

MICRO- AND SECONDARY-ELEMENTS IN TOBACCO

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I. Introduction

Tobacco plants (*Nicotiana tabacum* L.) have served as valuable subjects in many phases of pioneering research. The best known contributions are mineral nutrition, day length, virus and nitrogen metabolism.

Tobacco is probably the most frequently used plant in the study of mineral nutrition. Elements can hardly be classified as essential or nonessential, as in the past. The division into groups of major and minor, primary and secondary, and macro- and micro-elements is also arbitrary.

Current interest in the production of the tobacco crop in general, and in the study of leaf composition in particular, make it desirable to review the accumulated information.

Here I attempt to summarize and examine publications related to micro-elements and some secondary elements in tobacco, covering a period of nearly 30 years from 1935 to 1964. A few papers of special interest published prior to 1935 are also included. Occasionally, information from studies on other crops is cited in view of its general academic interest and its special value in tobacco research. Elements included are: Aluminum (Al), Arsenic (As), Barium (Ba), Beryllium (Be), Boron (B), Cesium (Cs), Chlorine (Cl), Chromium (Cr), Cobalt (Co), Copper (Cu), Fluorine (F), Gold (Au), Iodine (I), Iron (Fe), Lead (Pb), Lithium (Li), Magnesium (Mg), Manganese (Mn), Mercury (Hg), Molybdenum (Mo), Nickel (Ni), Platinum (Pt), Polonium (Po), Radium (Ra), Rubidium (Rb), Selenium (Se), Silicon (Si), Silver (Ag), Sodium (Na), Strontium (Sr), Sulfur (S), Thallium (Tl), Tin (Sn), Titanium (Ti), Uranium (U), Vanadium (V), and Zinc (Zn). No attempt is made to include all publications under the subject matter; in fact, some papers were eliminated intentionally to avoid duplication. A bibliography published in 1934 covering literature citations for the years 1921-33 on antimony, bismuth, cadmium, chromium, cobalt, copper, lead, manganese, mercury, nickel, thallium, tin, and zinc, may be consulted for additional information (Heffer and Sons, 1934). Omission of other micro-

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elements does not necessarily imply the absence of these elements in tobacco, but indicates a lack of information.

To interpret the effects of mineral nutrition in any biological system, one must recognize the interaction of elements and the role they play in organic metabolism. Studies in this area are quite involved, and explanations often differ. A review of publications in this area merely indicates the complexity of the biological system.

II. Individual Element

Aluminum

Aluminum was believed to be associated with development of the black color of cured tobacco, or so-called "black tobacco" (Le Compte, 1944). Although no single factor should be regarded as the only cause, it was found to be significantly related to soil Al concentrations in the first and second pickings of tobacco leaf.

Low concentrations of Al seemed to increase the growth rate slightly. An average of 24 analyses of Connecticut tobacco showed a content of 0.06% Al and no toxic effect of Al to tobacco was noted in solution cultures at concentrations of $\text{Al}_2(\text{SO}_4)_3$ of 4000 lb/A (Morgan and Street, 1938). However, definite toxic symptoms were observed in tobacco plants at Al concentrations as low as 0.00012M when Al citrate was used (Eisenmenger, 1935b). These differences may possibly be due to the interaction of acid radicles. The toxicity effects were usually indicated in the dry weight of top and roots (Eisenmenger, 1932). Stunted tobacco plants with short thickened roots were usually found in high Al conditions (Hiatt and Ragland, 1963). Ca and OH ions appeared to reduce such toxic effects to some extent, while the presence of phosphate could lower the ionic Al in soil (Eisenmenger 1933, 1935a, 1935b).

Arsenic

The occurrence of As in tobacco has been the subject of many studies (Popp, 1928). Tobacco plants are known to be among the most tolerant to it (Vincent, 1944). Yield of tobacco is not significantly affected by As treatments, nor have they any visible effect on growth (Small and McCants, 1962). The As content of tobacco increased with increasing rate of application. The factors contributing to the variation in concentration of As at different localities are texture and Fe content of the soils, the absorption having been higher from coarse-textured than from fine-textured soils and having increased as the Fe content decreased. In general, the As content of the tobacco was higher where P was added than when none was applied. The content of As was usually higher in the tobacco roots than in the leaves.

From 1917 to 1958 commercial tobaccos produced in the United States have been studied for their As content. The level of As_2O_3 rose steadily from 12 ppm in 1917 to a maximum of 57 ppm in 1951. Since then the As_2O_3 content has declined to 3–12 ppm (Guthrie *et al.*, 1959). In another study (Bailey *et al.*, 1957), the As content of 39 commercial tobaccos obtained from 18 countries during 1948 to 1956 was determined. It was found for a time that American tobacco had a high As content ranging up to 100 ppm of As_2O_3 . Later in the experiment the As content of American tobacco dropped considerably. The peak years for As content in American tobacco were in the early 1950's; then a decline began. Tobacco may obtain As either from treatments of arsenicals on the leaf or from soils which contain As as residues from previous applications of arsenicals to crops. This practice, however, has essentially ceased since 1952, and low As content in American tobacco resulted (Guthrie *et al.*, 1959).

Tobacco produced in foreign countries varied as to As content. In Great Britain, that of bulked samples of tobacco used for cigarette manufacture for the years 1939 to 1956 was 7–51 ppm As_2O_3 . There was an erratic but appreciable rise from 1939 to 1953, followed by a decline to below the 1939 value (Weber, 1957). In Canada, tobacco tested contained a trace to 10 ppm As (Lissack and Huston, 1959). The Italian tobacco was found to have from 15.1 to 46.2 ppm As_2O_3 ; some samples were reported to be free of it (Zanetti and Cutrufelli, 1961). In 14 typical samples of Turkish tobacco from 1949 to 1950, As_2O_3 varied between 0.1 and 0.7 ppm. Pipe tobacco blends contained 0.4 to 0.5 ppm, cigar blends 1.2 to 3.2 ppm, and waterpipe tobacco blends 0.3 to 0.4 ppm As_2O_3 (Aksu and Enercan, 1954). When tobacco is smoked, about 7 to 18% of the As is volatilized, while 60% remains in ash (Bailey *et al.*, 1957).

Barium

The occurrence of Ba in tobacco and other plants has been investigated by various authors (Artis and Maxwell, 1916; Knight, 1916; and McHargue, 1913). Flowering tobacco (*Nicotiana affinis*) was found to have a relatively high content of Ba (Headden, 1921). Ba was noted in the leaves, base, stalks, and roots of tobacco plants. The average amount found in seven dry leaf samples was 0.0399%; in eight stalk samples 0.0396% and in the root 0.115% (McHargue, 1913).

Barium content (As % of BaSO_4) in leaf and stem of tobacco plants obtained from different producing areas varied widely. Soil, climatic, and cultural practices all contribute to such a variation. The following percentages were reported (Knight, 1916): Havana tobacco from Cuba, leaf 0.0608, stem 0.072; Pennsylvania tobacco, leaf 0.098, stem 0.128; Sumatra tobacco, leaf 0.0308,

stem 0.0408; Wisconsin tobacco, leaf 0.0192, stem 0.028; tobacco grown in New York, leaf 0.0132, and stem 0.504.

Beryllium

Beryllium was identified and determined to be present in various tobacco types, including flue-cured tobacco 0.015 ppm, Burley 0.05 ppm, Turkish 0.07 ppm, and Maryland 0.075 ppm. Upon smoking cigarettes all of the beryllium was accounted for in the cigarettes and butts (Williams and Garmon, 1961).

Boron

Boron, probably the most frequently studied microelement in tobacco plants, is essential for tobacco growth (McMurtrey, 1929; Morgan and Street, 1938). It is important because of its participation in protein metabolism (Smirnov, 1930); alkaloid production (Steinberg, 1955); translocation (Van Schreven, 1934); interaction with major elements such as Ca and K (Swanback, 1946); and consequently its effects on yield and quality of the tobacco crop (Thomson and Monk, 1956). The effects of B deficiency on the major organic constituents of tobacco plants—including alkaloids, sugars, organic acids, and amino acids—both in free and hydrolyzate, and with special reference to the gradual development of the deficiency symptom—have been subjects of a series of studies (Tso and Engelhaupt, 1963; Tso and McMurtrey, 1960; Tso and Sorokin, 1963; Tso *et al.*, 1960 and 1962).

The B content of *Nicotiana rustica* at the beginning of flowering averaged 7.4 ppm of dried material. There is the least B (4–7 ppm) in roots and lower stalks, and the most (10.5–13.5 ppm) in the youngest leaves and inflorescence, except for the corolla which contains only 3.2 ppm (Bertrand and Silberstein, 1940).

