Agronomic Practices Affecting Nicotine Concentration in Flue-Cured Tobacco: A Review

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Core ideas:

- Proposed regulations may require lower tobacco nicotine concentrations.
- Production practices and timing significantly influence nicotine and leaf quality.
- Nitrogen fertility, crop density, growth regulation, and harvesting are paramount.
- Low density, high nitrogen, and increased maturity enhance nicotine accumulation.
- Flowers and axillary shoots are sinks that limit foliar nicotine concentrations.

Abbreviations: electronic cigarette, e-cigarette; electronic nicotine delivery systems, ENDS; heat-not-burn, HNB; maleic hydrazide, MH; plant growth regulator, PGR; potassium maleic hydrazide, KMH
ABSTRACT

Proposed regulations mandating lower nicotine concentrations in tobacco (*Nicotiana tabacum* L.) products will likely require changes in tobacco production to reduce nicotine while maintaining yield and quality. The agronomic practices used for tobacco production have a significant impact on the synthesis and accumulation of nicotine in flue-cured tobacco. Nicotine is the primary alkaloid in flue-cured tobacco and is one of the main reasons for its commercial production. Most agronomic practices that improve plant health and yield have a positive effect on nicotine production and accumulation. Some of the most important factors that affect nicotine concentrations are N fertilization, planting density, topping practices, sucker control, and harvesting practices. The amount of N available to the plant has a substantial effect on nicotine, as N is a primary component of the nicotine molecule. Factors leading to higher N uptake lead to higher nicotine concentrations. Plant and leaf densities within the field also have a significant effect on nicotine, where increasing densities leads to lower nicotine concentrations. Flowering and sucker production are both significant sinks of energy and other resources. Eliminating the inflorescence via topping and controlling suckers lead to higher nicotine concentrations. In fact, substantial nicotine synthesis and accumulation occurs in the days and weeks following topping. This comprehensive review discusses the agronomic factors affecting alkaloid production in flue-cured tobacco, and how these factors can be adjusted to manipulate the ultimate nicotine concentration.
INTRODUCTION

Tobacco synthesizes thousands of compounds, but the most economically important are the alkaloids, and specifically, nicotine (Bush, 1999). Nicotine is typically the primary alkaloid in tobacco (Wernsman and Matzinger, 1968), and concentrations can range from 5 to 80 mg g\(^{-1}\) depending on a number of agronomic, environmental, and varietal factors (Collins and Hawks, 1993; Collins et al., 1965; Leffingwell, 1999). Within flue-cured tobacco, average nicotine concentrations range from 15 to 35 mg g\(^{-1}\) (Collins and Hawks, 1993), with total alkaloid concentrations ranging 8 to 78.7 mg g\(^{-1}\) (Tso, 1990). Nicotine is synthesized in tobacco roots prior to being translocated to the leaves (Collins and Hawks, 1993; Wernsman and Matzinger, 1968; Weybrew et al., 1953). Most nicotine synthesis occurs later in the season after topping, but factors throughout the production cycle can ultimately affect foliar nicotine concentrations (Weybrew and Woltz, 1975). Cultivation, fertilization, planting density, topping, sucker control, and harvesting all affect overall plant growth and nicotine synthesis and accumulation (Bush, 1999). Thus, it is of interest to determine which practices can be manipulated by growers to successfully affect nicotine concentration while maintaining yield and quality.

The tobacco industry has experienced extensive changes in recent years, due in part to advances in electronic nicotine delivery systems (ENDS). Although most tobacco is grown for combustible products such as conventional cigarettes, electronic cigarette (e-cigarette) sales are increasing over time (Marynak et al., 2017). Between 2011 and 2015, US combustible cigarette consumption decreased while e-cigarette consumption increased; however, combustible cigarette sales were over 6,000% higher than e-cigarette sales by unit (Marynak et al., 2017). Combustible and electronic products both utilize nicotine but require tobacco with different characteristics. For instance, leaf quality serves a major role in combustible cigarette quality due to the
numerous chemical constituents also found in the leaf. In contrast, nicotine yield is most important for e-cigarettes because the nicotine is extracted directly from the leaf, and thus, the contributions of other chemical constituents become negligible. Like conventional cigarettes, heat-not-burn (HNB) products directly use the tobacco leaf, but they do not combust (Farsalinos et al., 2017), meaning leaf quality remains an important consideration. Therefore, it is essential for tobacco growers to optimize production to suit the needs of each respective nicotine delivery system.

