Nutritional Requirements for Florida Sugarcane

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In order for any crop to grow and remain healthy, certain elements (nutrients) must be made available to the plants from the soil and/or air. The "essential elements" include C, H, O, N, P, K, Ca, Mg, B, Cl, Cu, Fe, Mn, Mo, S, and Zn. In Florida and some other areas of the world, sugarcane production may also be markedly enhanced by the application of Si. This element qualifies as a "functional" or "beneficial" element since, in the absence of Si, the plant can still complete its entire life cycle, although production and general vigor may be reduced. In the Florida sugarcane industry, elements that are of nutritional concern include N, P, K, Mg, B, Cu, Fe, Mn, Si, and Zn. A deficiency or over-abundance of one or more of the above elements may limit yields. An over-abundance of one element may cause a deficiency of another. Growers striving to produce high crop yields should pursue management strategies that deliver a balanced supply of nutrients to the plant. Since relatively large quantities of N, P, K, S, Mg, and Ca are needed by plants, these are referred to as "macronutrients." The remainder of the required plant elements are usually called "micronutrients."

Most, if not all soils require some level of fertilizer or amendment input to produce high crop yields. Efficient fertilizer use is a primary component of a farmers Best Management Practice (BMP) program. BMPs are economically sensible practices shown by research to improve crop yields, conserve natural resources, and make the best use of all farm inputs. Fertilizer BMPs include soil testing, using split applications of nutrients when practical, and applying fertilizers at rates that are consistent with soil test results and realistic yield expectations.

Soil Testing

Today's high yielding crops put tremendous pressure on the soil for nutrients. This makes soil testing important for efficient sugarcane production. Applying the proper amount of nutrients saves the producer money, helps protect the environment, maximizes crop yields, conserves valuable resources, and prevents nutrient imbalances. More specifically, a well managed fertilizer program promotes rapid canopy development, increases crop resistance to disease and insects, and lowers irrigation.

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requirements. Rapid canopy development, in turn, aids in weed control.

Soil testing for fertilizer recommendations for sugarcane grown in Florida is conducted by the Everglades Soil Testing Laboratory at the UF/IFAS Everglades Research and Education Center (EREC), Belle Glade. These fertilizer recommendations are based on interpretations of field trial research that quantify plant growth responses to added fertilizer inputs.

**Collecting and Submitting Soil Samples**

A representative sample is a prerequisite for a meaningful and reliable soil test. The error associated with soil sampling is typically much greater than that associated with chemical analysis, so great care must be taken with the sampling procedure. Generally, fields to be sampled should be cultivated and ready for fertilization. It is recommended that samples are taken to a 6 inch depth starting about 100 feet from the road near one end of the field, and continue collecting samples in a zigzag fashion across the field, so as to have a minimum of 30 cores from a 40-acre block. These 30 cores should be composited and thoroughly mixed. To prevent contamination of the soil sample, use plastic rather than galvanized buckets for mixing. A subsample should be placed in a clean soil sample bag and labeled properly. Certain information must be provided with every sample submitted. Information sheets for providing this data are available from the Everglades Soil Testing Lab. Soil tests are available for pH, P, K, Ca, Mg, Si, and Fe. Contact your County Extension Agent for additional details regarding soil sampling, testing or sample submission procedures.

**Plant-Tissue Testing**

Recent work has shown plant-tissue testing, when used in conjunction with soil testing, can be a valuable tool for refining fertilizer recommendations and improving crop yields. Tissue testing is also useful in diagnosing, verifying or preventing future nutritionally related plant disorders or imbalances. In fact, detection of micronutrient imbalances is one advantage tissue testing has over soil testing. No suitable soil tests have been developed for accurate micronutrient recommendations for Florida sugarcane. Although many parts of the plant may be used for tissue sampling, the "top-visible dewlap" (TVD) leaf without its midrib is generally the tissue of choice for measuring the nutrient status of sugarcane in Florida (see EDIS publication SS-AGR-229 Sugarcane Leaf Tissue Sample Preparation for Diagnostic Analysis at http://edis.ifas.ufl.edu/SC076). Also, tissue sampling is usually done during the "grand growth period" when the crop is approximately 4 to 6 months of age.