The distribution of B in various vegetative organs of *Nicotiana tabacum* L. var. one sucker has also been reported. Leaves were found to accumulate the greatest concentrations of B. The lower, older leaves had significantly higher concentrations than the upper, younger leaves. There was a differential in accumulation among regions of a given leaf; the apical, marginal portion contained the highest and the midrib the lowest concentration. In contrast with leaves, the lower internodes of the stem contained the lowest concentrations. In a given internodal region of the stem the highest concentration was found in the outer chlorophyllous cells of the cortex and the lowest in the xylem (McIlrath, 1964a). There was no transport of B from one side of the leaf blade to the other (Scholz, 1960).

The optimum amounts as established in pot experiments with quartz sand were 1–2 mg of B per plant; that is 2–2.8 kg/ha of borax (Schmid, 1951).

Generally, B in the amount of 1.0 mg/kg of soil should be considered as normal. It can be reduced to 0.3 mg/kg of soil without any noticeable decrease in the yield, but 0.1 mg of B is not sufficient (Sokolov, 1938). Occasionally, due to soil and other nutrient status, 1 ppm of B in soil causes slight injury to the margins of lower leaves (Hirai and Hidaka, 1942; Lal and Tyagi, 1949). In solution culture, even 2 ppm boric acid gave optimal growth, while 400 ppm caused slight injury (Swanback, 1927).

The application of borax to soils at rates of 0.15 to 1.20 lb B/A, commonly used for tobacco cultivation, had little or no effect on yield. Young plants given B at rates of 0.9 to 1.20 lb/A showed evidence of B toxicity (Hutcheson and Woltz, 1956). In soils, the coarse, medium, and fine sands contained 0.03, 0.06, and 0.075 ppm B (Anon., 1939). In a typical light sandy tobacco soil symptoms of B deficiency became apparent. This was corrected by use of 5 lb/A of boric acid (Garner, 1935; Kuyper, 1930).

Boron was not absorbed by the clay or humus complexes of the soil, or made insoluble with Ca. Within certain limits, neither active Ca nor pH affected the uptake of B by tobacco plants. Growth of Turkish tobacco on a Norfolk sand appeared normal when the Ca/B ratio in the plants did not exceed 1340:1. A Ca/B ratio in the plants of 1500:1 was correlated with severe B starvation symptoms (Drake *et al.*, 1941). A functional relationship exists between B and Ca within the plant. This means that a high intake of one of these nutrients will increase the requirement of a plant for the other. Soil microbes are believed to have an important part in the fixation of B (Purvis and Davidson, 1948). The interaction between boron and K has also been observed (Swanback, 1946).

The first indication of B deficiency is a light green color of the bud leaves, especially at the base of the individual leaves; it is followed rapidly by more or less of a breakdown of tissue (McMurtrey, 1933, 1935). Tobacco plants having symptoms of B deficiency show weakness and discoloration of the root system; there is death of the growth point and axillary buds, and thickening, chlorosis, and wrinkling of the leaves. Starch translocations are impeded in plants suffering from B deficiency, indicating a brown discoloration and disorganization of the individual cells or cell groups in the apical and procambial regions (Van Schreven, 1934).

Turkish tobacco, of the type Xanti-Yaka, grown in B-treated plots contained less nicotine than controls (Leone, 1939). Boron deficiency caused excessive branching of roots of tobacco plants. This was interpreted as the cause of increased nicotine (Steinberg, 1955). However, other studies reported that formation of nicotine was hindered when no B, or only 0.01 ppm B, was supplied as compared to 0.5 ppm (Scholz, 1958).

No isotopic effects between B^{10} and B^{11} were observed in tobacco plants. There were no differences in the dry weights of leaf, stalk, and root between plants supplied with these isotopes. Without B, however, the weights were much lower (McMurtrey and Engle, 1953).

Boron plays an important role in the growth and development of the tobacco plant. Boron-deficient plants are richer in starch and sugars than normal ones, presumably because of the obstruction in transport through disorganized phloem. Secondary factors are the poisoning of the plants by the immobilization of sugars and proteins combined with the impaired absorption and distribution of the elements requisite for normal tobacco growth (Van Schreven, 1934). In tomato and soybean plants, QO_2 values were greater with B-deficient leaf tissue homogenates than with normal ones (MacVicar and Burris, 1948).

Boron in combination with sucrose, produced better pollen tube growth than either component alone and, in general, concentrations of the two producing best germination also produced longest pollen tubes. A medium containing 15% sucrose and 30ppm B should support good germination and tube growth of tobacco pollen (Dean, 1964). No significant alleviation of B-deficiency by indoleacetic acid was noted (MacVicar and Tottingham, 1947).

Boron was also observed to have some function in lignin production. whether it plays a direct role is not yet clear (McIlrath and Skok, 1964b). *Nicotiana tabacum* L., decapitated above the tenth node, was grown in plus- and minus-boron solutions for a study of its effect on lignin formation. The percentage and total quantity of lignin in the seventh and eighth internodes of tobacco stems were lowest in the minus-boron plants. In the ninth internode, however, these values were highest in the minus-boron plants. B may be involved in the polymerization of precursors into lignin.

Cesium

Cesium was found in Burley tobacco produced in Italy (Traetta-Mosea, 1913). Japanese tobacco was reported to have 0.003–0.025 ppm Cs in leaf web, and 0.022 ppm in whole leaf (Yamagata, 1950). In a study of the uptake and translocation of Cs by plants (Rediske and Selders, 1953), there was no significant redistribution of Cs from the older leaves. The younger leaves demonstrated translocation where concentration gradients existed. The concentration of Cs in the aerial portions of the plant was nearly proportional to the concentration of Cs added to the nutrient solution. The absorption of Cs tended to increase as the pH of the nutrient environment was decreased.

Chlorine

Small amounts of chloride, 2% in fertilizer, improve yield and certain

quality factors such as color, moisture content, elasticity, burning, and keeping-quality of tobacco (McEvoy, 1950). Larger amounts of chloride, however, decrease the fire-holding capacity of the tobacco leaf (Pal, *et al.*, 1963).

Early publications reported that Cl content in tobacco leaves varied from 0.422% in lower leaves to 1.011% in upper leaves (Selschotter, 1935). More recent studies, however, indicated that Cl contents in cured Burley of various stalk positions were fairly uniform (Moseley, *et al.*, 1951). The Cl content of flue-cured leaves is highest in the lower primings, gradually diminishing with successive primings, except for a slight increase in the tips (Moseley, 1965).

Russian tobacco was reported to have a low Cl content, 0.07–0.23% (Krevs, 1925). Chlorine tends to drop with time in the reproductive organs, while the anion is most actively taken up in the early stages of growth (Rogalev, 1962). If the content in Cl rises above 2.5%, the resulting tobacco is nearly incom-bustible. In certain Spanish tobaccos Cl was reported as high as 3% (Mira, 1955). With increasing Cl in the tobacco leaf, the equilibrium moisture increased, and the alkalinity of water-soluble ash and fire-holding capacity decreased (Pallister and Green, 1962; Neas, 1961).

Chlorine content of tobacco leaves was inversely related to soil pH. Additions of Ca reduced Cl content of leaf tissue. A correlation coefficient of 0.769 was obtained between the percentage of Cl in tobacco leaves and exchangeable H^+ (Reisensuen and Colwell, 1950). Various soil and fertilization conditions, as well as various types of tobacco and method of harvesting, may also contribute to the differences in the distribution of Cl with respect to stalk positions as well as total Cl content of the leaf.

As early as 1892, it was reported that fertilizers for tobacco should contain at least six parts of potash for every part of Cl at the disposal of the plant (Nessler, 1892). On light sandy and sandy loam soils a moderate supply of Cl in fertilizer was found to stimulate tobacco growth. The presence of Cl in soil enables the plant to absorb the necessary quantity of magnesium more readily (Garner *et al.*, 1930). Chlorine increased total ash in seedlings, but decreased N (Elliot and Back, 1963). Cl reduces the activity of N; the affect being more marked on light soils deficient in buffering power. It is suggested that the physiological cause of the bad effect of Cl is due to excess Ca absorption by plants accompanying excess Cl. This disturbs the normal K/Ca balance (Masaeva, 1936). Another theory is that in contact with the roots the potash replaces sodium in combination with Cl, potassium chloride being taken up by the plant while the salts of sodium remain in the soil. It has been observed that a decrease in the amount of nitrates in the soil is accompanied by an increase of Cl in tobacco, and vice versa (Pitchard, 1899).

Cl uptake has been inhibited by supplying tobacco plants with rich sources of N (Mira, 1955). Chloride interacts with the form of N in its effect on leaf configuration, tending to increase the extent of abnormality when NH_4^+ is supplied, but having little or no influence when NO_3^- is supplied (Skogley and McCants, 1963a).

