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Effect of topping height and ripeness on quality of flue-cured tobacco varieties

Utjecaj visine zalamanja i zrelosti na kvalitetu flue-cured sorata duhana

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ABSTRACT

Investigations of the topping height, ripeness and tobacco variety on the price and quality indices, as expressed by reducing sugars/nicotine, total nitrogen/nicotine and N-proteins/total nitrogen ratios, were conducted during 2004 – 2005 and 2007. Trial treatments included: (1) topping height (17 and 20 leaves for harvesting), (2) leaf ripeness at harvest (unripe, ripe and overripe) and (3) variety (HVT 1, VJ 1, DH 17). Investigation results showed that precipitation deficiency in 2004 resulted in a decrease of overripe tobacco prices, while water stress at the intensive growth stage in 2007 caused a significant price increase from unripe to overripe tobacco harvesting. In the first two trial years, the best reducing sugars/nicotine ratios were achieved by overripe tobacco harvesting. In 2007, delayed ripeness of tobacco led to irreparable disruption of the reducing sugars/nicotine ratio, below acceptable limits, irrespective of leaf ripeness at harvest. The total nitrogen/nicotine ratio was within the limits for good-quality tobacco in all trial years, while higher ratios were recorded in the climatically most favourable year 2005 also in tobaccos with higher topping. The N-proteins/total nitrogen ratio was in the range acceptable for that ratio in ripe tobacco leaves and in overripe tobacco leaves only in the extreme 2007. Significant impact of variety genetic variability on all the studied traits was established. It is also obvious from the results that irreparable impairment of the tobacco leaf chemical quality in 2007 was manifested only in the reducing sugars/nicotine ratio. Strong correlations were found between price and the reducing sugars/nicotine and N-protein/nitrogen ratios and weak correlations with the total nitrogen/nicotine ratio.

Keywords: N-proteins/nitrogen ratio, price, reducing sugar, reducing sugars/nicotine ratio, tobacco, total nitrogen/nicotine ratio

SAŽETAK

Proučavanja utjecaja visine zalamanja, zrelosti i sorte na cijenu i indekse kvalitete izražene omjerima reducirajući šećeri/nikotin, ukupni dušik/nikotin i N-bjelančevina/ukupni dušik provedena su tijekom 2004 – 2005. i 2007. godine. Postupci u pokusu bili su: (1) visina zalamanja (17 i 20 listova za berbu), (2) zrelost lista u vrijeme berbe (nedozreli, zreli i prezreli) i (3) sorta (HVT 1, VJ 1, DH 17). Rezultati istraživanja pokazali su da je nedostatak oborina u 2004. godini rezultirao padom cijene prezrelog duhana, a vodni stres u stadiju intenzivnog porasta u 2007. godini signifikantnim rastom cijene od berbe nezrelog do prezrelog duhana. U prve dvije godine istraživanja najpovoljniji omjeri reducirajući šećeri/nikotin postignuti su berbom prezrelog duhana. U 2007., godini s prolongiranim dozrijevanjem duhana, omjer reducirajući šećeri/nikotin bio je nepovratno narušen, ispod prihvatljivih granica i bez razlika obzirom na zrelost lista u trenutku berbe. Omjer ukupni dušik/nikotin je u svim godinama istraživanja bio u granicama koje označavaju sadržajan duhan, a veći omjeri ustanovljeni su u klimatski najpovoljnijoj 2005. i u duhanima s višim zalamanjem. Omjer N-bjelančevina/ukupni dušik ustanovljen je u prihvatljivom rasponu za taj omjer u listovima zrelog duhana a prezrelog samo u ekstremnoj 2007. godini. Značajni su bili utjecaji genetskog varijabiliteta sorata na sva svojstva u istraživanju. Iz rezultata je također razvidno da nepovratno narušavanje kemijske kvalitete lista duhana u 2007. godini pokazuje samo omjer reducirajući šećeri/nikotin. Ovim istraživanjem utvrđene su i jake korelacije između cijene i omjera reducirajući šećeri/nikotin i N-bjelančevina/dušik te slabe s omjerom ukupni dušik/nikotin.

