mg ha^{-1}), quality, and value occurred at OTRS-17 and UCPRS-17 locations (Figure 1a). Besides presenting the lowest yield, the LCPRS-16 environment also presented the lowest visual quality (Figure 1b), the lowest total alkaloids content (Figure 1c), the highest reducing sugars concentration (Figure 1d), and the highest reducing sugars and total alkaloids ratio (Figure 1e), resulting also in the lowest price (Figure 1f) and value (Figure 1g). The lowest reducing sugars concentration (Figure 1d) and the lowest reducing sugars and total alkaloids ratio (Figure 1e) were observed at the OTRS-17.

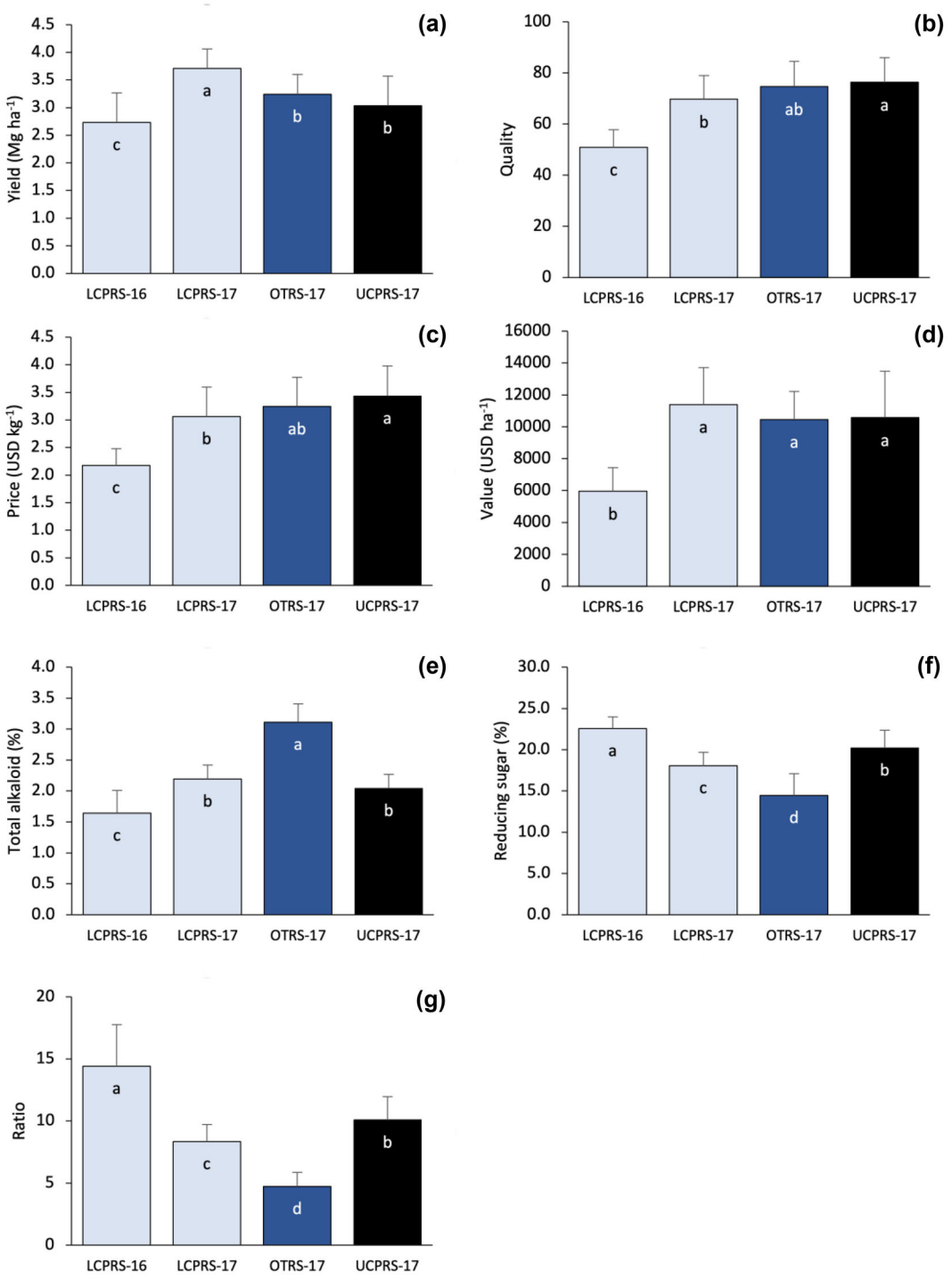

FIGURE 1 (a) Tobacco yield, (b) quality, (c) total alkaloids, (d) reducing sugars, (e) reducing sugars:total alkaloids ratio, (f) price, and (g) value, in four growing environments in NC state. Means followed by the same letter in columns comparing sampling times in each growing environment are not significantly different by Tukey's test at a significance level of $p < 0.05$. Each bar is an average of 44 observations (11 doses of Cl and 4 replicates). LCPRS-16, Lower Coastal Plain Research Station in Kinston, NC, in 2016; LCPRS-17, Lower Coastal Plain Research Station in Kinston, NC, in 2017; OTRS-17, Oxford Tobacco Research Station in Oxford, NC, in 2017; UCPRS-17, Upper Coastal Plain Research Station near Rocky Mount, NC, in 2017

TABLE 4 Significance of the effects of Cl rates on tobacco yield, quality, price, value, total alkaloids, and reducing sugars as resulting from analysis of variance (ANOVA) in four growing environments in NC

Variable	LCPRS-16	LCPRS-17	OTRS-17	UCPRS-17
Yield (kg ha^{-1})	0.7050	0.4713	0.2375	**0.0107**
Quality	0.1501	0.1390	0.4903	0.0702
Price ($US kg^{-1})	0.1919	0.1746	0.3975	0.0670
Value ($US ha^{-1})	0.6626	0.3484	0.0990	0.7365
Total alkaloids (%)	0.6860	0.7453	0.4025	0.1715
Reducing sugars (%)	0.7508	0.3759	0.6566	**<0.0001**
Ratio[a]	0.8475	0.6219	0.5681	**<0.0001**

Note: Bold values indicate significant effects at the $p < 0.05$ level.