The increase of Cl content in tobacco was in direct proportion to the amount of Cl added by irrigation water. No significant difference in nicotine content appeared when different amounts of Cl were added to water, but nicotine content was lower in irrigated than in unirrigated tobacco, and reducing sugars increased as Cl content of tobacco increased (Peele *et al.*, 1960).

Chromium

Trace amounts of Cr have been found in flue-cured tobacco (Kennaway and Lindsey, 1958), blend cigarette tobacco (Cogbill and Hobbs, 1957), and cigar tobaccos (Johnson, 1936). In a wide-range study of Canadian samples of soils and crops, Cr was found in all cases (Dingwall and Beans, 1934). It was also found in several important American soils studied, ranging from a trace to 0.025% (Robinson, 1914).

Generally, small amounts of Cr increase the yield of nearly all plants tested. More soluble sources are beneficial in low concentrations, but injurious in larger amounts (Gericke, 1943; Scharrer and Schropp, 1935).

Cobalt

Cobalt content in plants varies greatly (Nyrek, 1954). Co has been found in flue-cured tobacco (Kennaway and Lindsey, 1958) and various commercial tobaccos ranging from 0.90 to 1.54 ppm (Voss and Nicol, 1960). Extensive studies of Co content in food crops have been carried out (Hurwitz and Beeson, 1944; Nicholas and Thomas, 1953). It was found (Nicholas and Thomas, 1953) that the effects of Co were independent of dry-weight yields. Injurious effects of Co excess were less marked at higher levels of P fertilizer. At a high Co level, symptoms of Fe deficiency were readily produced. Cobalt was reported to reduce root yields and depress top yields (Nicholas, 1952).

Copper

Addition of Cu has been reported to give beneficial effects on tobacco yield and leaf quality from early studies in this country (Manns *et al.*, 1937; Russell and Manns, 1934; Swanback, 1950), and later in the U. S. S. R. (Lashkevich 1952).

Flue-cured tobacco contains 14.9 to 21.1 ppm Cu (Collins *et al.*, 1961). In a series of tests conducted in 6 Eastern States, the addition of 20–50 lb of

CuSO_4/A increased the weight of tobacco in 63 out of 89 cases, and also increased quality (Gilbert, 1948). The breakdown of tobacco leaves as they approach maturity has been corrected by use of CuSO_4 at 50 and 100 lb/A; 20 lb/A was ineffective, and more than 50 lb gave no worthwhile extra benefit. Cu-deficient leaves were rich in total and protein N and, when flue-cured, were low in sugars. Use of CuSO_4 reduces N and increases sugar content (Thomson and Askew, 1956). In other field experiments in North Carolina, South Carolina, and Virginia, addition of 50 lb CuSO_4 /ton of mixed fertilizer resulted in 33.85% increase in tobacco value (Churchman *et al.*, 1937). Copper sulfate produced a more even ripening of the leaf, increased the "body" of the leaf, and decreased the tendency of burning from hot sun in dry seasons. In an earlier study of tobacco in Delaware using the same rate of CuSO_4 , an increase of 10.4% in tobacco yield and much superior leaf quality was obtained (Russell and Manns, 1934). In addition, when CuSO_4 was supplied, tobacco plants produced a cleaner, healthier root system (Manns *et al.* 1936).

Such beneficial effects were also found in other types of tobacco. CuSO_4 applied to Havana seed cigar tobacco at 18-20 lb/A increased the crop value from 13 to 26%. Burn of tobacco was not retarded through the use of CuSO_4 ; nor were the potash and the chief mineral constituents of the tobacco leaf altered as compared with tobacco from check plots (Swanbeck, 1950).

Control of wilt organisms in tobacco soils increased with increase in application of CuSO_4 , but high applications of CuSO_4 (400 lb/ton of complete fertilizer) usually caused marked inhibition of the growth of tobacco plants (Manns *et al.*, 1936). CuSO_4 tended to delay the ripening process somewhat, but this effect was usually more than offset by the stimulating effect on the crop (Russell and Manns, 1934).

Other physiological effects of CuSO_4 on tobacco plants were reported. The addition of Cu was suggested to be related to the increase of nicotine content and the decrease of citric acid content of tobacco leaves (Kristof and Markovic, 1952). A decrease in ascorbic acid oxidase activity was noted in Cu-deficient tobacco leaves (Brown and Steinberg, 1953).

Despite the fact that Cu is essential for normal growth of tobacco and various beneficial effects cited here, the inclusion of Cu compounds in all tobacco fertilizer mixtures is not recommended until there is evidence indicating the deficiency of this element in soil. In normal agricultural soils, Cu occurs from 1 to 50 ppm (McMurtrey and Robinson, 1939).

Fluorine

Although F was reported to be present in cigarette smoke (Spira, 1948), there is no available data indicating the range of its content in tobacco leaf.

Soils may contain as much as 6,000 ppm of fluoride. Food crops usually range from 4.5 to 9.2 ppm in F content (Machle *et al.*, 1942). F content in irrigation water does not seem to be reflected in content of F in the crops (Pisareva, 1955). The application of 1000 lb/A of rock phosphate will add approximately 35 lb of F to the soil. About one-half this amount would be present if P were added in the form of superphosphate (Phillips *et al.*, 1933-34).

Sassafras sandy loam and loam soils were used in four series of studies to detect the concentration at which F becomes toxic to buckwheat and tomato plants. The point at which F became toxic varied with the type of soil, its lime and P levels, and the type of plant grown. In general, applications of lime with consequent increase in pH and increase in P_2O_5 in the soil decreased absorption and toxicity of F (Prince *et al.*, 1949).

In a study of some effects of F on peach, tomato, and buckwheat, when absorbed through the roots, about 10 ppm of F appeared to be the approximate threshold value where injury began to take place. Where actively growing plants were involved, injury first appeared on the tips of younger leaves, extending along the leaf margin and finally inward towards the midrib. At the highest F concentration, necrosis was preceded by a general wilting of the plant, probably as a result of root injury (Leone *et al.*, 1948).

A lower rate of fumigation, 0.048 ppm F, produced no injury on tomato foliage but a higher fumigation rate, 0.47 ppm F produced definite injury. The average F content of leaves fumigated at 0.048 ppm was 40 ppm, whereas, at 0.47 ppm the average was 477 ppm (Brennan *et al.*, 1950).

Fluoride was reported to inhibit oxidative processes in the cytochrome system of the cell. Fluoride is capable of inhibiting processes catalyzed by metallic ions or metal-containing enzymes (Borei, 1945).

Gold

Living organisms seem capable of absorbing gold. It is not quite clear whether the small quantities absorbed are favorable or unfavorable to the growth of these plants. Gold taken up by the roots of plants is distributed to various plant organs, especially to the seeds. Future generations show an enrichment when such seeds are grown in soil containing Au. Au content in tobacco is not known (Babicka, 1943). It appears to affect alkaloid formation of the tobacco plant (Tso *et al.*, unpublished).

Iodine

Iodine was reported to be present in various tobacco types ranging from 0.55 to 1.75 ppm (Schwaibold, 1930). Toxicity symptoms were pronounced when

K was supplied as chloride and I uptake was increased by the presence of Cl, but no effect of I on Cl uptake was observed (Newton and Toth, 1952).

Effect of I on the activity of tobacco mosaic virus is of special interest. At pH 5.5 to 6.0, I had slight effect on the activity of virus, causing greater reduction in activity at a higher or lower pH, with 100% inactivation at 4.5, 5.9, and 8.3. The amount of I associated with the virus protein was greatest at 4.5 and least at 5.9. Inactivation of the virus at pH values below 5.9 probably takes place through the association of I with the active protein groups by tautomeric rearrangement of double bonds (Agatov, 1945).

Denatured tobacco virus has a number of SH groups corresponding to its total S content (0.2%). The SH groups of the virus or their precursors can be abolished by reaction of the native virus with I. Turkish tobacco plants inoculated with active virus whose SH groups were abolished by I produced virus with the normal number of SH groups (Anson and Stanley, 1941).

Iron

Iron has been widely studied in various types of tobacco. The level of Fe content in tobacco varies according to type, grade, soil, culture, and climatic conditions. U.S. flue-cured tobacco has an average Fe content ranging from 132-595 ppm in 1952, 153-1013 in 1954 (Bacot, 1960). In Canadian flue-cured tobacco, dry year crop had 1220-1500 ppm, averaged year 110-320 ppm, burley tobacco 200-650 ppm, and uncured cigar tobacco 260-1560 (Ward, 1942). Japanese tobacco ranged between 1640-2620 ppm (Fesca and Imai, 1888). In cigar tobacco, depending on the source of tobacco leaf, Fe content varied from 560 to 8400 ppm (Frankenburg, 1950). Fe content of U.S. Connecticut shade cigar tobacco was 620 ppm (Vickery and Meiss, 1953); Connecticut "light" tobacco, 300-3000; and Connecticut "black" tobacco 600-3500 (LeCompte, 1946). Javanese cigar tobacco had the highest Fe content ever reported, 11,000-14,500 ppm (Coolhaas, 1930).