Ključne riječi: N-bjelančevina/ukupni dušik, cijena, reducirajući šećeri, reducirajući šećeri/nikotin, duhan, ukupni dušik/nikotin

INTRODUCTION

Tobacco quality depends on the balance between a number of physiological processes during the growing season up to the end of harvest that affect the agronomic traits and chemical composition of dried tobacco leaves Çakir and Çebi, 2010a, Weybrew and Woltz, 1975; Weybrew et al., 1983). The maximum qualitative potential of tobacco is reached at harvest time, since quality is not formed in the drying process but in the course of the growing season. The most common causes of poorer dried tobacco leaf quality are environmental factors, especially precipitation, mainly its distribution rather than its deficit during the growing season. It is a known fact that water stress and irregular distribution of precipitation during tobacco growth and development result in prolonged ripeness of tobacco, its high nicotine content and low content of reducing sugars (Weybrew and Woltz, 1975; Campbell et al., 1982; Weybrew et al., 1983). Severity of the consequences of drought stress for the quality depends on the duration and stage of tobacco growth when inadequate soil moisture occurs (Goenaga, 1989; Maw et al., 2009; Çakir and Çebi, 2010b).

Besides on climatic conditions, visual evaluation of tobacco leaf quality depends on the agrotechnical measures applied in the production as well as on variety adaptability to external conditions. Each agrotechnical measure affects quality in a special way. Several studies have shown that topping height did not have a significant effect on visually evaluated quality, namely, tobacco price (King, 1986; Čavlek et al., 1991), though higher prices in some studies were achieved with higher topping (Miner, 1980; Rao et al., 2003). The effect of leaf ripeness at harvest time on tobacco price was not consistent in the existing literature either. Depending on soil moisture during the growing season, postponement of harvesting resulted price increase until optimal ripeness and then its reduction (Suggs, 1986; Bowman, 2003).

Investigations done to date by other researchers allow the conclusion that no single constituent or group of constituents reflects adequately the chemical quality of tobacco. Considerable efforts have been made to find chemical quality indices that would represent the balance of essential characteristics. Different ratios of tobacco leaf components have been suggested for the evaluation of dried leaf chemical quality, such as the reducing sugars/nicotine ratio (Weybrew et al., 1983; Maw et al., 2009), total nitrogen/nicotine ratio (Tso, 1990; Maw et al., 2009) and N-proteins/total nitrogen ratio (Provost, 1959). According to the investigations done to date, the proposed ratios should be in the range of 6 – 10, 0.7 – 1.1 or 0.3 – 0.4, respectively.

All production factors that affect particular components of the chemical composition disrupt their interrelations as well. Campbell et al. (1982) reported an increase in the reducing sugars/alkaloids ratio with increased topping height. Based on his investigation results, Puangnak and Chotinuchit (1987) concluded that considering the ratio of reducing sugars /nicotine flue-cured tobacco should be harvested at the stage of optimal ripeness. Stocks (1991) and Zhong and Kefa (2003) reported that the reducing sugar/alkaloids ratio decreased with leaf ripeness. Weybrew et al. (1983, 1984), however, did not obtain consistent results and concluded that the reducing sugars/nicotine ratio was an indication of favourable climatic conditions and good agricultural practices. Inadequate soil moisture results in a low ratio while excessive moisture in a high ratio. The authors further maintain that the reducing sugars/nicotine ratio values cannot be applied to all soil types and at all latitudes. Stocks (1991) and Puangnak and Chotinuchit (1987) found a decrease in the total nitrogen/nicotine ratio due to delayed harvesting. Visual quality evaluation is in correlation with particular leaf chemical components and, consequently, also with their ratios (Gaines et al., 1983).

The goal of these investigations was (1) to establish the influence of height of topping, ripeness and variety on the reducing sugars/nicotine ratio, total nitrogen/ nicotine ratio and N-protein/nicotine ratio, and (2) to find out the most favourable ratios for dry farming of tobacco in the tobacco growing regions of Croatia.

MATERIALS AND METHODS

The trial was set up at the production areas of the tobacco enterprise Agroduhan d.o.o. at Slatina in 2004, 2005 and 2007. According to the soil map (Bogunović and Šalinović, 1974), the trial plot soil was luvisol on loess and Pleistocene sand. The soil is of loamy texture, slightly acid (pH 5.6), very poor in humus (0.83%) and moderately supplied with nitrogen (0.08%) in the plough-layer, and poorly supplied with nitrogen in the sub-plough layer (0.04%). It is very well supplied with available potassium (25.25 mg/100) and moderately supplied with available phosphors (11.58 mg/100 g).