Abbreviations: LCPRS-16, Lower Coastal Plain Research Station in Kinston, NC, in 2016; LCPRS-17, Lower Coastal Plain Research Station in Kinston, NC, in 2017; OTRS-17, Oxford Tobacco Research Station in Oxford, NC, in 2017; UCPRS-17, Upper Coastal Plain Research Station near Rocky Mount, NC, in 2017.

[a]Ratio of reducing sugars concentration verses total alkaloids concentration.

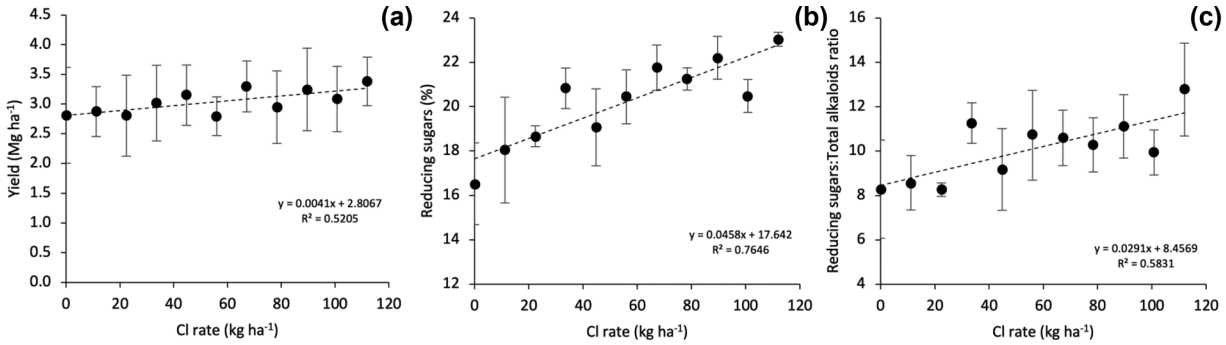

FIGURE 2 Effect of chloride application rates on (a) tobacco yield, (b) reducing sugars and (c) reducing sugars:total alkaloids ratio in the growing environment at the Upper Coastal Plain Research Station near Rocky Mount, NC, in 2017

TABLE 5 Significance of the effects of chloride application rates (C), sampling intervals (I), and the interaction of C × I to tobacco leaf concentration of N, P, K, Ca, Mg, S, Cl, and N–NO_3^- as resulting from analysis of variance (ANOVA) in four growing environments in NC

Site	Factor	N	P	K	Ca	Mg	S	Cl	N–NO_3^-
LCPRS-16	Chloride rate (C)	0.9928	0.0641	0.172	0.8688	0.0655	**<0.0001**	**<0.0001**	**0.0075**
	Sampling interval (I)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	C × I	**0.0383**	0.0601	**0.0008**	0.8550	0.2588	**0.0299**	**0.0197**	0.1403
LCPRS-17	Chloride rate (C)	0.2757	0.2375	**0.0294**	0.1433	0.1358	**<0.0001**	**<0.0001**	**0.0324**
	Sampling interval (I)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	C × I	0.0943	0.3404	0.5552	0.3893	0.3893	**0.0002**	**<0.0001**	**0.0345**
OTRS-17	Chloride rate (C)	**0.0323**	0.9454	**0.0303**	0.6262	0.4504	**<0.0001**	**<0.0001**	0.0970
	Sampling interval (I)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	C × I	0.1453	0.4613	0.8147	0.1524	0.1420	**0.0392**	**0.0003**	0.1090
UCPRS-17	Chloride rate (C)	0.1825	0.7663	0.6962	**<0.0001**	0.4085	**<0.0001**	**<0.0001**	**0.0012**
	Sampling interval (I)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	C × I	0.9405	0.8374	0.5253	0.3578	0.3130	0.7076	**0.0051**	**0.0253**

Note: Bold type indicates the *p*-value is <0.05.

Abbreviations: LCPRS-16, Lower Coastal Plain Research Station in Kinston, NC, in 2016; LCPRS-17, Lower Coastal Plain Research Station in Kinston, NC, in 2017; OTRS-17, Oxford Tobacco Research Station in Oxford, NC, in 2017; UCPRS-17, Upper Coastal Plain Research Station near Rocky Mount, NC, in 2017.