Efforts to interpret plant tissue testing results have led to two nutritional concepts - "Critical Nutrient Level" (CNL) and "Nutrient Balance." The CNL is defined as the nutrient concentration at which production losses reach 5 to 10% and may be considered the minimum acceptable level of nutrient concentration. At concentrations below the CNL, substantial losses in production would be expected. The CNL is in the marginal zone of nutrient concentration and slightly below the optimum or sufficient zone. The optimum zone of nutrient concentration is the preferred range for each nutrient but, increasing an element's concentration above the lower end of the optimum range is unlikely to appreciably affect the plant growth rate. In fact, excessively high concentrations of a given nutrient may promote nutrient imbalances or adverse toxicity symptoms.

The concept of "Nutrient Balance" is highlighted within the Diagnosis and Recommendation Integrated System (DRIS). A DRIS database is composed of a very large number of paired observations that relate plant tissue nutrient concentrations with observed plant yields. Optimum nutrient ratios (generally ranges) are then identified that correlate well with optimal yields. With respect to diagnosing plant tissue test results, when a tissue sample generates a sub-optimal nutrient ratio, this may indicate that one or more nutrients that compose the ratio are either deficient or out of balance. The details of DRIS are beyond the scope of this fact sheet, but are explained in greater detail in EDIS publication SS-AGR-129 Sugarcane Plant Nutrient Diagnosis at http://edis.ifas.ufl.edu/SC075.
A recent study determined that summer fertilizer supplements for Florida sugarcane based on spring leaf analysis were not cost-effective on a large scale. Growers are instead encouraged to use leaf analysis to make adjustments to amendments or fertilizer inputs that will be applied to the next season's crop rather than trying to estimate an additional fertilizer application to the current crop.

**Nutrient Mobility**

As the term "mobile" suggests, some elements can be readily moved through the plant from older plant parts to younger, actively growing plant tissues, while other elements become "fixed" (immobile) in the plant tissue during growth. N, P, K, Na, Mg and to some extent S are considered mobile. B, Fe and Ca are quite immobile, whereas Zn, Mn and Cu are generally considered immobile, but less rigidly so. Specific visual symptoms or patterns of plant nutrient deficiencies (chlorotic/pale striping along the leaf blade, "burning" at the leaf tip, etc.) as well as the location on the plant (old leaves, newly emerging growth, stalk, etc.) where these symptoms are found reflect, in part, the relative mobility (or immobility) of a given plant nutrient. Thus, a general understanding of nutrient mobility (or immobility) is important when trying to diagnose nutrient deficiency symptoms in sugarcane. When a nutrient is mobile, deficiency symptoms typically appear on lower older leaves, while if a nutrient is immobile, deficiency symptoms typically appear on new growth.

**Macronutrients**

**Nitrogen (N)**

General guidelines: No N fertilizer is recommended for sugarcane grown on muck soils. Under south Florida growing conditions on organic soils, N deficiencies are rarely seen in sugarcane. The high organic N contents in muck soils are continuously released during natural soil mineralization processes, and the resulting inorganic N compounds are readily available for plant uptake and growth. A deficiency may appear if organic N cannot be mineralized under unfavorable conditions, such as flood. For example, young sugarcane plants grown on shallow mucks during cool, wet periods have shown N-deficiency symptoms.

On the other hand, N deficiencies can readily occur in sugarcane grown on sandy soils. Multiple applications of N fertilizer are often required during the growing season to sustain adequate sugarcane production on mineral (sandy) soils, which lack the high organic N contents of muck soils. Failing to supply adequate N during critical growth periods can result in stunted plants, premature ripening, and reduced biomass and sugar yields.

UF/IFAS nitrogen fertilization recommendations for sugarcane grown on both organic and mineral (sandy) soils are not based on soil-testing. In fact, soil-testing for N is generally not a helpful tool since the soil-N pool can change rapidly depending on environmental (rainfall, temperature) and crop management (tillage, irrigation) factors. It has already been mentioned that the natural soil-N supply from south Florida organic soils is more than adequate for sugarcane crop growth. The remaining discussion addresses N fertilization and crop-N management issues relevant to sugarcane grown on sandy soils, particularly the "marginal" sandy soils that represent the areas in south Florida undergoing significant sugarcane expansion over the last few decades.