Investigations were conducted according to the split-split- plot method with four replications, with topping height as the main factor, degree of leaf ripeness at harvest as the sub-factor and flue-cured tobacco varieties as the sub-sub-factor. The main factor involved two gradations, namely, tobacco was topped to 17 and 20 harvesting leaves. The second factor, ripeness at harvest, involved three gradations, so that tobacco was harvested at three degrees of ripeness: unripe, optimally ripe and overripe. Three tobacco varieties, HVT 1, VJ 1 and DH 17, were the third factor. The main criteria for visual assessment of tobacco leaf ripeness were changes of blade and mid-rib colour, stalk friability and deflection from stalk according to Weybrew et al. (1984). Variety HVT 1 has a medium tall stalk, between 141 and 160 cm, and forms 22–25 leaves for harvesting. Varieties DH 17 and VJ 1 have stalks of 100 to 110 cm tall with averagely 21 leaves for harvesting. Varieties HVT 1 and VJ 1 belong to the group of medium early maturing, while variety DH 17 is of medium late maturing.

The trial plot was represented by one row with 25 tobacco plants. Spacing between rows, that is, trial treatments, was 100 cm while intra-row spacing of plants was 43 cm. Tillage and soil preparation in all three trial years were done in the way common in the flue-cured tobacco growing region of Croatia. Ploughing at 30 cm depth was applied in the autumn, and row ridges were prepared after the spring soil preparation about two weeks before planting. Two cultivations with the machinery were done in the growing season. Fertilization carried out by machine involved broadcasting, at row ridges preparation, of 400 kg/ha of complex mineral fertilizer NPK (0:5:30), and topdressing in bands in the first or second growth cycle of 250 kg/ha of calcium nitrate (14.4% N). Topping was carried out at the 25% flowering stage. Sucker growth was controlled by applying contact (fatty alcohols) and systemic (maleic hydrazide) suckericides.

Tobacco was harvested manually, from the stalk base towards the top, in six harvests. Two leaves were removed at the first harvest, three leaves at the second, third, fourth and fifth harvest each, and the leaves remaining on the stalk at the sixth harvest. Tobacco leaves were harvested unripe, ripe or overripe on the basis of visual evaluation of their ripeness. Tobacco was successively harvested manually in six harvests. Tobacco leaves removed in all treatments were dried in the same typical oven for flue-cured tobacco following the standard curing procedure for drying ripe tobacco. In this way, the fact that yellowing was not optimal for unripe and overripe tobacco could not be avoided.

Samples of dried tobacco leaves, about 100 g, from the fourth harvest (leaves 9, 10 and 11) were used for chemical analyses. Nicotine and reducing sugars were analysed according to HRN ISO 15152 and HRN ISO

15154. Total nitrogen was determined by the Kjeldahl method. These chemical analyses enabled quality evaluation according to internationally recognized criteria (quality indices), N-protein/total nitrogen (Provost, 1959), total nitrogen/nicotine (Tso, 1990) and reducing sugars/nicotine ratios (Weybrew et al., 1983). The correlation coefficient was calculated on the basis of all data from the three trial years, 216 input data in all.

Data were analysed according to the trial set-up method using the mixed procedure of SAS statistical software (SAS Institute Inc., 2004) with least significant difference (LSD) test for treatment comparison (Steel and Torrie, 1960), while Pearson's correlation coefficient was calculated for correlations.

RESULTS AND DISCUSSION

Climatic conditions in the three-year trial period were mainly defined by the amount of precipitation and especially by its distribution (Table 1). The data in Table 1 was obtained from the Croatian Meteorological and Hydrological service for the Virovitica meteorological station, which is about 25 km from the site of the experiment. Considering the known impact of water stress on the growth of tobacco plants (Maw et al., 2009), data are presented only for critical phases of tobacco development. Critical stages of flue-cured tobacco growth, development and ripeness under Croatian production conditions were studied from July to September. In the course of this period, 431.2 mm of precipitation was recorded in the climatically most favourable year 2005, 177.6 mm in the driest 2004, and 205.8 mm in 2007. Rainfall of 48.1 mm was recorded at the stage of intensive tobacco growth and development, stalk elongation, butonization, flowering and onset of ripening in 2004, while only 24.5 mm in 2007. However, the dry period continued in the second half of the growing season in 2004, while from the second decade of August 2007 to the end of the growing season, favourable soil water regime was re-established.