A proportionality existed between total Fe and chlorophyll content in the leaves of plants studied. Before chlorophyll occurs the total Fe content of the leaf must exceed a certain minimum level. This is determined by the species and growth conditions (Jacobson, 1945).

A high Fe concentration in the nutrient solution may reduce Ni uptake and toxicity symptoms therefrom (Brown and Steinberg, 1953; Crooke, 1955). Absorption of Ni and the intensity of necrosis increased with increase in the Ni-Fe ratio in the nutrient solution. Ni consistently reduced the Fe content of roots and tops. In the absence of Ni, Fe content of the roots was higher in healthy plants; increasing with Fe supply (Crooke, 1955).

The biting taste of burley tobacco leaves was studied in Japan in relation

to Fe content in nutrition (Fujiwara and Kurosawa, 1956); however, the results are too involved for a clear-cut conclusion.

Lead

The presence of Pb in tobacco and in smoke has been widely investigated. Pb content in blended tobacco varies from 0 to 200 ppm (Sund, 1956). The absorption of Pb by plants has also been the subject of many studies (Prat, 1927).

Lead toxicity in tobacco resembles an early symptom of frenching (David *et al.*, 1955). A comprehensive spectrochemical analysis was carried out on grouped portions of 6 tobacco plant samples. It was suggested that Pb may be a contributing factor in frenching of tobacco; however, this theory is no longer accepted.

The toxicity of soil treated with lead arsenate to vegetable plants varies with the soil type, being higher for some plants on sandy soil than on clay soils or those high in Fe or organic content (Johnson, 1943).

The presence of Pb does not affect plants too seriously, although there are indications that lead arsenate in soil reduces the germination of certain crops (Fleming *et al.*, 1943). The barrenness of Pb mine dumps and neighboring fields probably results from the windborne Pb dust which blocks the stomates of plants (Hooper, 1937). Lead nitrate as a source of nitrogen for fertilizing purposes is equal to sodium nitrate when applied. Its effect on the plant has been the production of a slightly broader leaf blade and a deeper shade of green as compared with the effect produced by sodium nitrate. No difference in root development has been observed (Berry, 1924).

Lithium

Only minute quantities of Li have been found in tobacco—0.013 to 1.2 ppm (Yamagata, 1950). Experiments on Kentucky tobacco supplied with varying amounts and proportions of K and Li salt appear to show that tobacco is able to utilize certain small portions (Ravenna and Maugini, 1912). Flowering tobacco has a relatively high content of Li (Heffer and Sons, 1934).

The Lithium content of the ash of Li-supplied tobacco plants was 2–3 times higher than that of control plants. During almost the whole period of the investigation photosynthesis was more intense in test plants. Lithium increased the hardness of the plant against drought and heat. The amounts of evolved CO₂ were much higher in test plants, indicating that Li stimulated respiration (Ezdkova, 1962).

The presence of Li in the ash of tobacco led to the investigation of

whether Li was poisonous to plants. It was found that for the Solanaceae, Li did not have a marked injurious effect (Ravenna and Zamorani, 1909).

Magnesium

Sanddrown, a chlorosis of tobacco involving both green and yellow pigments of chlorophyll, is due to Mg deficiency and is markedly aggravated by an increased sulfur supply (Garner *et al.*, 1922, 1923; Williams, 1937; Winters, 1924). Magnesium deficiency seldom occurs when Mg is a constituent of the fertilizer. MgSO_4 may be used in moderation, though excess will depress the uptake of K (Elliot and Vickery, 1954). Comparatively small amounts of available Mg in the soil will prevent sanddrown (Williams, 1927). When leaf tobacco contains about 0.15% Mg deficiency symptoms are usually evident, whereas leaves containing 0.25% of this element are generally free from such symptoms. High ash content is usually associated with Mg deficiency. The starch reserves of the green leaf are low when Mg deficiency is severe (McMurtrey, 1947). The response of tobacco plants to Mg in pot experiments is very pronounced. Absence of Mg reduced plant growth and development, impaired leaf color, and lowered seed stem, root, and leaf yields in that order. Adding Mg increased the fat content of seeds (Matusiewicz, 1964).

Magnesium, higher in the lowest leaves, decreased regularly in each successively higher leaf until the percentage at the top was about one-half that of the bottom leaves (Swanback, 1939b). A certain percentage of Mg in the leaf appeared essential for satisfactory combustion in the cigar, the optimum being nearly 2% (Anderson *et al.*, 1931). More than 2% decreased combustibility and made burning less uniform. Application of MgO with the fertilizer increases the total water-soluble carbohydrates and decreases albumins and nicotine. The yield of dry matter in the leaves is considerably increased by MgO (Kovalev, 1940). Tobacco soil should contain 30-40 ppm of easily replaceable Mg to avoid deficiency (Beaumont and Snell, 1935). Dolomite or magnesite application improved leaf texture of tobacco grown on soils low in Mg (Anon. 1944-45). Applications of MgO lower the burning quality of tobacco because MgO tends to repress K absorption by the plant (Popper *et al.*, 1960; Ward, 1938). In cigar tobacco, limestone can be used as a source of Mg (Swanback, 1942). Where calcite was applied sanddrown was worse than where no lime was used, indicating that the use of calcium alone seemed to hasten the exhaustion of the magnesia supply of these soils (Williams and Matthews, 1923). When magnesia is derived from dolomite, only small quantities should be used to avoid possible harmful effects from root diseases and loss of nitrogen which usually results when the soil is heavily limed for tobacco (Moss *et al.*, 1927).

Common crop plants differ greatly in their response to a deficiency of Mg

in the soil. The content of Mg in the plant is affected by the amount of precipitation during the growing season, being less in seasons of heavy rainfall. Heavy rains for two successive years apparently so depleted the available supply of magnesia that sanddrown was encountered even on light sand (Anderson and Swanback, 1929).

Manganese

Manganese has been reported in all tobacco and ranges from 140–700 ppm (Bacot, 1960). Tobacco plants require Mn for normal growth, but are sensitive to toxicity.

The characteristic effect resulting from Mn deficiency in tobacco plants is slowness in manifestation (McMurtrey, 1933); requiring from 4 to 5 weeks to become apparent. Chlorosis usually takes place only between the veins and follows outward to the minutest branches of the vascular system resulting in a checkered appearance of the tobacco leaf. Small necrotic spots develop on the chlorotic leaves in later stages, drying to a white or brownish color. Chlorosis symptoms can be corrected by the addition of Mn.

Tobacco plants begin to show symptoms of Mn toxicity during the early stages of growth in culture solution, when the tissue contains approximately 3000 ppm Mn. Growth is not materially reduced at concentrations up to 5000 ppm Mn (Hiatt and Ragland, 1963). Chlorosis usually resulted in plants grown in water cultures when Mn was present in concentrations of 15 ppm and became more severe with higher Mn concentrations (Bortner, 1935). In soil culture, abnormal physiological symptoms appeared in tobacco plants when the soils had a high acidity and a correlated high content of soluble Mn (Jacobson and Swanback, 1929). Mn under certain conditions has a regulating influence on the absorption of Ca (Swanback, 1939a). Liming very largely reduced Mn solubility (Murwin, 1929). The general correlation between extent of plant injury, percentage of Mn in the plants, and amount of Mn in the leachates indicates that chlorosis is due entirely to soluble Mn (Bortner, 1935).

High Mn contents of tobacco produced a dark, mud-colored ash. Brick-colored or ash muddy was not present in tobacco containing less than 0.04% Mn. The Mn content of tobacco may be controlled by keeping the pH of the soil above 5.0 (Anderson *et al.*, 1936).

Black tobacco is a dull, matte-surfaced, low-quality leaf that cures very dark brown with a blue-gray or purple-gray hue. Preliminary studies show that the black shade tobacco tested generally had greater average contents of Mn and Fe than shade leaf of light color. Such a high Mn and Fe content

seemed due to a deficiency of Ca and/or active P in the soil together with unfavorable acid soil reaction (LeCompte, 1941a).

Earlier investigations failed to show the significance of Mn on tobacco plants from pot or field tests (Mach, 1910). Later experiments demonstrated the possibility that Mn may perform a function of equal importance to that of Fe in plant metabolism (McHargue, 1923).

Mn toxicity symptoms developed much more readily at low temperatures than at high temperatures (Lohnis, 1950). Injury to the plants grown in Mn-treated soil decreased as light intensity decreased (McCool, 1935). The Vitamin C content of leaves and fruits of tomato plants fertilized with 150 g Mn/10 sq. m. was 12-30% higher than that of control plants (Bronstert, 1950). Increasing the Mn concentration in solution culture for Turkish tobacco increased the ascorbic acid content of tobacco leaves (Maton, 1947).