The tobacco industry also faces the challenge of increasing regulation. The US Food and Drug Administration began passing significant regulatory measures on nicotine delivery products during the mid-1990s (Kessler et al., 1996). Established in 2009, the Tobacco Control Act led to the development of further regulation which largely focused on developing science-based regulations for tobacco products (Ashley and Backinger, 2012). In more recent years, the focus has shifted to reducing nicotine dependency primarily via lowering nicotine content in tobacco products (Ashley and Backinger, 2012; Benowitz and Henningfield, 2013; Lewis, 2018). One method to achieve lower nicotine concentrations in tobacco products is by adjusting the blend. For instance, lower nicotine filler can be added to cigarette blends, resulting in a lower nicotine concentration (Fisher, 1999). Another method has been to design cigarettes with lower nicotine delivery due to increased ventilation and faster burn. However, this method does not necessarily have the intended effect, as consumers may compensate for the lower nicotine delivery by smoking more cigarettes (Benowitz and Henningfield, 2013). A third alternative is to produce tobacco filler that is naturally low in nicotine. In fact, the World Health Organization suggested developing cigarettes with ultra-low nicotine levels of 0.4 mg g\textsuperscript{-1} or 0.04% (World Health
Organization, 2015). This will likely require significant changes to the agronomic practices and varieties currently being used.

For over 50 years, US flue-cured tobacco quality has been largely controlled by the Regional Minimum Standards Program (Bowman, 1996). This program ensures that only desirable varieties are released and commercially produced by trialing potential new varieties for physical, chemical, and quality standards (Bowman, 1996). Current standards require candidate cultivars to exhibit chemical characteristics within certain ranges relative to selected check cultivars, but these standards will likely need to change in response to federal regulations (Lewis, 2018). Reducing sugars should typically be below 22%, with excessively higher and lower concentrations reducing leaf quality; however, maintaining a desirable sugar to nicotine ratio is more important (Tso, 1999). Therefore, it will be necessary to consider this ratio when altering nicotine concentrations in order to maintain quality.

Given current debate on potential mandated lowering of nicotine levels in conventional cigarettes (Lewis, 2018), it seems timely to provide a review of agronomic practices that can impact nicotine levels in cured tobacco leaves. A summary of such information should be useful for informing the tobacco regulatory community and developing tobacco production strategies to achieve desired nicotine concentrations.

**Influence of Variety Choice and Plant Genetics**

The most important agronomic decision affecting nicotine levels in flue-cured tobacco leaves is variety choice. Plant genetics have a major influence on nicotine accumulation and the degree with which various agronomic practices effect nicotine concentrations. Typical modern flue-cured tobacco varieties exhibit nicotine levels between 17 mg g$^{-1}$ and 34 mg g$^{-1}$ (averaged
over all stalk positions) (Lewis, 2018), with much of the observed variation being attributable to environmental variation. In the United States, a variety approval process that has existed since 1964 requires that commercial varieties exhibit nicotine levels within windows dictated by industryAccepted check varieties (Bowman, 1996). This Regional Minimum Standards Program has caused North Carolina flue-cured tobacco production to consist primarily of a few varieties. In fact, the top seven varieties in 2018 represented 87% of the total statewide production, and the most popular variety, NC 196, was grown on nearly half of the total tobacco acreage (Fisher, 2019).

Substantial naturally existing genetic variation for alkaloid accumulation exists amongst genetically diverse accessions of tobacco, however, with measured alkaloid levels ranging from 0.2 to 65.5 mg g\(^{-1}\) dry weight (Sisson and Saunders, 1982). Most of these low alkaloid materials do not have direct commercial utility because of poor agronomic and/or quality characteristics. Although nicotine accumulation in tobacco should be considered a quantitative trait controlled by numerous genes and highly influenced by the environment, several large-effect loci have been identified that might be used to reduce nicotine content. First, recessive alleles at the \(\text{Nic1}\) and \(\text{Nic2}\) (also designated as \(A\) and \(B\)) loci have been transferred from cigar to flue-cured tobacco to reduce nicotine levels to approximately 2 to 4 mg g\(^{-1}\) (Chaplin and Weeks, 1976; Lewis, 2018; Lewis et al., 2015). This variability has been associated with reduced cured leaf yields and quality, however, and no flue-cured cultivar containing this genetic variability is widely grown commercially at the current time. A second naturally occurring genetic mechanism that can be used to reduce nicotine content are genes encoding for active nicotine demethylase enzymes which can reduce nicotine to levels below 8 mg g\(^{-1}\). Such varieties may be viewed as
unacceptable, however, due to greater accumulation of nornicotine, a precursor to a carcinogen known as $N'$-nitrosonornicotine.