Decisive factors for the production of flue-cured tobacco are adequate nitrogen fertilization and the amount of precipitation in the growing season, with the emphasis on rainfall distribution (Weybrew et al., 1983). As no irrigation was applied in the trial, soil moisture

Table 1. Temperature and precipitation for the months of intensive growth and senescence of tobacco, Virovitica, 2004 - 2005 and 2007

	Mean monthly diurnal temperature (°C)				Precipitation (mm)		
Month	2004	2005	2007	Decade	2004	2005	2007
				I	23	47.6	13.1
July	21.3	21.3	22.3	II	0.7	41.6	2
				III	18.7	76	4.7
				I	5.7	91.3	4.7
August	21.0	18.9	21.1	II	16	53	38.2
				III	18.7	33.4	33.3
				I	3.8	1.4	64.8
September	15.6	16.7	13.7	II	25.9	56.5	31.1
				III	65.1	30.4	14.1
Total					177.6	431.2	205. 8

depended on the amount and distribution of precipitation. The first two years involved climatologically common alteration of weather conditions in a long-term period (insufficient quantities and "rather good" distribution of precipitation during the growing season in 2004 and satisfactory in 2005). The year 2007 was excessive and was characterized by a forty-day-long exceptionally dry period up to the second decade of August. After that "water stress" until the end of the growing season, precipitation was sufficient and soil water regime enabling plant nutrient uptake was established, which resulted in a prolonged growing season and delayed tobacco leaf ripeness. Last tobacco harvest was done 12 and 15 days, respectively, later than in previous years (data not shown); tobacco looked normal when harvested, with all the visual signs of the degree of ripeness at harvest.

Data from Table 2 shows that climatic conditions affected the price and quality indices of tobacco (reducing sugars/nicotine, total nitrogen/nicotine, N-protein/total nitrogen ratios). Influence of climatic conditions was manifested through significant interaction of ripeness and growing season as well as variety and growing season in the price and all the studied indices of tobacco chemical quality. Interaction of ripeness and variety was determined for the total nitrogen/nicotine and N-protein/total nitrogen ratios. Interaction of year by topping height by ripeness was significant only for the total nitrogen/nicotine ratio. Due to these interactions, the impact of year on the average values of studied characteristics should not be taken as such, but should be considered in the framework of interactions. For this reason, the table of average year impact values on the studied characteristics is not shown.

Significant effect of topping height was found only for average values of total nitrogen/nicotine ratio (Table 3). However, for the same reason as for the influence of

Table 2. ANOVA for tobacco price and quality index

Source of variance	Price		Reducing sugar/ nicotine ratio		Total nitrogen/ nicotine ratio		N-protein/ total nitrogen ratio	
	Fexp		Fexp		Fexp		Fexp	
Year (Y)	235.77	**	67.96	**	57.7	**	41.15	**
Toping height (TH)	0.17	ns	0.38	ns	6.53	*	1.69	ns
Y×TH	0.19	ns	0.27	ns	4.32	*	0.01	ns
Ripeness (R)	3.22	*	9.41	**	30.9	**	11.97	**
Y×R	7.71	**	5.96	**	23.14	**	30.57	**
TH×R	2.23	ns	0.87	ns	0.93	ns	2.95	ns
Y×TH×R	0.29	ns	1.34	ns	4.72	**	1.3	ns
Variety (V)	4.45	*	2.84	ns	3.8	*	2.22	ns
Y×V	9.7	**	2.79	*	5.64	**	3.57	**
M×V	0.65	ns	1.56	ns	2.5	*	4.67	**
TH×M×V	0.84	ns	1.29	ns	0.96	ns	1.41	ns

** Significant at P<0.01, * significant at P<0.05, ns not significant.

the year, the total nitrogen/nicotine ratio is considered only through double interaction. Tobacco price reflects the visually assessed quality of dried tobacco leaf. The non-significant effect of topping height on the price in this trial is in accord with the results of King (1986) and Čavlek et al. (1991). However, Miner, (1980) and Rao et al. (2003), significantly higher-grade index achieved with higher topping.

Based on the reducing sugars/nicotine and N-proteins/total nitrogen ratios, data in Table 3 show that effects of topping height were not significant. In previous studies, Campbell et al. (1982), King (1986) and Zulkifly et al. (1992) reported that reducing sugars/nicotine ratio increased with an increase in topping height, while Vuletić and Prpić (1980) established that better reducing sugars/nicotine and total nitrogen/nicotine ratios were obtained by lower topping.