Mercury

Traces of Hg were found in flue-cured tobacco (Kennaway and Lindsey, 1958). Hg vapor was toxic to germinating tobacco seeds as shown by reduced germination and stunted seedlings. The toxicity varies directly with the exposed area of liquid Hg, the volume of the enclosed space, and the temperature (Kincaid, 1936). HgCl_2 was found to inhibit infectivity of tobacco mosaic virus more at pH 7.0 than at pH 6.0 (Kassanis and Kleczkowski, 1944).

Some plant species were relatively resistant while others were susceptible to Hg. Injuries to plants caused by Hg vapor were indistinguishable from those caused by Hg compounds. There is no relation between the susceptibility of different species or varieties to injury by Hg and the amounts of Hg accumulated in the leaves (Hitchcock and Zimmerman, 1957). The poisonous action is manifested by the destruction of the chlorophyll-bearing organs, especially in young leaves, where it seems to check assimilation. The root system of plants seems to be affected to a lesser degree. Plants are able to grow in soils containing a considerable quantity of metallic mercury (Dafert, 1901). The total Hg content in tomatoes ranged from 0 to 0.04 ppm—those treated with phenylmercuric salicylate from 0.01 to 0.08 ppm (Stone and Clark, 1958).

Molybdenum

Molybdenum is present in trace amounts in tobacco (Kennaway and Lindsey, 1958). The main symptoms of Mo deficiency in tobacco are the appearance of small necrotic areas in young leaves and older leaves becoming chlorotic and bleached (Reddy and Mehta, 1958). Preliminary results indicate

that Mo deficiency causes a reduction in ascorbic acid content of several crops (Hiatt and Ragland, 1963).

Crops reacted differently in their response to Mo deficiency. In some cases Mo-deficiency symptoms appeared in the first crop; in other cases not until the second crop (Peterson *et al.*, 1961). The acidity of soil is another factor guiding the availability of Mo to plants. A low pH, especially in the presence of Mo, prevents the utilization of Mo from soil (Mulder, 1954a, 1954b).

When molybdate was provided to tomato plants, which had been cultured in the absence of Mo, changes in free amino acids were observed. Such Mo treatment was discussed in relation to pathway by which products of nitrate reduction are incorporated into proteins (Possingham, 1957).

The watering of plants with ammonium molybdate solution, at a rate of 2 g/10 kg soil, caused slower growth, earlier blooming, leaf yellowing, and reduction of tobacco roots and higher dosage intensified these symptoms. Plants susceptible to virus diseases, such as tobacco, were more sensitive to ammonium molybdate treatment; however, at a dose of 4 g of this material, tobacco became immune to tobacco mosaic (Kozłowska, 1947). Mo was believed to be associated with the multiplication of tobacco mosaic virus (Pirone and Pound, 1962).

Nickel

Nickel was indicated to be present only in minute amounts in tobacco (Kennaway and Lindsey, 1958). Recent application of neutron activation analysis confirmed the presence of less than 1 ppm of nickel in various types of cigarette tobacco and also the transfer of less than 0.1% of the element to mainstream smoke (Day, Bateman, and Cogbill, 1963). Methods of testing, however, were insufficient to decide whether Ni was present in the smoke as the carbonyl (Pailer and Kuhn, 1963).

Very young tobacco seedlings were sensitive to Ni toxicity; some developed extensive brown necrosis and many died. Older seedlings in sand culture were seriously affected by a nutrient solution containing 2.5 ppm Ni (Hunter, 1954). Various crops differ in their ability to absorb Ni from soil. Different plant species grown on the same soil varied greatly in total Ni content (Painter *et al.*, 1953). Nickel uptake is proportional to the acetic-soluble or exchangeable Ni content at a given soil pH, but is primarily related (inversely) to pH (Hunter and Vergnano, 1952). The addition of lime to Ni-treated soil counteracted the toxic of Ni on plant growth (Chang and Sherman, 1953). Total Ni content of soils varied from 3 to 26 ppm, and that of green leaves of various plants from 0.2 to 1.6 ppm dry weight (Shiha and Kodaira, 1952).

Nickel toxicity symptoms were less severe when the concentration of Fe

in the nutrient solution was high (Crooke *et al.*, 1954). The injurious effect of Ni was not alleviated by increasing fertilizer phosphate (Nicholas and Thomas, 1954). Reduction in the degree of necrosis was related to reduced Ni content in the leaf blades (Crooke *et al.*, 1954).

Nickel-toxic oat plants have higher Ca than normal plants; the reverse is true of tomato. Results suggest opposing phenomena to be occurring simultaneously: (A) roots are damaged and absorption by translocation of all major nutrients is reduced; and (B) Ni in the leaves by some unknown mechanism, increases absorption of Ca by the roots. In oats (B) predominates, while in tomatoes (A) predominates normally (Knight and Crooke, 1956).

Nickle and zinc sulfates have a physiological function of promoting both carotens and citrin formation in plant metabolism (Mitchell, 1945). Nickel is important as a regulator of oxidation processes occurring in plants (Porfir'ev and Troitskaya, 1937).

Platinum

Platinum was found to increase alkaloid content in tobacco plants (Tso *et al.*, unpublished). Beans grown in sand were inhibited when $\text{H}_2\text{PtCl}_6\text{H}_2\text{O}$ was added at concentrations of 15×10^{-5} , 9×10^{-5} and 3×10^{-5} M. These plants had smaller leaf areas, higher osmotic pressure, and lower transpiration rate. Treated plants resisted wilting much longer than controls, and were also less succulent. Tomatose grown under similar conditions were inhibited in growth, had chlorotic lower leaves, and resisted wilting longer than control (Hammer, 1942).

Polonium

The presence of polonium-210 in tobacco and tobacco smoke has been reported by several authors (Radford and Hunt, 1964; Tso *et al.*, 1964). Contents of Po-210 in leaf tobacco and tobacco soil varies with the source (Tso *et al.*, 1964); the difference resulting from production locality, culture or curing. Po seems not to be entirely derived from radium. The plant probably takes it up from the soil or air. The general range of Po-210 in tobacco leaf varies from 0.15 to 0.48 pc/g; in tobacco-growing soil, it varies from 0.26 to 0.55 pc/g (Tso *et al.*, 1964).

Radium

The amount of Ra-226 in tobacco-producing soil appeared to be related, to a certain extent, to phosphorus fertilization. Soils having high available P_2O_5 , continuously used for tobacco crops, usually have a high Ra-226 content; the range being 0.52 to 1.53 pc/g (Tso *et al.*, 1964). The presence of Ra-226 in

tobacco varies with type and culture conditions, ranging from 0.059 to 0.39 pc/g.

The total alpha activity, expressed as Ra-226 activity, was detected in tobacco of Australian origin. For cigarettes, the activity was 0.5 pc per cigarette, and for pipe tobacco the activity was 6.5 to 7.3 pc in the ash, representing a 25 g sample (Pallister and Green, 1962).

By adding 10^{-11} g of Rn to 6 liters of nutrient solution, the Ra content of the roots and stalks of plants increased greatly. This amount was considered optimum for plant growth (Drobkov, 1937).

Low doses of Ra were reported to stimulate the early stages of growth of tomato plants (Glushchenko and Drobkov, 1952). Ra content of reservoir water has a seasonal variation, varying with water depth (Brunovskii and Kunasheva, 1935).

Rubidium

The presence of Rb in tobacco and other plants has been reported in several publications (Grandeau, 1862; Kennaway and Lindsey, 1958; and Yamagata, 1950). It is present in a trace amount, averaging about 30 ppm.

Rubidium absorption and distribution by tobacco seedlings was studied relative to seedling size and also after decapitation of the plant (Skogley and McCants, 1963b). Results in the accumulation of Rb on roots suggested that, to a certain extent, the size of the plant was positively correlated with Rb accumulation. During a 6-hour experiment roots had a major influence on Rb accumulation. Decapitation made little or no difference. Salt treatments subsequent to Rb absorption indicated that distribution of Rb was dependent more on conditions during its absorption than on those imposed later. These observations are important for interpreting results from studies on absorption competition and metabolic ion transport.

Selenium

Selenium was found in tobacco only in trace amounts (Kennaway and Lindsey, 1958). Absorption of Se by tobacco was studied in sand cultures. Tobacco was injured more severely than were soybeans by Na_2SeO_3 . The addition of sulfate to the nutrient decreased Se absorption slightly (Martin *et al.*, 1933). There appeared to be a wide difference in the absorption of Se from Na_2SeO_4 by different crop plants, and this was directly correlated with corresponding differences in their respective S-absorbing capacities. Such parallelism suggests that the S requirement of the plant determines its tendency to absorb Se (Hurd-Karrer, 1937, 1938).