Hot south Florida growing conditions encourage the chemical transformation of applied N fertilizer sources into the nitrate (NO$_3^-$) form, which is readily available for uptake by plant roots. The problem is that these negatively charged nitrate NO$_3^-$ molecules also resist being "held" by negatively charged soil organic matter (OM). Since sugarcane biomass accumulation (and N requirements) are greatest during the hot-humid summer months of the rainy season, there is a very real likelihood that some fraction of the applied N fertilizer will be lost either due to leaching below the root zone of these marginal sandy soils or due to denitrification, and thus become unavailable to the sugarcane plant. Estimating these losses and designing a sugarcane N-nutrition management response to account for these potential losses is highly problematic. Sandland sugarcane growers are encouraged to apply their N fertilizer in multiple split-applications in order to minimize N losses and maximize the N-supply to plant roots. The frequency and method of fertilizer applications, however, will ultimately be determined by economic
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Factors such as increasing labor and fuel costs, as well as other practical considerations such as an inability to enter a field due to increasing crop canopy height during the growing season.

Mineralization of soil OM releases plant-available N to the growing crop. Soil OM mineralization rates are generally much higher during hot summer weather, and although this coincides with the grand growth period of sugarcane, it also coincides with the rainy season, which again influences the movement of N (released from soil OM) through the soil profile. Additionally, south Florida mineral (sandy) soils typically have very low soil OM contents, so the soil-N supply from native soil OM can be expected to be very low, and crop availability of this N supply will likely be diminished by soil-N movement during frequent south Florida rainfall events.

It is also widely recognized that the sandy soils cropped to south Florida sugarcane are variable in terms of native soil OM contents, cation exchange capacities (CEC), and to some degree, soil pH. These factors affect native soil-N supply, and can vary considerably across adjacent 20-acre fields, and even within any given 20-acre field block. Regardless of this variability, the native soil OM and CEC for any of these sandy soils is considered low.

The discussion presented above highlights the difficulties south Florida growers face regarding efforts to manage the N supply to sugarcane grown on sandy soils for optimal sugarcane growth. In response to these concerns, the Sugarcane Focus Area Team has created a task force to design and conduct N fertilization studies to assess sugarcane biomass yield and sucrose accumulation in response to different N fertilization rates and application timings across a wide spectrum of different south Florida sandy soil types.

In the meantime, current UF/IFAS nitrogen fertilizer recommendations during a 1-year crop cycle for sugarcane grown on south Florida sandy soils is 180 lb N/A. There is the expectation that growers will use split-applications of N fertilizer sources, but recommended management options in response to untimely high rainfall events (which could result in low N-use efficiency) remains uncertain. This uncertainty has led to an increased interest in the use of slow release N fertilizer sources for sugarcane, which should also be the subject of future research efforts. Nitrogen recommendations for mucky sands are 110 lb N/A and for sandy mucks, the recommendation is 30 lb N/A. Again, for organic muck soils, no N is recommended for sugarcane.

Nitrogen has the greatest influence on cane ripening of all the nutrient elements. Cane will store a higher percent of sucrose when N is limited 6 to 8 weeks prior to harvest. Although a late-season N deficiency can actually promote improved sugarcane ripening, this scenario is unlikely to be achievable on organic soils.

Deficiency symptoms: Since N is a mobile nutrient, N-deficiency symptoms are first observed on older leaves (since N is mobilized from older tissues in favor of supporting growth in new tissue), although deficiency symptoms can eventually be seen over the entire plant. Leaf blades become uniformly pale-green to yellow, stalks become short and slender, internode growth is reduced, and vegetative growth is reduced. If the deficiency is prolonged, the tips and margins of older leaves become necrotic.

Phosphorus (P)

General guidelines: Phosphorus is likely to be deficient in organic and mineral soils in Florida. Careful control of available P levels is essential for high yields of sugarcane and sucrose. Root development is slow when P is limited and results in inadequate utilization of available moisture and nutrients. Deficiency is much more common in ratoon crops, and deficiency symptoms tend to increase with age of planting. Amounts of P recommended by the Everglades Soil Testing Laboratory range from 0 to 33 lb P/A (0 to 75 lb P₂O₅/A) for plant cane and first ratoon crops, 0 to 31 lb P/A (0 to 70 lb P₂O₅/A) for second ratoon, and 18 lb P/A (40 lb P₂O₅/A) for subsequent ratoons.

Deficiency symptoms: Since phosphorus is a mobile nutrient, deficiency symptoms typically appear first in older tissues. P deficiency causes an overall retarding of plant growth. Stalk internode length, total stalk length and stalk diameter are
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red. At first, distinct foliar symptoms may not be observed. Later, leaves may become slender and blue-green in color. Red or purple color may also be seen, particularly at tips and margins exposed to direct sunlight. Eventually, leaf foliage dies back from the leaf tip and along the leaf margins.