Mutation breeding, gene editing, and genetic engineering can also be used to create new genetic variation for lowering nicotine content. A series of genes have been identified that are involved in nicotine biosynthesis (Dewey and Xie, 2013) and methods to decrease or stop their expression can result in reduced nicotine accumulation (Lewis, 2018). For example, inactivation of the $BBL$ gene family via mutation breeding or genetic engineering has been shown to reduce nicotine content to approximately 4 mg g$^{-1}$ (Lewis et al., 2015). Commercial growing of varieties developed using gene editing or genetic engineering is complicated by varying regulations throughout the world, however. Regardless of the agronomic practices that can be altered to influence leaf nicotine concentrations, the effect of variety is ultimately the most critical factor for commercial flue-cured tobacco production.

**Cultivation, Tillage, and Weed Control**

Sufficient aeration and drainage is essential for optimal growth of flue-cured tobacco. Loose soil structure is best for growth, so sandy and sandy-loam soils are ideal (Tso, 1999). Studies comparing conventional tillage with no-till systems have had inconsistent results depending on the year (Moschler et al., 1971). Moschler et al. (1971) found that flue-cured tobacco grown in a no-till system exhibited nicotine concentrations higher (1.53% compared to 1.09%), similar (2.48% compared to 2.50%), and lower (2.44% compared to 2.96%) than those grown in conventional tillage systems over the course of 3 yr. These results indicate that the tillage methods have a significant effect on nicotine, but that effect is not predictable. Additionally, the no-till system resulted in a 9.3% reduction in yield (2,863 kg ha$^{-1}$ compared to...
2,600 kg ha\(^{-1}\)) and a 10.1% reduction in value (Moschler et al., 1971). Subsoiling, a method of tillage that breaks up the lower layers of the soil, has been shown to result in similar alkaloid concentrations as non-subsoiled tobacco (Vepraskas and Miner, 1987). In some instances, alkaloid concentrations were lower in subsoiled tobacco, but total alkaloid production remained similar due to differences in yield (Vepraskas and Miner, 1987).

Weed control via herbicides has been shown to have little effect on foliar nicotine concentrations as well as the sugar:nicotine ratio in flue-cured tobacco (Rodriguez and Worsham, 1973; Walls et al., 1974). In a study by Rodriguez and Worsham (1973), only one field site had significantly different nicotine levels in the herbicide treated plot compared to the control. In this instance, plants from the control plot had lower levels of nicotine that were primarily attributed to higher weed pressure and competition with weeds for water and nutrients (Rodriguez and Worsham, 1973). In general, judicious use of approved herbicides for broad-spectrum weed suppression would not be expected to influence final nicotine concentration in cured tobacco, unless severe injury or stunting from excess application and/or poor growing conditions were observed.

**Transplanting**

Transplanting practices can affect the ultimate nicotine content based primarily on their effects on growth. For instance, excessively large transplants tend to flower prematurely and will not achieve the optimal number of leaves per plant (McCants and Woltz, 1967). This is one reason why transplants are commonly trimmed prior to being moved to the field. The act of clipping itself does not affect yield or nicotine content (Miner et al., 1983). Additionally, taller transplants tend to be damaged more, limiting the potential yield (Splinter and Suggs, 1959).
Transplants with intact roots typically have less transplant shock when moved into the field and exhibit greater levels of growth than transplants with damaged or excessively disturbed roots; however, this has not been found to affect the ultimate nicotine concentration in mature plants (Flower, 1999). The time of planting can have a significant effect on the total alkaloid concentration. A study by Hawks et al. (1976) demonstrated that plants transplanted earlier in the season had the lowest alkaloid levels at harvest, while those planted 2 wk later had significantly higher alkaloids and those planted 4 wk later had intermediate levels. Another study by Miner (1980) reported higher alkaloids from plants transplanted 4 to 6 wk later than usual. Wilkinson et al. (2008) reported inconsistencies with cured leaf total alkaloid concentration in a modern study designed to evaluate various transplanting dates and practices. For example, total alkaloid concentration was similar among a wide range of transplanting dates and methods in seasons characterized by above average precipitation but was sometimes increased when growing conditions were improved and transplanting was delayed by 2 to 5 wk (Wilkinson et al., 2008).

In contrast, Congleton et al. (1980) observed no significant differences in alkaloid concentrations when flue-cured tobacco was planted 4 to 8 wk later than usual. Ultimately, it appears that environmental conditions and agronomic practices implemented after transplanting largely dictate nicotine concentration.