In 2004, harvesting of ripe tobacco led to a significantly higher price compared to overripe tobacco, while the differences in price were not significant in the following year (Table 4). In 2007, each harvest delay resulted in a significant price increase. The obtained results are in accord with the studies of Suggs (1986) and Bowman (2003). In 2005, the year with adequate quantities and distribution of precipitation, no significant differences in price were recorded between the varieties under study. In 2004, the season with insufficient precipitation, variety VJ 1 achieved the lowest dried leaf price. In 2007, however, characterized by a forty-day-long excessively dry period up to the second decade of August followed by retrovegetation of the same variety, the significantly highest price was achieved. In 2007, all the three varieties achieved a significantly lower price compared to the two preceding years. Adaptability of varieties to the production conditions with maintenance and/or improvement of qualitative traits, as attained in this trial, was reported earlier by Gaines et al. (1983), Kozumplik et al. (1989), Beljo et al. (1999) and Bowman (2003).

Contents of reducing sugars and nicotine contribute most to the quality of smoking. The optimal ratio of reducing sugars/nicotine ranges from 6 to 9 (Weybrew et al., 1983). Maw et al. (2009) report the range of 6 – 10, preferably closer to 10. According to so far findings, if reducing sugars and nicotine have the mentioned ratio, other chemical components are balanced as well (Weybrew et al., 1983; Weybrew et al., 1984). This ratio is used to evaluate the balance of hydrocarbons and nicotine in flue-cured tobacco leaves. A high reducing sugars to nicotine ratio (over 9) marks less substantial

Table 3. Effect of topping height on price, reducing sugars/nicotine ratio, total nitrogen/nicotine ratio and N-proteins/total nitrogen ratio of flue-cured tobacco

Topping height	Price, kn/kg	Reducing sugar/nicotine ratio	Total nitrogen/nicotine ratio	N-protein/total nitrogen ratio
17	8.8[a]	9.38[a]	0.97[a]	0.46[a]
20	8.91[a]	8.99[a]	0.92[b]	0.45[a]

Average values marked by the same lowercase letter are not statistically different within the same column (P<0.05).

Table 4. Effect of interactions between year and ripeness, and between year and variety on price of flue-cured

Year	Ripeness			Variety		
	Unripe	Ripe	Overripe	HVT 1	VJ 1	DH 17
2004	10.3[abA]	10.44[aA]	9.67[bB]	10.7[aA]	9.46[bB]	10.25[aA]
2005	10.38[aA]	10.53[aA]	10.6[aA]	10.6[aA]	10.5[aA]	10.41[aA]
2007	5.16[cB]	5.89[bB]	6.52[aC]	5.88[bB]	6.36[aC]	5.33[cB]

Average values marked by the same lowercase letter are not statistically different within the same year (P<0.05). Average values marked by the same uppercase letter are not statistically different within the same ripeness and variety (P<0.05).

tobacco, relatively mild smoke, and if the ratio is under 6, tobaccos are course and over-nicotinized, strong and acrid when smoked.

Average values of the reducing sugars/nicotine ratio in the leaves of unripe and overripe tobacco were significantly lower in 2004 compared to ripe tobacco, whereas in the following year the lowest ratio was found in overripe tobacco leaves (Table 5). In 2007, no differences were found in the reducing sugars/nicotine ratio for different leaf ripeness at harvest. The obtained results are in agreement with those of Gaines et all. (1983) and Weybrey et al. (1984) who did not get consistent results for reducing sugars/nicotine ratios in their investigations either. Puangnak and Chotinuchit (1987) achieved the optimal reducing sugars/nicotine ratio at the stage of optimal leaf ripeness, while Stocks (1991) and Zhong and Kefa (2003) reported reduction of the sugar/alkaloid ratio with the progress of ripeness.

Genetic variability of the studied varieties resulted in different responses to the weather conditions during the trial period (Table 5). Significantly lower ratio of reducing sugars/nicotine was recorded in the first trial year for variety HVT 1 compared to the other two varieties and for variety VJ 1 in the following year. In 2007, significantly lower reducing sugars/nicotine ratio was determined for all the three varieties compared to the two preceding years, in which no significant differences were found.