**Potassium (K)**

**General guidelines:** Sugarcane utilizes large quantities of potassium. Deficiencies are commonly observed on well-drained, coarse, sandy soils. In comparison to other nutrients, sugarcane response to K fertilization is usually most immediately apparent. Fertilizer K recommendations by the Everglades Soil Testing Laboratory range from 0 to 208 lb K/A (0 to 250 lb K\(_2\)O/A) for plant cane and first ratoon crops, and 0 to 125 lb K/A (0 to 150 lb K\(_2\)O/A) for second ratoon and all subsequent ratoon crops.

**Deficiency symptoms:** A potassium deficiency can result in depressed growth, slender stalks, and "firing" (an orange or reddish-brown discoloration) on older leaves. Because K is readily mobile, deficiencies are first observed in older plant leaves. Young leaves are generally all dark green. The most distinguishing characteristic of this deficiency is a red discoloration on the upper surface of the leaf blade midrib. Discoloration on both sides of the midrib may indicate a fungal disease. Under severe deficiency, the leaf spindle will distort, producing a characteristic "bunched top" or "fan" appearance. Poor bud germination and decreased drought and disease resistance are associated with K deficiency.

**Calcium (Ca)**

**General guidelines:** Florida sugarcane is unlikely to suffer calcium deficiencies since this element is abundant in the soil-water environments where sugarcane is traditionally grown. Nonetheless, Ca is a macronutrient that is vitally important for plant growth. An acute shortage of Ca will lead to rapid deterioration and plant death. Most of the organic soils in Florida overlay limestone bedrock, and Ca is moved into the surface soil profile by diffusion and mass flow with soil water.

**Deficiency symptoms:** Ca deficiency is not a concern for sugarcane production in Florida. A Ca deficient plant, however, is characterized by limited top and root growth and slender stalks. Older leaves acquire a "rusty" appearance and show signs of mottling and chlorosis. The spindle leaf browns and dies, and young leaves can become distorted. If the deficiency is severe, the terminal bud and plant may die. Calcium is considered an immobile plant element.

**Magnesium (Mg)**

**General guidelines:** Magnesium deficiency is sometimes seen in sugarcane grown on acidic sandy soils and organic soils. Magnesium is a component of the chlorophyll molecule and therefore is essential for photosynthesis. High K fertilization can promote Mg-deficiency symptoms. Although Mg is not routinely recommended for application on organic soils, leaf analysis can be used to determine if Mg application is needed. For sands and mucky sands, a 6-lb Mg/A application is recommended when the acetic acid extractable soil test value is less than 100 lb (soil-test Mg)/A. Because Mg was determined to be a major limiting factor on sandy mineral soils in a recent leaf nutrient survey of commercial sugarcane fields in Florida, a Mg amendment such as dolomite (or calcium silicate containing Mg) should also be considered at planting for fields with low soil-test Mg or for fields with plants exhibiting low leaf tissue Mg concentrations in the previous season's crop.

**Deficiency symptoms:** Magnesium deficiency is distinguished by intense "rust-like" red freckling that is especially prevalent on older leaves. Severe Mg deficiency may cause the stalk to become stunted and severely "rusted" and brown. Magnesium is considered a mobile plant element.

**Sulfur (S)**

**General guidelines:** Sulfur deficiencies are unlikely to occur in sugarcane grown on organic soils since the quantity of S supplied directly from the muck soil is more than sufficient to satisfy the crop nutrient requirement of sugarcane. Soil microorganisms can oxidize elemental S and organic S compounds present in the soil into the sulfate forms that are available for plant uptake. Also, some popular fertilizer blends used to deliver N, P, K, or Mn to the crop may also contain appreciable amounts...
of S. Finally, S is also present in the atmosphere as sulfur dioxide and sulfur trioxide, which enters the soil-water system via rainfall. For these same reasons, S deficiency is also unlikely to be a problem for sugarcane grown on mineral soils. The Everglades Soil Testing Laboratory does not perform a S soil-test and has never offered S fertilizer recommendations for the purpose of delivering S to the sugarcane crop. However, when soil sample pH values exceed 6.6, the Everglades Soil Testing Laboratory has traditionally offered a suggested input of elemental S directly into the sugarcane furrow at planting. Elemental S is a soil acidifying source, and by reducing soil pH (making more acidic) within the furrow, the availability of micronutrients should be enhanced. Thus, when soil pH exceeds 6.6, the suggestion has been to apply (in the furrow) 500 lb S/A for muck and sandy mucks, 300 lb S/A for mucky sands, and no S for sands. The economics underlying this use of S should be assessed by the grower. Elemental S rates are currently under study for sugarcane on organic soils. Environmental issues should also be considered since environmental S has been linked to sulfate-reducing bacteria (i.e., they use specific sulfur compounds as an energy source) that catalyze mercury transformations in wetland ecosystems.