**Planting Density**

The chemical composition of tobacco is considered a function of the planting density (Tso, 1999; Weybrew and Woltz, 1974). To produce flue-cured tobacco, populations of 15,000 plants ha\(^{-1}\) have been found to result in optimal yield and quality in the United States. As planting density increases, leaves are unable to expand to their potential maximum (Weybrew et
Additionally, higher planting densities will lead to greater competition for resources including N. This can ultimately restrict nicotine synthesis and the foliar nicotine concentration (Weybrew et al., 1953). Lowering planting densities and increasing plant spacing increase nicotine concentrations (Elliot, 1970a; Miner, 1980). Higher nicotine concentrations can be maintained at higher plant populations if the number of leaves per plant is reduced (Figure 1). However, higher number of leaves per plant typically leads to relatively lower nicotine concentrations even at lower planting densities (Figure 1).

Figure 1. Effect of tobacco plant (11,861, 15,815 and 19,768 plants ha$^{-1}$) and leaf (12, 16, and 20 leaves per plant) densities on the average foliar alkaloid concentration [adapted from Tso (1990)].

Although planting density is known to affect overall nicotine levels, only the mid and upper leaves tend to be affected. Lower leaf nicotine content is not significantly affected by plant
population, but higher plant populations will decrease the nicotine concentrations in leaves at the mid- and upper-stalk positions (Flower, 1999). The optimal leaf population for conventional flue-cured tobacco production is 296,520 leaves ha\(^{-1}\) (Collins and Hawks, 1993). Higher leaf populations decrease the total N and nicotine concentrations while lower leaf populations increase nicotine (Collins and Hawks, 1993). The constant total leaf number in a field will result in similar N and nicotine content, regardless of plant population (Tso, 1999).

**Fertilization and Irrigation**

Mineral nutrition is important for the growth and development of tobacco, as well as the synthesis and accumulation of nicotine. Each essential nutrient plays an integral role in the development of tobacco, but none are as important for nicotine production as N (Collins and Hawks, 1993). Nicotine and N levels are closely associated with each other and follow similar patterns of accumulation (Weybrew et al., 1953). As N uptake and accumulation increases, so does the nicotine concentration (Elliot and Court, 1978; Miner, 1980). Thus, higher N fertilization leads to higher nicotine concentrations (Figure 2). Within the plant, the upper leaves accumulate higher levels of both N and nicotine than the lower leaves. In fact, N and nicotine concentrations typically increase with higher node positions moving up the plant under conventional systems of flue-cured tobacco production (Collins and Hawks, 1993; Rogers and Mitchem, 1976; Tso, 1999); however, this trend may be inverse for non-topped tobacco plants (Shmuk, 1953). Nicotine and plant growth both increase with increasing N fertilization, but growth will eventually reach a maximum while nicotine continues to increase (Weybrew et al., 1953).
Figure 2. Increase in nicotine concentrations with increasing nitrogen (N) fertilization [adapted from Collins and Hawks (1993)].

Fertilization is affected by the nutrient form, rate, timing, and placement of application (Tso, 1999). Fertilizer placement and distribution has a significant effect on nicotine concentrations, as uniform (i.e. broadcast) nutrient applications typically result in lower N uptake and consequently limit nicotine content (Wilkinson et al., 2008; Weybrew and Woltz, 1975). In contrast, side-dressing fertilizer increases N uptake, leading to higher nicotine levels (Flower, 1999). Differences between pre-transplanting broadcast N applications and post-transplanting side-dress applications are most likely due to increased N use efficiency. In early investigations, granular N fertilizers were sometimes found to result in higher alkaloids while liquid fertilizers result in lower alkaloids (Maw et al., 1995). However, more recent investigations have not documented these differences. Parker (2009) evaluated granular calcium nitrate, granular ammonium nitrate, and liquid urea ammonium-nitrate and ultimately concluded that fertilizer
source did not influence agronomic measurements or cured leaf chemistry. If N is limited early in development and sufficient N is applied to relieve the nutrient deficiency, foliar N and sugar levels will recover, but nicotine concentrations will not (Raper and McCants, 1970). When entering a state of N deficiency, nicotine synthesis stops, depriving the developing leaves of nicotine (Raper and McCants, 1970). In contrast, the nicotine concentration in mature leaves is affected very little by the onset of N deficiency (Raper and McCants, 1970).