Se injury to plants grown in soil in the greenhouse can be inhibited by the addition of excess S either in the form of sulfates or as elemental S (Hance, 1938). In water cultures containing no sulfate, Se concentration even as low as 0.1 ppm produced distinct injury (Hurd-Karrer, 1934).

Silicon

Silicon in flue-cured tobacco varies from 0.94 to 1.41% (Collins *et al.*, 1961). Silica at the rate of 8,000 lbs/A decreased the toxic effect of 8,000 lb/A MgO to tobacco, and still greater effect was produced by 32,000 lbs. This decreased toxicity was apparently caused by a chemical combination between the magnesia and silica. All of the added magnesia had changed to either the carbonate or silicate form after harvesting the second crop (MacIntire *et al.*, 1925).

Silver

Silver is present in a minute quantity in flue-cured tobacco (Kennaway and Lindsey, 1958) as well as in various cigar tobaccos (Johnson, 1936). Tobacco mosaic virus, in suspensions, was treated with AgNO_3 and then inoculated. Such treatment caused a progressive increase in lesion number from no effect at 6×10^{-7} M to a maximum effect (2.75 times the control) at 8×10^{-4} M (Gill and Yarwood, 1964). The effect of AgNO_3 is attributed to Ag^+ .

Sodium

Sodium content in flue-cured tobacco ranges from 240 to 330 ppm (Bacot, 1960). The Na content of cigar tobacco was much higher, from 400 to 4500 ppm (Frankenburg, 1950). The highest Na content, 14,700 ppm, was reported in Japanese tobacco (Yamagata, 1950).

Where an insufficiency of potassium occurred, Na could partially replace K. In the absence of Na a depression of 30% in the growth of seedlings was caused by partially withholding potassium, which indicated a direct beneficial action of Na. A portion of the benefits arising from the use of Na on plants in the field is believed to be due to indirect action, although the culture solution experiments only indicate a direct beneficial effect (Hartwell and Damon, 1919; Holt and Volk, 1945; McEvoy, 1955).

Na_2SO_4 , at 150 ppm, showed a slightly stimulating effect on growth characteristics of tobacco (Gupta). NaCl at 200 ppm was slightly toxic to tobacco plants. A dose of 300 ppm NaCl killed 50% of the plants 3 days after treatment, while 600 ppm killed 71.7%, and 1000 ppm killed 84% (Gupta, 1962-63).

Plants treated with NaNO_3 consistently gave better yields than those receiving $\text{Ca}(\text{NO}_3)_2$. Leaf analyses indicated that Na was readily utilized by, and decreased the K requirement of, the plant (Verona and Stefanelli, 1951).

In a similar experiment, tobacco was grown in soil and river sand for comparison of NaNO_3 with $\text{Ca}(\text{NO}_3)_2$. Results indicate that plants were favorably influenced by Na and need of K was decreased (Popper *et al.*, 1960; Verona, 1951).

Applications to tobacco at the rate of 12 kg, 18 kg, and 24 kg/A of N as $(\text{NH}_4)_2\text{SO}_4$ and NaNO_3 were made. The highest grade of leaf was obtained from application of NaNO_3 (Corbett, 1945-46).

Guaicol peroxidase reaction in tobacco, in the Presence of NaNO_3 , showed an increased effect. The enhanced peroxidase reaction is ascribed to the direct action of NaNO_3 on peroxidase, and not to the suppression of catalase by NaNO_3 (Ostrovskaya, 1950).

The effect of sodium on (A) fresh weight, (B) CO_2 assimilation, (C) respiration, and (D) pigment formation in spinach and tomato plants grown in liquid culture has been investigated. Full or partial substitution of Na for K resulted in decreased fresh weight of spinach. Only complete substitution of Na for K caused a decrease in fresh weight of tomato plants. Without Na, increasing amounts of K increased CO_2 assimilation in spinach and addition of Na increased it still more. Complete substitution of Na for K resulted in an increase in CO_2 assimilation in tomato. In both plants respiration increased with addition of Na over that with K alone. In pigment formation only the content of chlorophyll (A) and (B) was affected by Na (Schmidt, 1959).

Strontium

The occurrence of Sr in plants was first reported in 1897 (Trimble, 1897). Flowering tobacco showed a relatively high content of Sr (Headden, 1921). Various commercial tobacco contains 19-500 ppm Sr (Ward, 1949). Sr-90 in tobacco has increased progressively in the last 10 years: Connecticut cigar wrapper had 0.62, 2.6 and 4.9 pc/g in samples produced in 1956, 1959, and 1963, respectively; Tennessee Burley had 1.7, 2.2, and 3.8 in samples of 1961, 1962, and 1963, respectively; and Florida cigar wrapper had 2.3 and 3.8 in 1962 and 1963, respectively (Tso *et al.*, unpublished).

Most crops were harmed by only the largest amount of Sr. Smaller amounts were definitely stimulating to maximum growth with 1 milliequivalent. In general, Sr is less toxic than Ba. Partial substitution of Sr for Ca was frequently slightly stimulating, especially to maize in germination experiments in sand; but in solution-cultures this crop was invariably harmed (Scharrer and Schropp, 1937).

When tomato plants were grown in nutrient solutions containing Ca/Sr ratios of 2:1 to 4000:1, the shoots did not distinguish between the elements, but the roots absorbed low concentrations of Sr preferentially (Bowen and Dymond, 1956).

Sulfur

Sulfur content in tobacco varies with the type and cultured, generally between 0.2 to 0.7% (Bacot, 1960; Collins *et al.*, 1961; Ward, 1942). When there is insufficient S, the tips of upper tobacco leaves are yellow, and when cured they are much lighter in color than the remainder of the leaf (Anon., 1934). S-deficient tobacco plants differ from normal plants in their organic constituents, including alkaloids, sugars, organic acids, and amino acids (Tso *et al.*, 1960; Tso and McMurtrey, 1960). The effect of S was detrimental to cigar tobacco plants (Gilmore, 1954).

The S content of the tobacco leaf is influenced by S in the fertilizer. The use of superphosphate increases the S content of the leaves as much as 48% over that given by a low-S triple superphosphate (Albert and Lunn, 1935).

As the sulfate content of the fertilizer was increased, there was a decrease in the duration of burn. There is also evidence that increased fertilizer sulfate caused a decrease in alkalinity number of the ash (Neas, 1953).

Of 31 varieties of tobacco tested in Kentucky, only two contained less S than phosphorus; in some cases twice as much. It was found that when S was added to the soil it was rapidly oxidized to the sulphate, the oxidation proceeding more rapidly in a fertile soil than in a poor soil (Shedd, 1914).

Lower tobacco leaves contain 20 times as much sulfate as upper leaves grown with nitrate. This is explained by the theory that S metabolism is dependent upon a reduction process, and in presence of NH_4 , there is no competition for energy from respiration between reduction of sulfate and nitrate. When N is supplied as nitrate, an excess of sulfate must be taken up to maintain the equilibrium (Heiserich, 1935).

Thallium

Only trace amounts of Tl have been found in tobacco (Kennaway and Lindsey, 1958). Study of the toxic effects of 33 elements on young tobacco plants showed that only Tl salts produced the symptom characteristics of frenching: TlNO_3 and Tl_2SO_4 produced similar effects. A concentration of 0.067 ppm TlNO_3 induced chlorotic symptoms of tobacco in 5 days in water cultures. The strap leaf symptom was induced in 12 days at a concentration of 0.13 ppm. The minimum toxic concentration in a nonfrenching soil was about double that in a soil where frenching of tobacco occurred (Spencer, 1937).

Although the appearance of plants in the Tl experiment was similar to that of frenched plants, investigations did not indicate that all frenching was due to Tl toxicity (Van der Veen, 1938). Turkish tobacco tested in fields with

and without added TiNO_3 suggested that frenching and known Tl injury are two distinct physiological diseases. A water extract of nontoxic soil collected near a natural frenching area did not produce frenching until supplemented by the additive effect of nontoxic amount of Tl. It is suggested that the toxic action of Tl may be exerted on the root (Spencer and Lavin, 1939). In many instances, however, the stems of test plants were killed at the surface of the soil, possibly because the material was applied in solution and did not penetrate to any considerable extent from the surface. In solution cultures, 1 ppm Tl either killed the plants outright or slowed the growth, and produced symptoms of chlorosis (McMurtrey, 1932). The possible relationship between Tl and B toxicity was suggested (Shear and Schnell, 1940). The injurious effect of Tl was most noticeable at the higher moisture content.

The weaker the nutrient solution the smaller amount of Tl necessary to produce a toxic symptom. Tl is fixed in plant tissue in such a way as to produce a toxic symptom and a gradient decrease in roots of the youngest leaves. The plant must have a continuous supply of available Tl if toxicity symptoms are to continue to appear on the new growth as it is produced (Shear and Ussery, 1940).