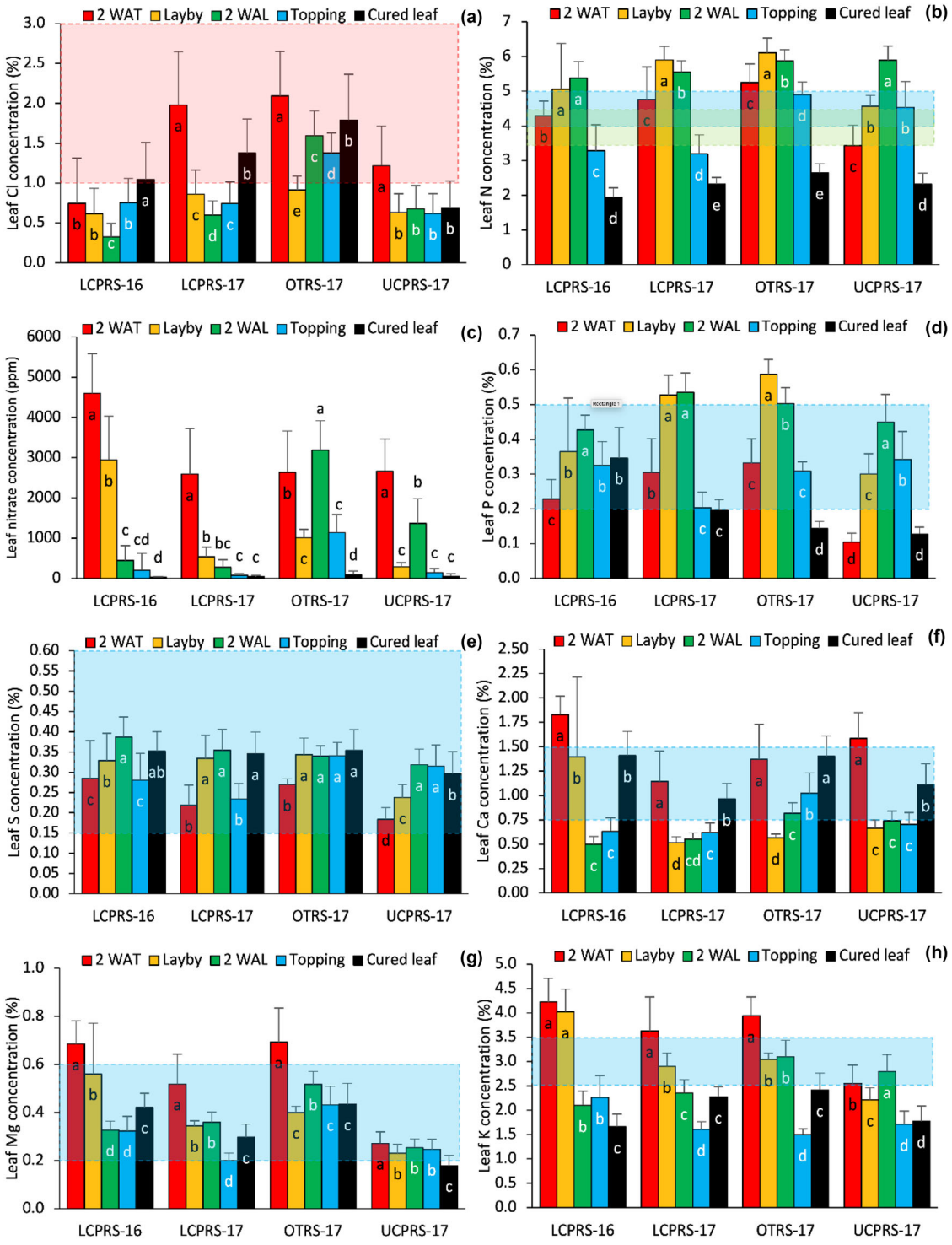

FIGURE 3 Variation of (a) Cl, (b) N, (c) N-nitrate, (d) P, (e) S, (f) Ca, (g) Mg, and (h) K concentration in tobacco leaf at different sampling intervals in four growing environments in NC. Means followed by the same letter in columns comparing sampling times in each growing environment are not significantly different by Tukey's test at a significance level of $p < 0.05$. Each bar is an average of 44 observations (11 rates of Cl and 4 replicates). Blue band indicates adequate nutrient content for early growth, and green band indicates adequate N content for flowering according to Campbell (2013). The red band indicates the 1% Cl content in tobacco leaf for excellent quality classification according to Zehler et al. (1981). LCPRS-16, Lower Coastal Plain Research Station in Kinston, NC, in 2016; LCPRS-17, Lower Coastal Plain Research Station in Kinston, NC, in 2017; OTRS-17, Oxford Tobacco Research Station in Oxford, NC, in 2017; UCPRS-17, Upper Coastal Plain Research Station near Rocky Mount, NC, in 2017

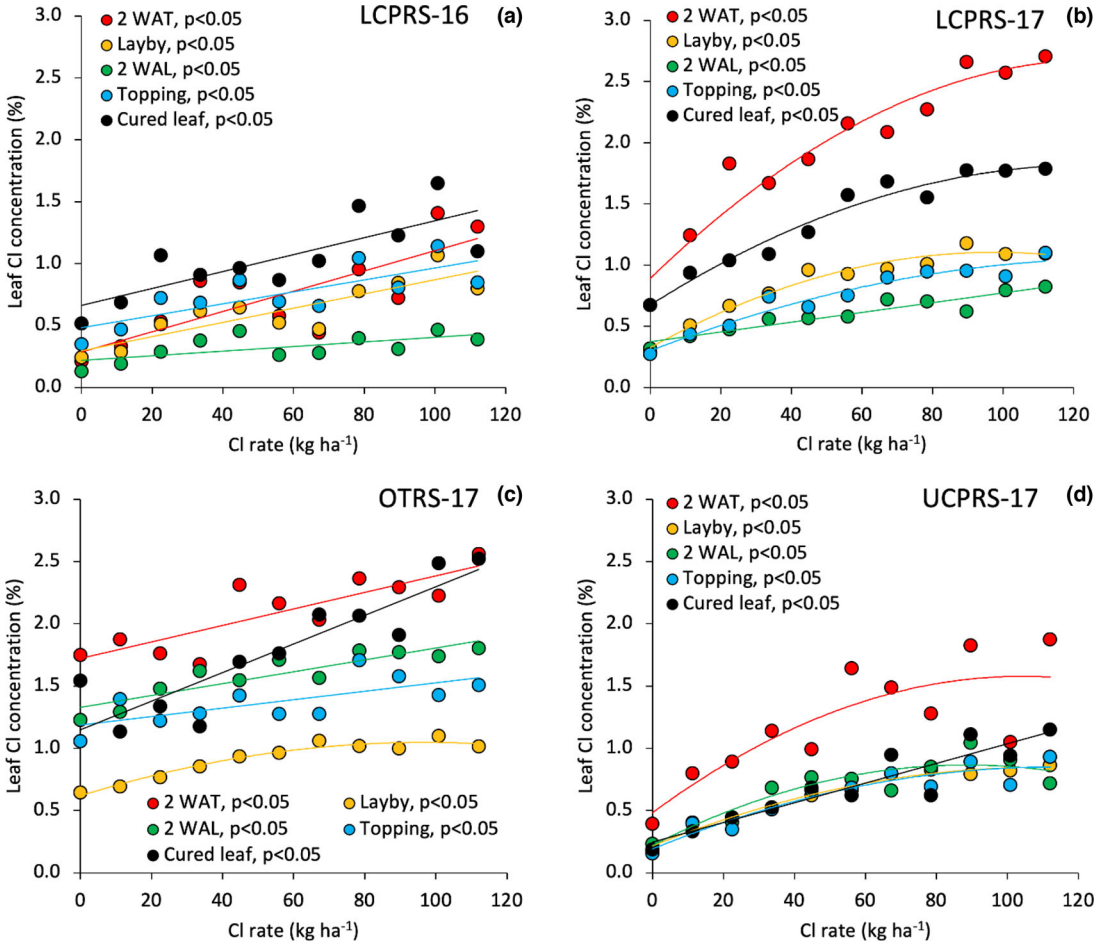

FIGURE 4 Effect of Cl application rates in different sampling intervals on Cl concentration in tobacco leaf in four growing environments: (a) LCPRS-16, Lower Coastal Plain Research Station in Kinston, NC, in 2016; (b) LCPRS-17, Lower Coastal Plain Research Station in Kinston, NC, in 2017; (c) OTRS-17, Oxford Tobacco Research Station in Oxford, NC, in 2017; (d) UCPRS-17, Upper Coastal Plain Research Station near Rocky Mount, NC, in 2017. Regression equations are given in Table S1. 2WAL, 2 weeks after layby; 2WAT, 2 weeks after treatment application; Topping, at flowering