Nitrogen uptake is positively correlated with nicotine production and negatively correlated to sugar and starch accumulation (Flower, 1999). The optimal N source or source ratio is a topic of much debate. Ultimately, the ideal N source for growth has more to do with the environmental conditions, specifically the amount of precipitation (Fisher, 2019). During wet seasons, ammoniacal (NH₄⁺) -N sources tend to be more efficient due to less leaching. In contrast, nitrate (NO₃⁻) -N sources are particularly prone to leaching, and thus, are more efficient during dry seasons (Flower, 1999). Typical annual N fertilizer recommendations for flue-cured tobacco are 50 to 90 kg ha⁻¹ with 30 to 50% in the form of NO₃⁻ (Fisher, 2019; Peedin and McCants, 1977).

A study by Drake et al. (2015) observed the effects of applying different amounts of N at different stages of growth between transplant and 8 wk after transplant. It was found that applications of 50%, 50%, and 25% of the recommended N rate (80 kg ha⁻¹) applied 0, 4, and 8 wk after transplant, respectively, resulted in the highest nicotine concentrations (Drake et al., 2015). In contrast, the lowest nicotine concentrations were from three applications of 25% of the recommended N rate, regardless of timing (Drake et al., 2015). The N application treatments with the highest nicotine concentrations all involved applying N at transplant and 8 wk after
transplant (Drake et al., 2015). The lowest nicotine concentrations typically came from treatments that did not have any N applied 8 wk after transplant (Drake et al., 2015).

Underfertilization and leaching caused by excessive precipitation lead to lower nicotine levels. In contrast, overfertilization and drought conditions lead to higher levels of nicotine (Flower, 1999). Excessive fertilization can also affect nicotine primarily due to its effects on plant growth. Although high N fertilization increases yield, it can also be detrimental, causing delayed ripening and reducing quality (Tso, 1999). Excessive K can also delay ripening, while high levels of P can cause leaf thickening and ultimately reduce yield (Tso, 1999). Excessively high levels of K will not affect yield but will limit nicotine production (Chaplin and Miner, 1980; Vann et al., 2012), and soil P content does not affect nicotine (Lolas et al., 1979; Parups et al., 1960). Studies involving B, Ca, Cl, and Fe indicate that these nutrients do not affect nicotine concentrations (Gaines et al., 1976; McCants and Woltz, 1967; Neas, 1961). However, hydroponically induced deficiencies of N, P, K, Mg, S, and Zn led to alkaloid levels that were significantly lower than those observed in nutrient sufficient control plants (Gaines et al., 1976). Nicotine concentrations will not be significantly affected if sufficient but not excessive levels of all other essential nutrients are available (Weybrew et al., 1953). Although nicotine is not affected directly by applications of Ca-containing fertilizer, it has been shown that increased liming can significantly increase alkaloids (Peedin and McCants, 1977).

Irrigation practices vary by country. In the United States, flue-cured tobacco is not typically irrigated unless it is unusually hot and dry. Maintaining leaf turgidity is important for optimal growth, and thus is important for nicotine production (Tso, 1999). However, mild drought stress in the roots has been shown to increase nicotine synthesis (Parups et al., 1960). Ismail and Long (1980) demonstrated that the effects of drought stress and higher fertilizer N
rates could be combined to significantly increase nicotine concentrations. Tobacco uses different quantities of water throughout the season, with the greatest water requirement 7 to 9 wk after transplant (Flower, 1999). Thus, supplemental irrigation can be most beneficial during this period. If irrigation is implemented, frequent, light irrigations are ideal for production. Applying soluble N fertilizers through driplines can help to optimize irrigations by replacing leached N, leading to higher nicotine levels (Maw et al., 1997; Rideout et al., 1998). High humidity during the day has a positive impact on nicotine levels, which may provide additional reason to supply supplemental irrigation to raise field humidity and increase nicotine levels (Long and Woltz, 1977).

Soils with high moisture levels limit N and nicotine accumulation, largely due to N leaching from the soil (Figure 3) (Tso, 1999). In such cases, excessive irrigation or precipitation can be detrimental to nicotine production, especially when water does not readily move through the soil. In fact, as little as 4 hr of flooding can have a negative effect on growth, damaging plants and limiting the potential yield (Tobacco Research Board, 1978; Tso, 1999). In contrast, excessively dry conditions lead to higher nicotine accumulation (Figure 3) (Weybrew et al., 1953). Much of the year to year variation in nicotine content is due to differences in rainfall, all else being equal (Weybrew et al., 1953). Flue-cured tobacco cultivars with lower natural nicotine concentrations tend to be less affected by soil moisture, while high nicotine cultivars are more sensitive to high or low soil moisture (Weybrew et al., 1953).
Figure 3. Average nicotine concentrations for flue-cured tobacco grown under low to high soil moisture [adapted from Collins and Hawks (1993)].