Tin

In various commercial tobaccos, 0.5 to 13.0 ppm of Sn have been found (Ward, 1941). As tin chloride, 0.01 and 0.05 ppm of Sn stimulates root growth of sunflower. Concentrations of Sn in 5 ppm or more, either as chloride or sulfate, showed toxic effects upon many crops (Cohen, 1940). Tin has a very marked influence in promoting germination and growth. It apparently acts upon the reserve material of the seed in much the same manner as diatase or other ferments (Micheels, 1906).

Titanium

Trace to 20 ppm of Ti was found in flue-cured tobacco (Kennaway and Lindsey, 1958; Sund, 1956). From 85 to 270 ppm Ti in various commercial tobaccos was reported (Ward, 1941). Titanium occurs rather generally in soils. The content of Ti oxide varied from traces to 1% and, in general, can be reckoned to vary from 0.3 to 0.6%. Analyses of plants indicate the presence of Ti oxide in almost all cases, the content being greatest in the green parts of plants (Geilmann, 1920). Experiments with sodium titanate showed no influence on plant production (Blanck and Atten, 1924).

Uranium

Certain phosphate rocks contain U; for example, clastic apatite from the

land pebble phosphate field in Florida ranges between 0.011 and 0.032% in pellets (Altschuler, *et al.*, 1958). In igneous rock, 3.3 pc/g of U was reported (Moore, 1914). Fertilizers containing U will supply this element or its daughters to plants. The high level of Ra-226 and Po-210 in certain tobacco soils and in tobacco leaf is probably contributed by such fertilization (Tso *et al.*, 1964). Intake of U by plants increases during blooming and maturation (Drobkov, 1949).

Vanadium

Between 0.2 to 20 ppm of V has been reported in cigar tobacco (Sund, 1956), traces in flue-cured (Kennaway and Lindsey, 1958), and 0.15 to 6.1 ppm in commercial tobaccos (Ward, 1941).

Vanadium accelerates the growth of *Aspergillus niger* (Bertrand, 1941a). Plants ranged from 0.27 to 4.2 mg V/kg of dry matter. Seeds of legumes were particularly poor in V (Bertrand, 1941b).

Zinc

The average content of Zn in various commercial tobaccos is between 51 to 84 ppm (Ward, 1941). Plants receiving 0.0005 ppm or less Zn in culture solution exhibited Zn-deficiency symptoms, *ciz.*, a cessation of meristematic activity in the root tips and cambium, necrosis of leaf tissue, and precocious maturation of tissues. Small tumors developed behind the growing root tips similar to those formed after treatment with growth-regulating substances. Zinc deficiency had more effect on secondary development, which was retarded, than on primary tissues (Carlton, 1943; McMurtrey, 1952).

In experiments with subsoil from a "little-leaf" (rosette) orchard and with solution cultures without Zn, symptoms resembling those of "little-leaf" were evident in tobacco. These symptoms could be prevented by addition of Zn to the medium (Hoagland *et al.*, 1936).

A significant decrease in water content in Zn-deficient plants as compared with control and retardation of growth occurred simultaneously. Only two days after the addition of Zn the water content had increased, accompanied by an increase in growth. The osmotic pressure of the tops of affected plants gradually increased from 5 to 9 atm. as the experiment progressed, while that on the control varied between 5 and 6 atm (Tsui, 1948a).

A decrease in auxin in Zn-deficient plants occurred before any symptoms were noticeable. When Zn was added to the nutrient solution, free auxin and enzyme-digestible bound auxin increased within two days. Zinc is required directly for the synthesis of tryptophan and indirectly for the synthesis of auxins (Tsui, 1948b). It also has a function in promoting both carotene and citrin formation in plant metabolism (Lo, 1945).

The synthesis of nicotinic acid has been studied relative to Zinc (Mothes, 1964). There appeared to be some relation between the level of Zn and the level of chlorophyll in plants (Deschreider and Van Collie, 1952). In hydroponic cultures, Zn is well tolerated and stimulates growth up to 49 ppm. Zinc stimulates germination and probably activates the enzymic processes of mobilizing the food reserves of seeds. Growth of tissues is accelerated. Formation of the flower bud and inflorescence is made earlier. More flowers are formed and more fruit develops; the color is normal. No injury to roots was observed up to 100 ppm Zn, although some inhibition of root formation was noted (Banfi and Gronasoli, 1957).

III. Interaction

In a biological system such as the tobacco plant, the presence of elements of various physical and chemical nature, and also of different physiological roles, would result in a complex phenomena of interaction. Studies regarding such interactions are very involved and interpretations are difficult. Antagonistic phenomena and cation absorption in tobacco in the presence or absence of manganese and boron has been reported (Swanback, 1939a). In that report it was stated that K is antagonistic to Ca, but Ca exhibits only pseudoantagonism toward K. The same is true of Ca vs. Mg. Ca depresses the uptake and translocation of Fe and is antagonistic to Na. Mn, under certain conditions, has a regulating influence on the absorption of Ca, while Ca is antagonistic to Mn. B aids absorption and utilization of Ca.

This kind of interaction also exists among other elements. For example, a 3-way Fe-Mn-Mo interaction has been observed in tobaccos (Kirsch *et al.*, 1960). Added Fe counteracted the yield-depressing effect of added Mn. The amount of Fe required for maximum yield increased as Mo was increased. At lower Fe levels, Mo decreased yield, increasing it at higher Fe levels. Fe reduced total Mo uptake, stimulating translocation from roots to leaves. Mo in toxic levels accumulated in roots at low, but not high Fe levels. At low Fe and Mn levels, added Mo had little effect on Mn uptake, while at lower Fe and higher Mn levels, Mo decreased Mn uptake. At higher Fe and Mn levels, Mo increased Mn uptake. Another example is the absorption of Ni by oat plants (Crooke, 1955). Ni absorption increased with increasing pH for a fixed Fe supply. Nickel uptake and toxicity symptoms were reduced when Fe concentration in the nutrient solution was high.

Phosphorus fertilization has been demonstrated to affect plant Zn nutrition (Burleson *et al.*, 1961). Excessive amounts of phosphate fertilization depress Zn, however, addition of Na_2SO_4 together with Zn to the same soil decreased the

P uptake and left the tobacco leaf Zn contents unvaried (Fortini and Morani, 1960). High concentration of phosphorus also decreased Mg in tobacco plants (Takahashi and Yoshida, 1957).

The influence of Fe and Na on growth of tobacco tissue cultures has been studied. Ferric tartrate was a better source of Fe than $\text{Fe}_2(\text{SO}_4)_3$. The omission of sucrose, Fe, and NaH_2PO_4 , resulted in poor growth of tobacco tissue cultures in the first passage (Hildebrant *et al.*, 1946).

Black shade leaf was higher in Fe and Mn content than light leaf from the same farm. On the basis of dry weight, black shade leaf averaged 0.19% Fe_2O_3 and 0.16% Mn_2O_4 , while light shade leaf averaged 0.09% Fe_2O_3 and 0.05% Mn_2O_4 . Graduation in color of leaves from light to black from the same fields was accompanied by graduation of Fe and Mn contents, the concentration of the metals being almost regularly greater as colors darken (Le Compte, 1941b). Generally the greatest foliar concentration of Fe and Mn was found below the seventh node (Le Compte, 1943).

In solution-cultured burley tobacco, growth of plants and quality of leaf were both strongly retarded by the presence of high concentrations of Fe and Mn under low P conditions. High Fe caused a brownish color and lowered leaf quality. In the case of high P, an increase in Fe gave better growth and quality. Adequate oxidizing conditions were needed to attain a normal result (Fujiwara and Kurosawa, 1956).

NaI plus KI and $\text{Co}(\text{NO}_3)_2$ in 0.2% solution were sprayed on tomato plants at the time of full blooming, first on I-sprayed plants, then on Co-sprayed. Damage to leaves was noted and the yield of fruit was less than on controls but Cu, Mn, Ni, Zn, and Fe content was higher. Plants sprayed with I and Co showed increased resistance to fungal infection (Terent'eva, 1962).

Tobacco plants produced in limed and nonlimed fields under various fertilizations were analyzed for Si, Mg, Fe, and Al oxides, S, Cl and P or organic constituents (Darkis *et al.*, 1937). Total ash is smaller from limed tobaccos including Si, Cl, and S. Rainfall affects the content of microelements in tobacco; decreased rainfall increases Fe, Al, Cl, and S in cured tobacco.

The addition of Hoagland solution with microelements including Li, Cu, Zn, Ti, B, Al, Sn, I, Mn, Ni, Co, and Br increased the weight of tobacco 100-180% (Gessner, 1939).