Significant decrease in the reducing sugars/nicotine ratio in the leaves of overripe tobacco in the first two trial years can be ascribed to the distribution of precipitation over the growing season. Those two years, though differing in the amount of precipitation, were characterized by similar precipitation distribution from the intensive growth stage to the end of the growing season (Table 1). Such climatic conditions resulted in normal ripeness of tobacco and, due to dissimilation processes, in an expected decrease of the reducing sugars/nicotine ratio in overripe leaves. Significantly lower reducing sugars/nicotine ratio in 2007, compared to the two preceding years, was a consequence of a forty-day-long water stress, which also encompassed the beginning of tobacco leaf maturing, and which was accompanied by establishment of a favorable water regime starting with the second decade of August. Consequences of drought, namely, insufficient soil moisture, are limited absorption and prolonged nitrate availability in soil, and consequent prolonged reduction of nitrates in leaves. Ripeness is delayed under such conditions, which was also the case in 2007. Also, starch accumulation starts after completion of nitrate reduction, though they are not separated. Hence, the interruption in continuous utilization of soil nitrogen results in higher nicotine content and lower content of reducing sugars in tobacco leaf, and thereby lower reducing sugars/nicotine ratio. A singularly unfavourable ratio was determined for all leaf ripeness degrees at harvest and for all varieties. These results show that the long-lasting water stress, estimated on the basis of the amount of precipitation in the critical stage of growth and development, caused a disturbance in tobacco

Table 5. Effect of interactions between year and ripeness, and between year and variety on reducing sugars/nicotine ratio of flue-cured tobacco

Year	Ripeness			Variety		
	Unripe	Ripe	Overripe	HVT 1	VJ 1	DH 17
2004	11.63^{bB}	15.15^{aA}	12.25^{bA}	11.58^{bA}	13.54^{aA}	13.91^{aA}
2005	14.59^{aA}	14.02^{aA}	9.33^{bB}	13.26^{aA}	11.26^{bA}	13.42^{aA}
2007	2.84^{aC}	1.5^{aB}	1.37^{aC}	1.96^{aB}	1.46^{aB}	2.29^{aB}

Average values marked by the same lowercase letter are not statistically different within the same year (P<0.05). Average values marked by the same uppercase letter are not statistically different within the same ripeness and variety (P<0.05).

metabolism, which had irreversible consequences. Similar conclusions, based on their respective trials, were also reached by Weybrew and Wolz (1975), Ismail and Long (1980), Weybrew et al. (1983), Goenaga et al. (1989), Maw et al. (2009), Çakir and Çebi (2010b).

It is generally taken that the flavour and taste of smoke are in correlation with nitrogen components. Total nitrogen/nicotine ratio is presumed to provide some kind of chemical balance in the leaf. Maw et al. (2009) maintain that the total nitrogen to nicotine ratio from 0.7 to 1 and Tso (1990) from 0.8 to 1.1 marks substantial and rounded-up flue-cured tobaccos for smoking. Tobaccos with low ratios are usually pale-coloured, with smooth tissue and without desirable physical characteristics. Lower ratios indicate heavier, fuller tobaccos while tobaccos with a high index (over 1) are light and emptier. Extremely high total nitrogen/nicotine ratios are less desirable, since such flue-cured tobaccos have poor flavour and smoke odour, and are also less suitable for natural ripeness. Too low values of the total nitrogen/nicotine ratio are considered undesirable, the smoke has heavy odour due to a high nicotine content and low content of reducing sugars.

In 2004, the highest total nitrogen/nicotine ratio was found in leaves of overripe tobacco topped to 17 harvesting leaves (Table 6). In previous research Puangnak and Chotinuchit (1987) reported optimal total nitrogen/nicotine ratios at the stage of optimal tobacco ripeness at harvest. However, the decrease of the nitrogen/nicotine ratio due to delayed harvest in 2004 in tobacco topped to 20 harvesting leaves as well as for both topping heights in the two subsequent years is consistent with research of Stocks (1991). Higher total nitrogen/nicotine ratio was found in the leaves of overripe tobacco topped to 17 harvesting leaves in the first trial year, and in in the leaves of unripe tobacco topped to 20 harvesting leaves in the following year. Double interaction also shows that the highest total nitrogen/nicotine ratio in unripe and ripe tobacco leaves, for both topping heights, was recorded in 2005 compared to the other two trial years. Significantly higher total nitrogen/nicotine ratio in overripe leaves of plants topped to 17 leaves was recorded in 2004 compared to 2005 and 2006, while total nitrogen/nicotine ratio in plants topped to 20 leaves was not affected by the year.

Table 6. Effect of interaction among year, topping height and ripeness on total nitrogen/nicotine ratio of flue-cured tobacco

Year	Topping height	Ripeness		
		Unripe	Ripe	Overripe
2004	17	0.85bA	0.92bA	1.07aA
	20	0.84abA	0.92aA	0.8bB
2005	17	1.21aB	1.05bA	0.87cA
	20	1.36aA	1.15bA	0.86cA
2007	17	1aA	0.83bA	0.79bA
	20	0.96aA	0.8bA	0.78bA

Average values marked by the same lowercase letter are not statistically different within the same year and topping height (P<0.05). Average values marked by the same uppercase letter are not statistically different within the same year and ripeness (P<0.05). Average values marked by the same α, β and δ letter are not statistically different within the same topping height and ripeness.