**Topping**

Topping practices can significantly affect nicotine concentrations in flue-cured tobacco. For instance, topping earlier and lower on the stalk will lead to higher levels of nicotine (Bush, 1999; Collins and Hawks, 1993; Tso, 1999). After approximately 60 d of vegetative growth (Collins and Hawks, 1993), tobacco plants produce a terminal panicle inflorescence that is removed so nutrients and other resources are put into the leaves rather than the flowers (Tso, 1999). Topping is believed to encourage root growth and also strongly increases the expression of genes involved in nicotine biosynthesis and transport (Bowman, 1996; Hibi et al., 1994;
Weybrew et al., 1953), leading to greater subsequent nicotine accumulation in the leaves (Bush, 1999; Flower, 1999). Thus, topping is associated with a significant increase in nicotine concentrations (Long and Weybrew, 1981; Raper and McCants, 1966).

Flue-cured tobacco is typically topped at 18 to 22 leaves (Flower, 1999) when it has reached the button stage (Peedin and McCants, 1977). This leaf number results in moderate levels of nicotine and the postponement of topping past the button stage can reduce yield by 16 to 30 kg ha\(^{-1}\) d\(^{-1}\) (Peedin and McCants, 1977). Stocks and Whitty (1994) reported higher average losses in yield of 54 kg ha\(^{-1}\) d\(^{-1}\) when topping was delayed over 30 d in 5 d increments. This loss equates to a 34% reduction in yield, from 4,183 kg at the earliest topping date to 2,743 kg 30 d later (Stocks and Whitty, 1994). Decreasing topping height to 14 leaves leads to significantly higher nicotine concentrations in the leaves (Elliot, 1970a; Miner, 1980). Plants that are not topped have significantly lower levels of nicotine than topped plants, and the longer topping is delayed, the greater the decrease in nicotine (Elliot, 1975; Marshall and Seltmann, 1964; Stocks and Whitty, 1994). Lower leaf numbers contribute to higher nicotine concentrations while higher leaf number lead to lower nicotine concentrations (Bush, 1999; Campbell et al., 1982; Elliot, 1970b). These effects of topping are more pronounced in the mid and upper foliage than in the lower foliage (Flower, 1999). Approximately 2 wk after topping, lower leaves have increased levels of nicotine, but this increase is short-lived and will rapidly decrease to lower levels (Bush, 1999).

Chemical methods of topping have been investigated. Some of these chemicals include potassium maleic hydrazide [KMH (potassium;1,2-dihydropyridazine-3,6-dione)], flumetralin {N-[(2-chloro-6-fluorophenyl)methyl]-N-ethyl-2,6-dinitro-4-(trifluoromethyl)aniline}, and Off-Shoot-T (octanol, decanol, and dodecanol). These chemicals have been found to successfully
inhibit terminal bud growth but led to lower nicotine concentrations than manually topped plants (Long and Woltz, 1977). This may have been due to a dilution effect because the chemically topped plants continued to develop small leaves past the point of manually topped plants (Long and Woltz, 1977).

Current topping practices were developed for the optimal combination of yield, quality, and chemical composition. The proposed nicotine regulations will change the desired characteristics, potentially changing the optimal topping practices. If lower nicotine concentrations are required, a higher topping height could reduce foliar nicotine concentrations by as much as 0.3% while simultaneously increasing yield (Elliot, 1970a; Gršić et al., 2014). Therefore, topping higher may become a beneficial agronomic practice in the future. Although manipulating topping practices can significantly affect nicotine concentrations, it is not currently recommended to deviate from current practices due to potential losses in yield.

**Sucker Control**

Once plants reach reproductive maturity and begin to form flower buds, the plant directs most of its energy and resources toward the developing flower. However, topping results in the development of axillary shoots, commonly referred to as suckers (Weybrew et al., 1953). Removal of the inflorescence breaks apical dominance, causing suckers to develop. Suckers act as sinks, redirecting nutrients and other resources from other plant organs, such as the roots and leaves. Thus, sucker development limits leaf expansion and development, nicotine synthesis, and nicotine accumulation. In fact, total alkaloid concentrations typically increase with increasing sucker control efficacy (Tobacco Research Board, 1996). Allowing suckers to develop dilutes the
potential nicotine concentration in the leaves because the suckers will accumulate nicotine that would otherwise be translocated to the leaves (Weybrew et al., 1953).