3.2 | Effect of Cl rates on yield, leaf chemistry, quality, and value of tobacco

Tobacco leaf yield was not affected by the Cl rates in the LCPRS-16, LCPRS-17, and OTRS-17 growing environments (Table 4). This result agrees with findings of Moore et al. (2011) and Pace et al. (2020). Moreover, in these growing environments, Cl rates did not affect tobacco quality, concentration of total alkaloids and reducing sugars or their ratio, price, or value (Table 4).

On the other hand, in the growing environment UCPRS-17, there was a linear increase in tobacco leaf yield with the increasing Cl rates applied (Table 4; Figure 2a). Tobacco leaf yield increased on average 4.1 kg kg−1 of Cl applied (Figure 2a). Similar results from NC were found in the southern United States by McCants and Woltz (1967) and Warren et al. (1990), with increases in flue-cured tobacco yield with rates of Cl ranging from 22 to 45 kg ha^{-1}. Although reducing sugars (Figure 2b) and ratio (Figure 2c) also increased with Cl rates, tobacco quality and value were unaffected. The increase in reducing sugars due to Cl application was also observed by Karaivazoglou et al. (2005). These results indicate that in years with rainfall below the average where the potential for Cl leaching is low, the amount of Cl applied by tobacco growers using soil fumigant such as chloropicrin (CCl_3NO_2) (28–84 kg ha^{-1} of active ingredient, corresponding to 18–54 kg ha^{-1} of Cl; Becker et al., 2005) may be enough to meet the crop's demand for the nutrient.

3.3 | Effect of Cl rates and sampling intervals on nutrient concentration in tobacco leaves

The concentrations of all nutrients in the tobacco leaves were influenced by sampling time in every environment (Table 5).

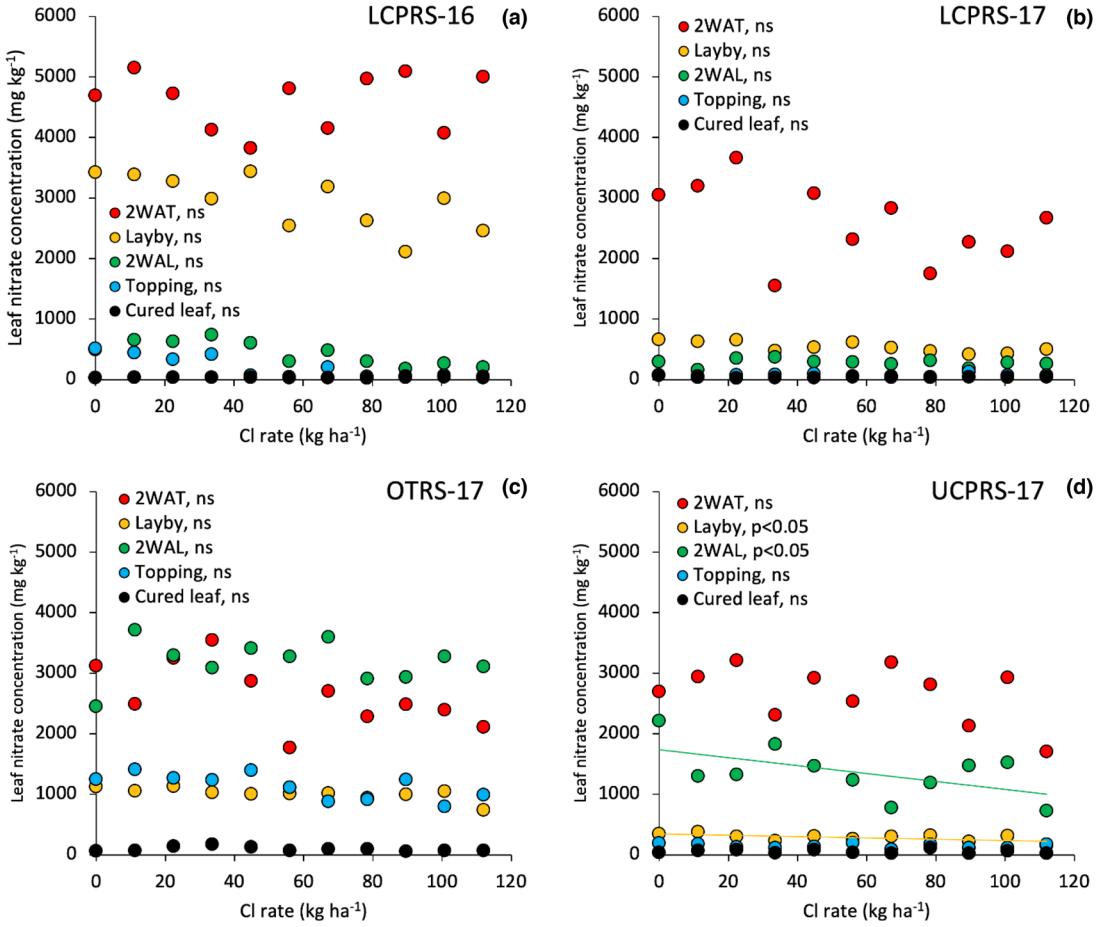

FIGURE 5 Effect of Cl application rates in different sampling intervals on nitrate concentration in tobacco leaf in four growing environments: (a) LCPRS-16, Lower Coastal Plain Research Station in Kinston, NC, in 2016; (b) LCPRS-17, Lower Coastal Plain Research Station in Kinston, NC, in 2017; (c) OTRS-17, Oxford Tobacco Research Station in Oxford, NC, in 2017; (d) UCPRS-17, Upper Coastal Plain Research Station near Rocky Mount, NC, in 2017. Regression equations are given in Table S3. 2WAL, 2 weeks after layby; 2WAT, 2 weeks after treatment application; Topping, at flowering

In contrast, Cl rate primarily changed the concentration of Cl, S, and NO_3^- (Table 5).