Significantly lowest total nitrogen/nicotine ratio was found in variety HVT 1 in 2004, and the following year in the leaves of variety VJ 1, in which lower ratio compared to HVT 1 was found also in 2007 (Table 7). Significantly highest total nitrogen/nicotine ratio was found in all varieties in 2005, the climatically most favourable year for the growth, development and ripeness of tobacco leaf.

Table 7. Effect of interactions between year and variety on total nitrogen/nicotine ratio of flue-cured tobacco

Year	Variety		
	HVT 1	VJ 1	DH 17
2004	0.85bC	0.93aB	0.93aB
2005	1.12aA	1.05bA	1.08bA
2007	0.93aB	0.77bC	0.88abB

Average values marked by the same lowercase letter are not statistically different within the same year (P<0.05). Average values marked by the same uppercase letter are not statistically different within the same variety (P<0.05).

As regards tobacco ripeness at harvest, differences between the varieties were detected only in unripe tobacco leaves. Harvesting of unripe tobacco variety VJ 1 resulted in lower total nitrogen/nicotine ratio compared to varieties HVT 1 and DH 17 (Table 8). The highest total nitrogen/nicotine ratio in all varieties was found in the leaves of unripe tobacco, while variety differences were determined for ripe and overripe tobacco harvesting. In variety HVT 1, a significant decrease of the ratio was obtained by harvesting ripe tobacco, without its significant decrease in overripe leaves, while the lowest ratio in VJ 1 was found in overripe tobacco leaves. In variety DH 17, each delay of harvesting resulted in a decrease of the total nitrogen/nicotine ratio.

Table 8. Effect of interactions between ripeness and variety on total nitrogen/nicotine ratio of flue-cured tobacco

Year	Variety		
	HVT 1	VJ 1	DH 17
Unripe	1.09^{aA}	0.95^{bA}	1.07^{aA}
Ripe	0.94^{aB}	0.94^{aA}	0.95^{aB}
Overripe	0.87^{aB}	0.85^{aB}	0.87^{aC}

Average values marked by the same lowercase letter are not statistically different within the same ripeness (P<0.05). Average values marked by the same uppercase letter are not statistically different within the same variety (P<0.05).

Smoke quality is very low without some free proteins. The presence of some proteins in tobacco is necessary to increase its strength and bitterness and improve the taste of smoke, at least for some smokers (Davis and Nielsen, 1999). Provost (1959) proposed the ratio of N-proteins to total nitrogen as a criterion for evaluation of tobacco quality. He emphasized the importance of the N-proteins/total nitrogen ratio for Virginia tobacco, which should range from 35 to 40% providing that the total nitrogen content is 1.4 to 1.8%, while the content of total sugars may be up to 20% with 50% fructose. Individual components of leaf chemical composition important for this ratio were described in a previous publication (Gršić et al., 2014). It is worth mentioning here that the content of nicotine and reducing sugars in the first two trial years was slightly higher according to the methodology proposed by the authors. In 2007, nicotine content was extremely higher while that of reducing sugars was lower than that required by the methodology.

Differences between N-proteins/total nitrogen ratios in tobacco leaves of different ripeness were not significant in 2004 (Table 9). In 2005, the highest N-proteins/total nitrogen ratio was found in ripe tobacco leaves, and the lowest in overripe leaves. In 2007, the year characterized by water stress, the lowest N-proteins/total nitrogen ratio was found in ripe tobacco leaves.

Significant differences between varieties were recorded in 2005 and 2007 (Table 9). varieties HVT 1 and VJ 1 had significantly higher N-proteins/total nitrogen ratios in 2005 compared to variety DH 17, and variety HVT 1 in comparison with VJ 1 and DH 17 in 2007. Significantly highest N-proteins/total nitrogen ratio in the three-year trial was found in all the three varieties in 2005, the climatically most favourable year, and significantly the lowest in variety VJ 1 and DH 17 in 2007, the water stress characterized year.

The impact of ripeness on the N-proteins/total nitrogen ratio in the leaves of varieties under study is shown in Table 10. When harvesting unripe tobacco, lower average N-proteins/total nitrogen ratio was recorded in the leaves of variety VJ 1, while the differences between varieties in the leaves of ripe and overripe tobacco were not significant. Significantly lower N-proteins/total nitrogen ratio in varieties HVT 1 and DH 17 was found in the leaves of overripe tobacco compared to ripe and unripe tobacco, and in VJ 1 in the leaves of unripe and overripe tobacco compared to ripe tobacco.