Chemical controls such as maleic hydrazide (MH) are often used to control sucker development by inhibiting cell division and subsequent sucker proliferation and fatty alcohols work by desiccation. Additionally, suckers can be controlled by manual removal. The method of sucker control affects nicotine concentrations (Tso, 1999). For instance, applications of MH will typically, but not always, increase sugar and decrease nicotine (Gaines, 1959; Marshall and Seltmann, 1964; Parups and Richard, 1962), while manual control of suckers leads to higher nicotine and alkaloid levels (Tso, 1999). In fact, a study by Vickery (1959) demonstrated a reduction of 0.32% total alkaloids in MH treated plants compared to plants receiving manual sucker control. Of note is the fact that other chemical suckercides do not affect the chemical composition of the leaf, so the effects of MH are unique to that specific suckercide (Tso, 1999). Other suckercides including Chiptac, Delete, Emtrol, Emtrol Ten, and Pfizol-10 (all mixtures of octanol and/or decanol) were not found to affect nicotine levels in flue-cured tobacco (Rosa and Caughill, 1991).

Parups and Richard (1962) attempted to use quinones to negate some of the undesirable effects of MH, and found that 2,3-dichloro-1,4-naphthoquinone and 1-2-naphthoquinone applied with MH helped to maintain a more desirable sugar:nicotine ratio. In addition, applications of these quinones without MH significantly increased nicotine concentrations (Parups and Richard, 1962). Although MH decreases foliar nicotine concentrations (2.29% down to 2.08% for control and MH treated plants, respectively), the effects of not topping are significantly more detrimental to nicotine (Gaines, 1959). For instance, topped and non-topped plants treated with MH had nicotine concentrations of 2.08% and 1.63%, respectively (Gaines, 1959). Seltmann and Peedin
(1972) found that MH applied in the morning or early afternoon resulted in better sucker control than when applied in the evening; however, timing did not affect nicotine levels. Another chemical sucker control is flumetralin. Jones and Rideout (1986) demonstrated that flumetralin provides significant sucker control and can even increase nicotine concentrations when applied at higher rates. Flumetralin rates of 91 mg plant$^{-1}$ resulted in nicotine concentrations as high as 3.12%, while rates of 57 mg plant$^{-1}$ resulted in 2.54% nicotine (Jones and Rideout, 1986). Using a combination of chemical suckercides and manual control provides the best control of suckers (Tobacco Research Board, 1996).

**Leaf Ripeness and Harvest Timing**

As with fertilization practices, harvest timing is considered one of the most important factors for the ultimate quality of tobacco (Tso, 1999). Flue-cured tobacco is typically harvested in stages, and the first harvest begins approximately 10 d after topping (Collins and Hawks, 1993). Approximately 30% of flue-cured tobacco grown in North Carolina was harvested manually while 70% was harvested mechanically (Vann et al., 2015). Average nicotine concentrations in flue-cured tobacco differ significantly based on their location within the plant (Rogers and Mitchem, 1976; Tso, 1999). Nicotine concentrations in the upper leaves are commonly 200 to 300% higher than in the lower leaves (Bush, 1999). For instance, reported nicotine in the lower, middle, and upper thirds of the plant average 18.7, 26.5, and 32.6 mg g$^{-1}$, respectively (Tso, 1999).

Tobacco is considered “ripe” once it reaches the early stages of senescence, which is generally about 12 d after becoming physiologically mature (Weybrew, 1984). For proper ripening, soil N levels should be depleted around topping to avoid greening late in the season.
Immature leaves that are not yet ripe are difficult to cure and do not exhibit desired leaf characteristics. Leaf characteristics indicating ripeness include moving to a horizontal position perpendicular to the stem, chlorophyll degradation, velvety appearance, and small mounds between the veins that are referred to as grain (Peedin and McCants, 1977). Nicotine concentration typically increases with increasing leaf maturity (Gršić et al., 2014; Long and Weybrew, 1981; Moseley et al., 1963); however, overly mature leaves that are past optimal ripeness can have decreased levels of nicotine (Tso, 1999). Suggs (1986) reported a 15% increase in nicotine between leaves harvested 2 wk before optimal ripeness and those harvested 3 wk after (Figure 4). Thus, harvesting leaves at the proper stage of ripeness is important not only for optimal quality, but also for controlling nicotine concentrations.