At all environments except the LCPRS-16, leaf Cl concentration was higher at 2WAT than other sampling intervals (Figure 3a). As expected, the leaf Cl concentration increased with increasing Cl rates in all sampling intervals and all growing environments (Figure 4). In the LCPRS-16, Cl rates ranging between 34 and 67 kg ha^{-1} resulted in Cl concentration oscillating close to 1% (Figure 4a), whereas rates above 78 kg ha^{-1} seem to consistently result in Cl levels above this threshold for cured leaves considered excellent quality (<1% of Cl) (Zehler et al., 1981). On the other hand, in the second year in this environment (LCPRS-17), Cl rates ≥34 kg ha^{-1} resulted in Cl concentration in cured leaf being higher than 1% (Figure 4b). Interestingly, in the OTRS-17 growing environment, the Cl^- concentration in cured leaves was above the 1% threshold for all rates, including the control treatment (Figure 4c). By contrast in the UCPRS-17 environment, this threshold was only surpassed at rates higher than 90 kg ha^{-1} (Figure 4d), whereas the rates between 90 and 112 kg ha^{-1} were on average, only 0.1% above the 1% threshold.

The leaf N content in tobacco was only affected by Cl rates at the LCPRS-16 (significant interaction between sampling interval and Cl rate) and OTRS-17 environment (single effect of Cl rate) (Table 5); however, the lack of significance in regression did not allow a mathematical model in both cases. Comparing the sampling intervals, the leaf N concentration presented the same trend in all environments as growth occurred. Averaged over environments, N increased from the first sampling period (4.4% at 2WAT) and approached maximum content at layby (5.4%) and 2WAL (5.7%) (Figure 3b). After that, the N content decreased about 30% at topping (4.0%), with a further decrease in cured leaves, resulting in a low of 2.3% (Figure 3b). For the early growing season except for the UCPRS-17 growing environment at 2WAT, the leaf N content was above the critical level of 4.0% (Table 3). At topping, the N content was above (OTRS-17 and UCPRS-17) or slightly below the critical level of 3.5% (Table 3). These

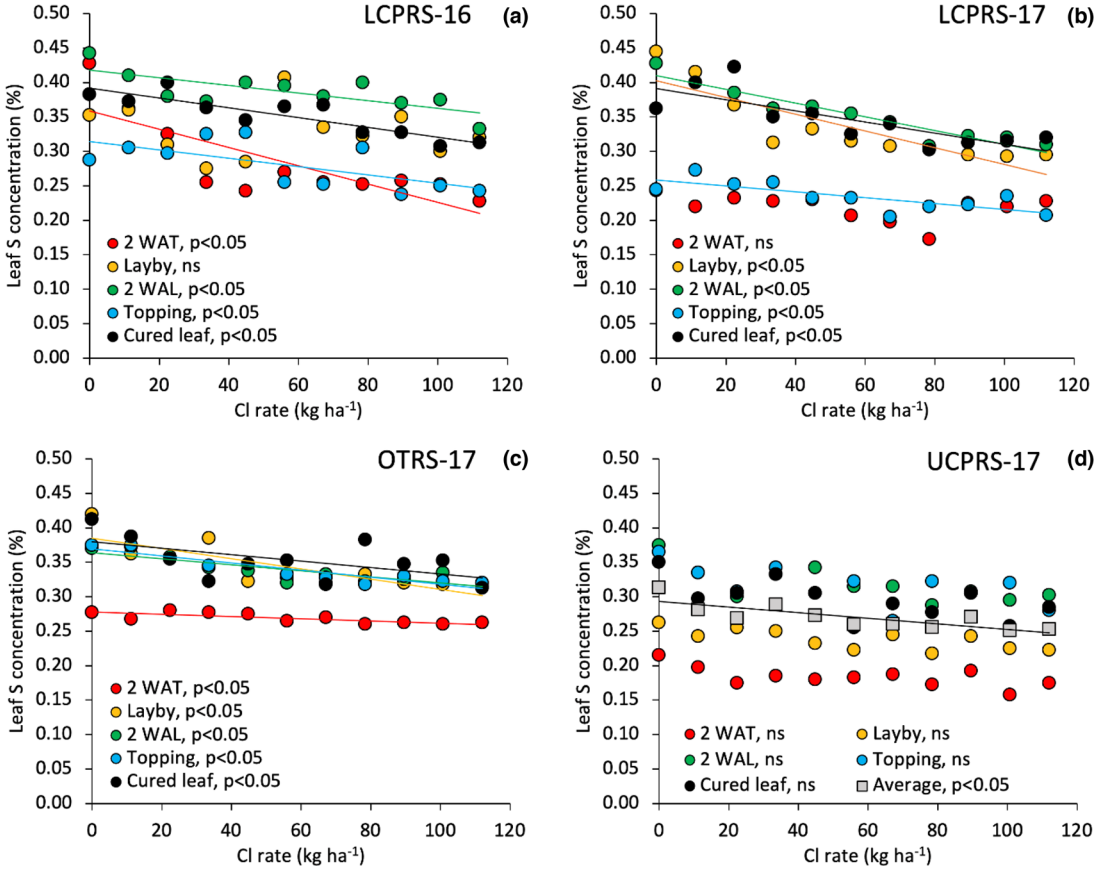

FIGURE 6 Effect of Cl application rates in different sampling intervals on S concentration in tobacco leaf in four growing environments: (a) LCPRS-16, Lower Coastal Plain Research Station in Kinston, NC, in 2016; (b) LCPRS-17, Lower Coastal Plain Research Station in Kinston, NC, in 2017; (c) OTRS-17, Oxford Tobacco Research Station in Oxford, NC, in 2017; (d) UCPRS-17, Upper Coastal Plain Research Station near Rocky Mount, NC, in 2017. Regression equations are given in Table S2. 2WAL, 2 weeks after layby; 2WAT, 2 weeks after treatment application; Topping, at flowering

results indicate that N was probably not a limiting nutrient in the four growing environments. Moreover, the N content was above the maximum optimal range of 5% in the layby and 2WAL sampling intervals, which may indicate luxury consumption (Table 3).