Field tests with Fe, Mg, Cu, Zn, Mn, Co and B added individually to the control fertilizer indicated that, with the exception of B, Zn, and Mn, they all had depressing effects on leaf value (Smith *et al.*, 1939). These results differ from many other studies since the soil and culture conditions may have been entirely different.

IV. Physiology and Metabolism

Two different ways are used to express the changes in various constituents of a plant. They are: (A) amounts per 100 g dry tissue, and (B) per individual plant. It appears that method (B) gives a more satisfactory picture of the changes (Valadesecu, 1934a). In *Nicotiana tabacum*, dry weight, organic matter and Ca increase both absolutely and relatively during the first 30 days after germination. Other elements and total N steadily decrease by method (A), while they increase by method (B) during the same period. The decrease is most marked for Fe, Si, and Mn. The elements determined were assimilated with decreasing ease as follows: Ca, K, N, P, Mg, Si, Mn, and Fe.

S, Mg, and Cl seem to be absorbed in large quantities at the beginning of the tobacco growing period, while P and Mn are absorbed rapidly toward the end of the season (Ward, 1941). There is an apparent slowing up of physiological activity in the bottom portions of the plant after bottom leaves have matured; in some cases, decreasing the quantity and percentage of constituents in these leaves. A translocation of minerals is more evident in flue-cured tobacco than cigar tobacco. Mineral absorption with respect to various stages of plant development was also investigated (Valadesecu, 1934b). The total dry weight of tobacco plants reaches the maximum at time of flowering (about 45 days), then decreases about 20%, rising again to a higher maximum than before and again decreasing at the time of appearance of the lateral buds. The second maximum is also shown, to a lesser extent, in plants when buds are removed. Si, Fe and Mn show a steady increase with age, and no decrease, probably because present in insoluble form.

Alterations in the flowering and fruiting of *Nicotiana rustica* produced by deficiency of the fundamental mineral elements was interpreted as an indirect action, possibly through a metabolic effect or growth alterations related to auxin formation (Nebot, 1959). The effect of trace elements on some physiological processes and on yield of tobacco has been studied (Kalekenov, 1962). Addition of B, Cu, and Mn fortified the growth of tobacco leaves and stems, increasing the dry matter content during the entire vegetative period as well as intensifying the photosynthetic process (max. with B). This effect was somewhat lower during the flowering period. All these trace elements show a definite effect on the chemical composition of leaves. B increased mostly the N content (protein N by 17.7%) and the nicotine content to a smaller degree. All added elements increased the content of cellulose and water-soluble sugars.

Application of MH-50 mixture, consisting of 13 trace elements (B, Mn, Cu, Zn, I, Br, Ti, Sn, Li, Ni, Co, Mo and Cr), increased the content of monoglucides in leaves by 28.3% and diglucides by 34.6%, but had no substantial

effect of total N, nicotine, or total ash content (Pastyrik and Priehradny, 1956).

Although there were some effects of 2,4-D on the accumulation of some mineral elements in tobacco plants, no difference was noted for B, Cu, Fe, Mg, Mn, Na, or Zn (Wildon *et al.*, 1957).

B deficiency led to a large increase in total alkaloid, while deficiencies of Fe, Zn, Cu, Mn, or Mo led to decrease. Excesses led to decreased alkaloid with Fe, Cu, or Mn, but to increased alkaloid with Mo or Zn (Steinberg and Jeffrey, 1956).

Soil temperature was reported to affect the content and distribution of B, Mn, Zn, Fe, Al, Ti, Cu, Ni, Pb, Mo, Ag, Cr, Ca, V, Sn, and Co in certain crops (Paribok and Kuznetsova, 1963).

Symptoms of Mg, S and Fe deficiency can be reported experimentally in the tobacco plant by withholding the element from a portion of the root system. Failure to cross transfer nutrients does not always manifest distinctive effects unilaterally on the individual leaf. In some instances an entire leaf may be normal, while an adjacent leaf bilaterally manifests symptoms of deficiency. A twisted or one-sided growth results when a portion of the root system fails to receive distribution of the elements. Root and top growth does not always take place when the roots are divided, thus applying a given element to one-half of the root system and withholding it from the other (McMurtrey, 1937).

Tobacco mosaic virus (TMV)-infected plants grown in liquid nutrient solutions containing various concentrations of Mo developed characteristic deficiency symptoms at low levels of the element and toxicity symptoms at high levels. TMV concentrations were lower in plants grown at concentrations of Mo supra-suboptimal for plant growth. The reduction in virus concentration caused by Mo deficiency could be overcome by supplying the plants with high levels of ammonium N. Deficiency symptoms were lessened somewhat by this treatment. The addition of supplemental Fe to plants supplied with excess Mo alleviated somewhat the chlorosis produced by excess Mo, but had no effect on virus concentration (Pirone and Pound, 1962).

There is a positive effect of trace nutrients B, Cu, and Mn on growth processes and development of the tobacco plant. Definite increase in the intensity of photosynthesis, accumulation of chlorophyll, contents of free and combined water in leaves, and accumulation of N products have been observed (Darkanbeau and Kalekenov, 1963). In another study, B, Mn, Zn, Cu, Mo, Co, and Al had a positive effect on the rate of photosynthesis; this effect being associated with their capacity to increase heat resistance and decrease the negative effect of high temperatures on enzyme activity, activity of plastids, and migration of assimilation material (Shkol'nik, 1961).

The foliar absorption of sulfate, Rb, and Cl by leaves of tobacco plants may affect the mechanisms and kinetics of ion uptake, binding of ions on cuticular leaf surfaces, penetration of cuticular membranes by ions, and ion uptake by isolated cells of the leaf (Wittwer *et al.*, 1964).

Micronutrient deficiency, except that of Cu, led to an increased content of nitrate and free amino acids in the tobacco leaf and a decrease in protein. Diminished growth caused by deficiency led to decreased ascorbic acid. Deficiency of B and Mo drastically reduced ascorbic acid (Steinberg *et al.*, 1955).

Tobacco plants with deficiencies of Ca, Mg, or P contain increased proportions of polyphenols; the chlorogenic acid content being lowered. Deficiencies were also associated with increased accumulations of substances which were probably derivatives of cinnamic acid and/or of coumarin. These substances occur in the flower and in leaves immediately below the flower shoots of normally grown plants (Loche and Chouteau, 1964).

Mineral nutrition and enzymic activity of plants, particularly on *Nicotiana tabacum*, has been summarized in a review (Tombesi, 1958) and shall not be repeated here. Some of the enzymic patterns appeared in leaves prior to, or in the absence of, visual symptoms of tobacco (Brown and Steinberg, 1953).

The activities of ascorbic acid oxidase, catalase, and peroxidase in tobacco plants, deficient in specific micronutrients, showed an enzymic pattern in the leaf lamina characteristic of each type of deficiency (Brown and Steinberg, 1953). Tobacco grown on calcareous soil (CaO-induced chlorosis) gave the same type of enzymic pattern as when grown in Fe-deficient solutions. Growth on Cu-deficient organic soil gave the same type of pattern as in Cu-deficient solutions. Fe-deficient plants had a lower peroxidase activity than B- and Zn-deficient plants. Catalase plants decreased in ascorbic acid oxidase activity, this decrease being greater in Cu-deficient plants with high catalase activity.

V. Summary

This review covers thirty-six microelements related to various areas of tobacco research, including agronomy, botany, physiology, and biochemistry. Despite advances in the field of biological science in recent years, the phenomena of plant growth, development, and organic metabolism remains to be understood. Various interpretations of similar findings are purposely included in this review in order to stimulate further investigations.

The presence of certain micro-elements in tobacco plants may merely be the resultant of the circumstance of site, season, and variety, and therefore have no physiological or agronomic significance. The wide distribution of tobacco plants in various locations of the world under different climatic, soil, and culture conditions made it impossible and impractical to summarize into

a general conclusion the micro-and secondary-element requirements in tobacco. Any discussion of a certain element regarding its level, distribution, physiological role, as well as the fertilizer and soil requirements, may well not be applicable to that element under different circumstances. In this review the gathered information, which is often contradictory, can only be meaningful when interpreted with such an understanding.

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菸草中之微量元素及次要元素

左 天 覺

植物生理研究工作，常應用菸草爲材料。植物營養學之發展，菸草貢獻尤多。近年世界各國改進菸草生產，着重菸葉化學成份。微量元素及次要元素，影響菸草成長，調節生物化學變化，而決定菸葉品質。近三十年來（1935-1964）之文獻，達三百餘篇。其中有性質相同，試驗重複；或結果相似，而理論懸殊。本文擇要加以歸納整理，並加評述。計包括三十七種元素，其對植物成長發展，元素間之相互關係，植物生理，新陳代謝作用，及菸葉品質等等都分別中論，以供參考。

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