Figure 4. Five-year mean flue-cured alkaloid concentrations based on ripeness at time of harvest [adapted from Suggs (1986)].
Harvesting of flue-cured tobacco, often referred to as priming, is conducted in multiple stages that correspond to leaf ripeness, from the bottom to the top of the plant (Peedin and McCants, 1977). Practices can vary by region, with approximately two to 10 total primings (Peedin and McCants, 1977). Fewer primings are typically associated with reduced labor costs (Peedin and McCants, 1977); however, more primings can result in leaves being harvested at optimal ripeness. In theory, more primings should lead to higher nicotine levels, due to more uniform ripeness, but the research does not always support this. For instance, one study concluded that three primings did not affect nicotine levels or yield compared to six primings (Gooden et al., 1976). In contrast, other research reported that fewer primings still did not affect nicotine concentrations but could reduce yield by 5 to 38% (Chaplin, 1975; Collins and Hawks, 1993). A 38% reduction in yield was associated with a single harvest; however, a single harvest was also found to increase nicotine by approximately 20% (Chaplin, 1975). Additionally, total alkaloids can be affected by the interaction of the number of primings and the topping height. Gooden et al. (1976) reported that topping plants at 15 leaves with two primings or 12 leaves with one priming resulted in significantly higher alkaloids than when topping at 18 leaves with three or six primings.

In comparison to the number of primings, the harvest timing appears to have a more consistent and significant effect on nicotine (Peedin and McCants, 1977). Nicotine concentrations increase moving up the stalk, so the upper leaves which are harvested at later primings typically have higher nicotine concentrations than lower leaves harvested at the first priming (Figure 5). Additionally, leaves that are bruised during harvest typically have lower alkaloid levels than those that are not bruised (Suggs and Howell, 1972). Care should be taken
while harvesting and preparing leaves for the curing process to limit bruising; however, some bruising is unavoidable, especially when utilizing mechanical harvesters.

![Figure 5. Average flue-cured nicotine concentration based on priming. Higher priming number correspond to later harvests of upper leaves [adapted from Tso (1990)].](image)

Chemical plant growth regulators (PGRs) have also been used for their effects on ripening. For instance, ethephon (2-chloroethylphosphonic acid) has been used to enhance leaf ripening, being referred to as a yellowing agent due to induction of leaf senescence (Cutler and Gaines, 1971). Ethephon works by releasing gaseous ethylene, but this process is highly dependent on solution pH. Thus, Cutler and Gaines (1971) conducted a study to observe the effects of ethephon applications with different pH levels on leaf ripeness and chemical composition. They found that ethephon had a greater effect on ripening of topped plants than non-topped plants but did not significantly affect total alkaloids (Cutler and Gaines, 1971).
However, Cutler and Gaines (1971) found that the solution pH had a significant effect on total alkaloids, where higher pH typically led to lower foliar alkaloid levels. Miles et al. (1972) conducted further studies and found that ethephon treated plants had lower alkaloid concentrations than non-treated plants.

**CONCLUSION**

Recent and proposed changes in the tobacco industry may require more precise management of tobacco quality and nicotine concentrations moving forward. This review is intended to provide a comprehensive summary of how agronomic practices can be manipulated to alter tobacco nicotine concentrations as well as the associated effects on yield and quality. It is our hope that this information will provide scientific evidence with which governing bodies may develop new regulations.

Agronomic production practices including N fertilization, irrigation, ethephon application, and the number of leaves per plant can be successfully manipulated by growers to influence nicotine concentrations. Practices such as cultivation, tillage, topping, and sucker control can also influence nicotine concentrations, but either achieve inconsistent results or significantly impede leaf development and quality. Ultimately, variety selection is the most important aspect to consider for achieving the desired nicotine concentration, yield, and leaf quality. After considering variety, certain agronomic practices may be implemented to encourage nicotine concentrations to increase or decrease as desired. Furthermore, agronomic practices such as irrigation and leaching adjustments can be made in response to changing environmental conditions within a season. Precise production management practices will be necessary for tobacco growers to meet the evolving requirements set forth by governing bodies and the tobacco
industry. To achieve the ultra-low nicotine concentrations suggested, modified production practices must be used in conjunction with varieties and genetic potential for reduced nicotine accumulation.

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Alkaloid concentration (mg g⁻¹)

Leaves per plant

Plants per hectare

- 11,861
- 15,815
- 19,768

77x75mm (600 x 600 DPI)
Nicotine concentration (mg g⁻¹) vs. Fertilizer N (kg ha⁻¹)

- 0.0 kg N ha⁻¹: nicotine concentration is 12 mg g⁻¹
- 33.6 kg N ha⁻¹: nicotine concentration is 18 mg g⁻¹
- 67.3 kg N ha⁻¹: nicotine concentration is 30 mg g⁻¹

81x75mm (600 x 600 DPI)