Differences in leaf NO_3^--N were found among sampling intervals (Figure 3c). At all site-years except OTRS-17, concentrations were highest at 2WAT, with a value as great as 4598 mg kg^{-1} being found in LCPRS-16. At LCPRS-16 and LCPRS-17, levels declined during the growing season and ranged from 34 to 93 mg kg^{-1} in cured leaves (Figure 3c). In the OTRS-17 and UCPRS-17, an increase in NO_3^- concentration at 2WAL (Figure 3c) was observed, resulting from the second application of N to the crop, with a subsequent continual decline at topping to harvest of cured leaf. Although NO_3^- concentration in tobacco leaves was significantly affected by Cl rates in all growing environments, except OTRS-17 ($p = 0.0970$) (Table 5), there was no clear trend for this relationship (Figure 5). These results differ from Fuqua et al. (1974) that found an inhibition of NO_3^- absorption by Cl^-.

Leaf P concentration was not affected by Cl rates in all growing environments (Table 5). Like N, leaf P concentration presented the same trend in all growing environments, increasing from the first sampling period (0.24% at 2WAT) and reaching the maximum content at layby (0.44%) and 2WAL (0.48%) (Figure 3d). After that, the leaf P content decreased about 38% at topping (0.29%), and then, in cured leaves, it further decreased to a P content of 0.20%. Except for the first sampling period in the UCPRS-17 environment, the leaf P content remained above the critical level in all sampling periods and sites (>0.20%; Table 3). These results indicate that P probably was not a limiting nutrient for tobacco.

The Cl application rates directly influenced the leaf S concentration in tobacco in all growing environments (Table 5). Like P and N, the leaf S concentration increased from the first sampling interval (2WAT) and reached the maximum concentration between layby and 2WAL, while generally maintaining levels through topping and harvest (Figure 3e). At the LCPRS-16, LCPRS-17, and OTRS-17, the leaf S content decreased with the increasing Cl application rates in all sampling intervals, except for layby in LCPRS-16 and 2WAL in

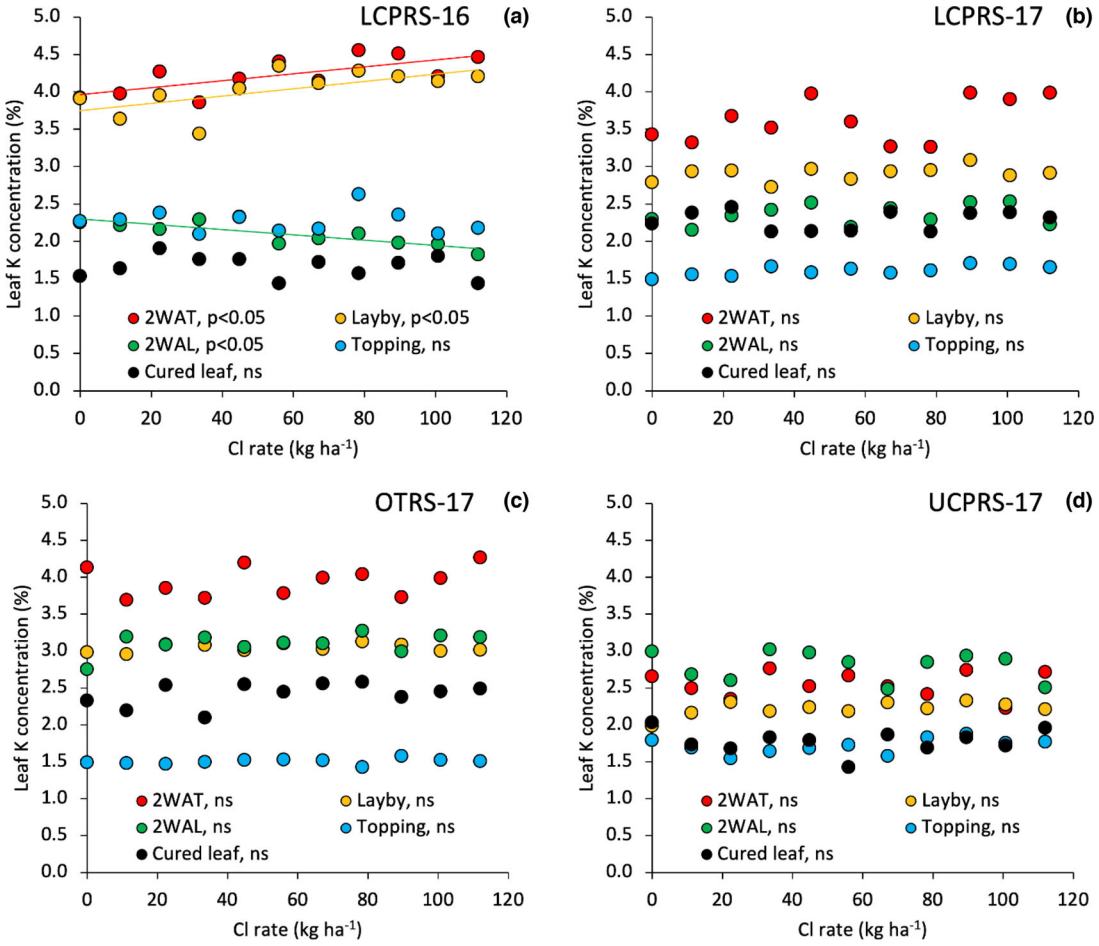

FIGURE 7 Effect of Cl application rates in different sampling intervals on K concentration in tobacco leaf in four growing environments: (a) LCPRS-16, Lower Coastal Plain Research Station in Kinston, NC, in 2016; (b) LCPRS-17, Lower Coastal Plain Research Station in Kinston, NC, in 2017; (c) OTRS-17, Oxford Tobacco Research Station in Oxford, NC, in 2017; (d) UCPRS-17, Upper Coastal Plain Research Station near Rocky Mount, NC, in 2017. Regression equations are given in Table S4. 2WAL, 2 weeks after layby; 2WAT, 2 weeks after treatment application; Topping, at flowering

LCPRS-17 (Figure 6). At the UCPRS-17, there was no interaction between the sampling times and the Cl rates applied, but in the average of all the sampling periods evaluated, the S content in the leaf decreased with the Cl rates. Wedin and Struckmeyer (1958) also found that S uptake was depressed by an increase in Cl in tobacco leaves. However, in all cases evaluated in our study, the leaf S concentration was never below the optimal range (0.15%–0.60%, Table 3) (Figures 3e and 6), indicating that S was not a limiting nutrient.