Correlation coefficients for the correlation of price with the studied indices of leaf chemical quality, characterizing tobacco leaf quality, are given in Table 11. Significant correlation coefficients showed a strong correlation of price with the reducing sugars/nicotine ratio and the N-proteins/total nitrogen ratio and a very weak correlation with the total nitrogen/nicotine ratio. Positive correlations of the price with the reducing sugars/nicotine ratio and the total nitrogen/nicotine ratio are in agreement with the results obtained by other authors (Gaines et al., 1983).

Table 9. Effect of interactions between year and ripeness, and between year and variety on N–proteins/total nitrogen ratio of flue-cured tobacco leaves

Year	Ripeness			Variety		
	Unripe	Ripe	Overripe	HVT 1	VJ 1	DH 17
2004	0.44[aB]	0.45[aB]	0.45[aA]	0.44[aB]	0.46[aB]	0.45[aB]
2005	0.49[bA]	0.56[aA]	0.46[cA]	0.52[aA]	0.52[aA]	0.5[bA]
2007	0.44[aB]	0.37[cC]	0.39[bB]	0.41[aB]	0.39[bC]	0.4[bC]

Average values marked by the same lowercase letter are not statistically different within the same year (P<0.05). Average values marked by the same uppercase letter are not statistically different within the same ripeness and variety (P<0.05).

Table 10. Effect of interaction between ripeness and variety on N–proteins/total nitrogen

Year	Variety		
	HVT 1	VJ 1	DH 17
Unripe	0.47[aA]	0.44[bB]	0.46[aA]
Ripe	0.47[aA]	0.47[aA]	0.45[aA]
Overripe	0.43[aB]	0.44[aB]	0.43[aB]

Average values marked by the same lowercase letter are not statistically different within the same ripeness (P<0.05). Average values marked by the same uppercase letter are not statistically different within the same variety (P<0.05).

Table 11. Correlation coefficients between price and chemical quality indexes, 2004 – 2005 and 2007 (n=216)

Chemical quality indexes	Price (kn/kg)
Reducing sugar /nicotine ratio	0.701**
Total nitrogen /nicotine ratio	0.204**
N-protein / total nitrogen ratio	0.503**

*Significant at P<0.05, **significant at P<0.01.

CONCLUSIONS

Investigation results have shown that the predominant effect on the price was that of the amount and even more distribution of precipitation during the growing season. In the year with insufficient precipitation, higher price was achieved by the harvesting of unripe and ripe tobacco, and in the normally humid year with non-significant differences in price with regard to leaf ripeness at harvest. In the year with water stress, the highest price was achieved by overripe tobacco harvesting. Varieties differences were determined in varieties responses to the amount and distribution of precipitation during the growing seasons, whereas topping height had no impact on tobacco price in any trial year.

Tobacco leaf chemical quality was evaluated by quality indices, which show the balance of some leaf chemical components and thereby the suitability of tobacco as raw material for smoking. Interactions between year and ripeness, year and variety indicate dependence of tobacco smoking characteristics, expressed by quality indices (reducing sugars/nicotine, total nitrogen/nicotine and N-proteins/total nitrogen ratios) on weather conditions during the growing season. In the years with insufficient and inadequate quantities of precipitation, the most favourable reducing sugars/nicotine ratios were achieved by overripe tobacco harvesting. In 2007, the reducing sugars/nicotine ratio was far below acceptable limits, without differences with regard to leaf ripeness at harvest, with irreversible implications of tobacco retrovegetation on the leaf chemical composition. In the year deficient in precipitation and the year marked by water stress and retrovegetation, all total nitrogen/nicotine ratios were within acceptable limits despite the recorded effects of trial factors. N-proteins/total nitrogen ratio in the leaves of ripe and overripe tobacco of all varieties was within the acceptable limits for this ratio only in the extreme 2007, the most unfavourable year for tobacco production.

The found strong correlations between price and the reducing sugars/nicotine ratio and N-proteins/nitrogen ratio and weak correlations with the total nitrogen/ nicotine ratio indicated a significant relation of visual evaluation and that based on indices of tobacco leaf chemical quality. However, it was only in case of the reducing sugars/nicotine ratio that a significant and irreversible disruption of the tobacco leaf chemical composition was established in 2007, which was in full agreement with the visual evaluation of leaf quality.

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