The leaf Ca content was affected by the Cl rates only at the UCPRS-17 (Table 5), but the regression model was not significant. Among the sampling intervals, there was a similar variation in the Ca content of tobacco leaves in the four growing environments; Ca content was highest in the first evaluation (1.48% at 2WAT) (Figure 3f) and above the minimum content of the appropriate range (0.75%–1.50%) for tobacco development (Table 3). In the following sampling intervals (layby, 2WAL, and topping), leaf Ca content decreased to values near the minimal concentration (0.78%, 0.65%, and 0.74%, respectively; Figure 3f). Interestingly, there was an increase in Ca content in the cured leaf compared to the green leaf at latter growth stages (2WAL and topping) in all environments (Figure 3f).

The leaf Mg content was not affected by Cl rates in all growing environments (Table 4). In all sites, the leaf Mg content decreased over the sampling intervals (Figure 3g), indicating a dilution effect with tobacco growth. In LCPRS-16, LCPRS-17, and OTRS-17, the leaf Mg content remained in the middle of the appropriate range for tobacco development (0.2%–0.6%; Table 3) in most sampling intervals (Figure 3h). In fact, in the first sampling interval at LCPRS-16 and OTRS-17, the Mg content was above this range. These results indicate that Mg was probably not a limiting nutrient in these three environments. By contrast, in the UCPRS-17, the leaf Mg concentration was 0.27%, 0.23%, 0.25%, and 0.25% at 2WAT, layby, 2WAL, and topping, respectively (Figure 3g). These values are close to the minimal content of the optimal range. Moreover, in the first sampling interval, the leaf Mg

concentration in UCPRS-17 was about 2.0–2.5 times lower than in the other environments. These results indicate that Mg may have been a limiting nutrient for tobacco development in UCPRS-17.

Although the effect of Cl application rates on leaf K content was significant in the LCPRS-17 and OTRS-17 growing environments (Table 5), the regressions provided no significant relationship (Figure 7). On the other hand, in the LCPRS-16, there was a significant interaction between Cl rates and sampling interval (Table 5). In this growing environment, leaf K content increased with Cl rates at 2WAT and at layby, decreased with Cl rates at 2WAL, and was not affected by Cl rates at flowering and harvest of the cured leaf (Figure 7a). Like Ca and Mg in green leaves, the leaf K concentration was maximized in the first sampling interval and then decreased over time, except where K was highest at 2WAL in UCPRS-17 (Figure 3h), indicating a dilution effect of the nutrient with plant growth. In the LCPRS-16, LCPRS-17, and OTRS-17 growing environments, the leaf K content was above the adequate range (2.5%–3.5%; Table 3) at 2WAT and remained within this optimal range until layby (LCPRS-16 and LCPRS-17) and 2WAL (OTRS-17) (Figure 3h). These results indicate that K was probably not a limiting nutrient in these three environments. However, in the UCPRS-17, the leaf K concentration was barely above the minimum concentration required, and on average was 35% lower than in the other growing environments in the first sampling interval. These results indicate that K could be a limiting nutrient for tobacco development in UCPRS-17.

4 | CONCLUSION

The application of Cl rates up to 112 kg ha^{-1} did not reduce the productivity or quality of flue-cured tobacco in any of the four growing environments evaluated. The Cl rate to reach the established threshold for Cl content in cured leaf of 1.0% was site-specific, being surpassed even in the control treatment in OTRS-17, or with Cl rates higher than 34 and 90 kg ha^{-1} in LCPRS (both years) and UCPRS-17, respectively.

In one (UCPRS-17) of the four growing environments, the Cl rates increased tobacco yield, probably due the direct effect of Cl as a nutrient. Although the increasing Cl rates also increased the reducing sugars concentration and altered the reducing sugars:total alkaloids ratio, tobacco quality was unaffected. Regardless of the increased yield and nutrient uptake recorded in this environment, Cl applications above 34 kg ha^{-1} should not be recommended by extension agronomists, due to the potential negative impact the nutrient may have on combustibility or smoke quality, flavor, and aroma.

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AUTHOR CONTRIBUTIONS

Tales Tiecher: Validation; visualization; writing – original draft; writing – review & editing. **Cara Ruth Pace**: Conceptualization; data curation; formal analysis; investigation; methodology. **Luke Gatiboni**: Supervision; Validation; Visualization; Writing – review & editing. **Matthew Vann**: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – review & editing. **David Hardy**: Formal analysis; resources; validation; visualization; writing – review & editing. **Loren Fisher**: Conceptualization; funding acquisition; methodology; resources; supervision; validation; writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Akehurst, B. C. (1981). Usability and some properties important for the manufactured product. In *Tropical agricultural series: Tobacco* (pp. 611–617). Longman.

Becker, J., Chism, W., Donaldson, D., Kaul, M., & Kiely, T. (2005). *Overview of the use and usage of soil fumigants*. U.S. Environmental Protection Agency, Office of Pesticide Programs. https://archive.epa.gov/pesticides/reregistration/web/pdf/soil_fumigant_use.pdf

Bowman, D. T. (1996). History of the regional minimum standards program for the release of flue-cured tobacco varieties in the United States. *Tobacco Science*, *40*, 99–110.

Bowman, D. T., Tart, A. G., Wernsman, E. A., & Corbin, T. C. (1988). Revised North Carolina grade index for flue-cured tobacco. *Tobacco Science*, *32*, 39–40.

Broyer, T. C., Carlton, A. B., Johnson, C. M., & Stout, P. R. (1954). Chlorine—A micronutrient element for higher plants. *Plant Physiology*, *29*, 526–532. https://doi.org/10.1104/pp.29.6.526

California State Transportation Agency (2014). *Methods of testing soils, concrete patching materials, and waters for chloride content* (California Test 422). Division of Engineering Services.

California State Transportation Agency. https://dot.ca.gov/-/media/dot-media/programs/engineering/documents/californiatestmethods-ctm/ctm-422-apr2020-a11y.pdf

Campbell, C. R. (Ed.). (2013). *Reference sufficiency ranges for plant analysis in the Southern region of the United States* (Southern Cooperative Series Bulletin #394, pp. 41–42). North Carolina Department of Agriculture and Consumer Services. http://www.ncagr.gov/agronomi/saaesd/scsb394.pdf

Chen, W., He, Z. L., Yang, X. E., Misrha, S., & Stofella, P. J. (2010). Chlorine nutrition of higher plants: Progress and perspectives. *Journal of Plant Nutrition*, *33*, 943–952. https://doi.org/10.1080/01904160903242417

Colmenero-Flores, J. M., Franco-Navarro, J. D., Cubero-Font, P., Peinado-Torrubia, P., & Rosales, M. A. (2019). Chloride as a beneficial macronutrient in higher plants: New roles and regulation. *International Journal of Molecular Sciences*, *20*, 4686. https://doi.org/10.3390/ijms20194686

Davis, R. E. (1976). A combined automated procedure for the determination of reducing sugars and nicotine alkaloids in tobacco products using a new reducing sugar method. *Tobacco Science*, *20*, 139–144.

Franco-Navarro, J. D., Brumós, J., Rosales, M. A., Cubero-Font, P., Talón, M., & Colmenero-Flores, J. M. (2016). Chloride regulates leaf cell size and water relations in tobacco plants. *Journal of Experimental Botany*, *67*, 873–891. https://doi.org/10.1093/jxb/erv502

Fuqua, B. D., Leggett, J. E., & Sims, J. L. (1974). Accumulation of nitrate and chloride by burley tobacco. *Canadian Journal of Plant Science*, *54*, 167–174. https://doi.org/10.4141/cjps74-026

Garner, W. W., McMurtrey, Jr., J. E., Bowling, J. D., & Moss, E. G. (1930). Role of chlorine in nutrition and growth of the tobacco plant and its effect on the quality of the cured leaf. *Journal of Agricultural Research*, *40*, 627–648.

Honda, N. (1963). The uptake and distribution of chlorine in tobacco plant. *Bulletin Kagoshima Tobacco Experimental Station*, *11*, 6–11.

Ishizaki, U., & Akiya, T. (1978). Effects of chlorine on growth and quality of tobacco. *Japan Agricultural Research Quarterly*, *12*, 1–6.

Karaivazoglou, N. A., Papakosta, D. K., & Divanidis, S. (2005). Effect of chloride in irrigation water and form of nitrogen fertilizer on Virginia (flue-cured) tobacco. *Field Crops Research*, *92*, 61–74. https://doi.org/10.1016/j.fcr.2004.09.006

Liu, X., Hu, C., Zhu, Z., Riaz, M., Liu, X., Dong, Z., Liu, Y., Wu, S., Tan, Z., & Tan, Q. (2021). Migration of chlorine inplant—soil—leaching system and its effects on the yield and fruit quality of sweet orange. *Frontiers in Plant Science*, *12*, 744843. https://doi.org/10.3389/fpls.2021.744843

Marschner, P. (2012). *Mineral nutrition of higher plants* (3rd ed.). Academic Press.

McCants, C. B., & Woltz, W. G. (1967). Growth and mineral nutrition of tobacco. *Advances in Agronomy*, *19*, 211–265. https://doi.org/10.1016/S0065-2113(08)60736-1

Mehlich, A. (1984). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis*, *15*, 1409–1416. https://doi.org/10.1080/00103628409367568

Moore, J. M., Bafalluy, R., LaHue, S. S., & Troxell, C. E. (2011). Evaluation of growth, yield, grade and chloride content of flue-cured tobacco fertilized with various rates of KCl and K-Mag as potassium sources [Paper presentation] *2011 CORESTA agronomy/phytopathology meeting, Santiago, Chile. 06-10 Nov. Paper AP02*. https://www.coresta.org/sites/default/files/abstracts/2011_AP02_Moore.pdf

Myhre, D. L., Attoe, O. J., & Ogden, W. B. (1956). Chlorine and other constituents in relation to tobacco leaf-burn. *Soil Science Society Proceedings*, *20*, 1956. https://doi.org/10.2136/sssaj1956.03615995002000040024x

Pace, C. R., Vann, M. C., Fisher, L. R., & Hardy, D. H. (2020). Implications of chloride application rate and nitrogen fertilizer source to flue-cured tobacco. *Agronomy Journal*, *112*, 2916–2927. https://doi.org/10.1002/agj2.20251

Plank, C. O. (1992). *Plant analysis reference procedures for the southern region of the United States* (Southern Cooperative Series Bulletin 368). Georgia Agricultural Experiment Station. http://www.ncagr.gov/agronomi/pdffiles/sera368.pdf

Rosales, M. A., Franco-Navarro, J. D., Peinado-Torrubia, P., Díaz-Rueda, P., Álvarez, R., & Colmenero-Flores, J. M. (2020). Chloride improves nitrate utilization and NUE in plants. *Frontiers in Plant Science*, *11*, 442. https://doi.org/10.3389/fpls.2020.00442

Saso, J. K., Parkin, G. W., Drury, C. F., Lauzon, J. D., & Reynolds, W. D. (2012). Chloride leaching in two Ontario soils: Measurement and prediction using HYDRUS-1D. *Canadian Journal of Soil Science*, *92*, 285296. https://doi.org/10.4141/CJSS2011-046

Sierra, F. A. (1966). *Interrelationships of sulfur, chloride, and potassium on certain chemical and physical properties of flue-cured tobacco* [PhD dissertation, North Carolina State University].

Warren, R. L. (1990). *Effects of nitrogen source and chloride rate on some agronomic and chemical characteristics of flue-cured tobacco*. [Master's thesis, North Carolina State University].

Wedin, W. F., & Struckmeyer, B. E. (1958). Effects of chloride and sulfate ions on the growth, leaf burn, composition and anatomical structure of tobacco (*Nicotiana tabacum* L.). *Plant Physiology*, *33*, 133–139. https://doi.org/10.1104/pp.33.2.133

Xu, G., Magen, H., Tarchitzky, J., & Kafkafi, U. (2000). Advances in chloride nutrition of plants. *Advances in Agronomy*, *68*, 97–150. https://doi.org/10.1016/S0065-2113(08)60844-5

Zehler, E., Kreipe, H., & Gething, P. A. (1981). *Potassium sulfate and potassium chloride—Their influence on the yield and quality of cultivated plants* (pp. 63–64) International Potash Institute Research Topics.

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