**Deficiency symptoms:** Sulfur is somewhat mobile within the plant. Sulfur deficiency is characterized by a general chlorosis and yellowing of leaves, especially younger leaves. The young leaves may develop a faint purplish tinge on their margins. In time, plants lack vigor, leaves are shorter and more narrow, and stalks are small in diameter.

**Micronutrients**

**Boron (B)**

**General guidelines:** Boron deficiency has been observed on Florida sugarcane grown on sandy soils. Boron is readily leached from the root zone. Boron deficiency is accelerated under extremely dry soil conditions. The recommended application rate for B, based on apparent crop requirement, is 1 lb B/A (for mucks and sandy mucks) and 0.5 lb B/A (for mucky sands and sands). The B application should be delivered directly into the furrow at planting. No recommendations are made for ratoon cane.

**Deficiency symptoms:** Boron deficiency is very distinctive since it is relatively immobile (not readily translocated from older tissues to young growth). Young leaf blades contain clear lesions (or "water sacks") sometimes accompanied by exudation of water droplets from the upper leaf surface. Young leaves become distorted and necrotic, leaf blades become narrow and brittle, leaf tips may desiccate (dry out and look burned), and young plants become bunched with many tillers. The spindle leaves turn white, dry out and eventually appear dead. This spindle leaf symptom may appear similar to those associated with the fungal disease "pokkah boeng" (a Javanese term that describes a malformed, twisted, or disturbed top) as well as symptoms associated with damage from some herbicides.

**Copper (Cu)**

**General guidelines:** Copper deficiencies were a major problem when the organic soils of the Everglades were first cleared and planted to agricultural crops. Copper is an immobile nutrient, therefore meristematic tissues and young leaves are first affected. Excessive P fertilization can accentuate Cu deficiency. Copper deficiency of relatively mature cane is characterized by a wilted appearance of the younger leaves. Most deficiencies have been observed on organic soils and are easily corrected through foliar sprays or fertilizer. Copper recommendations, based on apparent crop requirement, is 2 lb Cu/A (for mucks and sandy mucks) and 1 lb Cu/A (for mucky sands and sands). The Cu application should be delivered directly into the furrow at planting. No recommendations are made for ratoon cane.

**Deficiency symptoms:** Dark-green splotches appear on a slightly chlorotic and wilted leaf blade. If unchecked, poor stool development and "droopy top" can result. Although no necrosis is seen at first, severe Cu deficiency may cause leaf necrosis, shortened internodes, reduced tillering and vigor, and reduced growth and yields. On the other hand, in mild deficiencies, the crop may "out-grow" the Cu deficiency after the young plant develops a more mature root system.
Iron (Fe)

**General guidelines:** Iron deficiency has not been of much concern in Florida sugarcane production. The rare Fe deficiencies that have been found were geographically localized. A recent survey of Florida sugarcane fields suggested that Fe may be a minor concern on mineral soils. Poorly developed and physically damaged root systems resulting from alkaline soils, or soil insect pests can induce Fe deficiency. Plants normally out-grow Fe deficiency symptoms, but with excessive root damage and low soil Fe conditions, the symptoms may persist. Iron foliar sprays can be used to correct chlorotic leaf symptoms, but they may not increase or affect yields. Further research is needed on Fe, particularly in mineral soils. Although there is no official UF/IFAS Fe recommendation for sugarcane, an application of 5 lb Fe/A at planting is suggested if leaf tissue Fe concentrations were low in the previous season's crop.

**Deficiency symptoms:** Many times plants showing deficiency occur randomly among plants that appear normal. Since Fe is relatively immobile, deficiency symptoms typically appear in young, actively growing plant tissues. Young shoots may emerge in a completely chlorotic condition. Deficiency symptoms in young leaves include light green striping between parallel leaf veins along the full length of the leaf blade. The stripes are similar to those seen in Mn deficiency (although Mn deficiency symptoms generally involve striping from the tips to the middle of the leaf blade). In severe deficiencies, the areas between the veins become increasingly chlorotic to white.

Manganese (Mn)

**General guidelines:** Manganese deficiencies are commonly seen on both high-pH (above 6.5) organic and sand soils. High pH soils limit plant availability of Mn. Deficiency is also associated with soils high in Mg, Ca, and N. An effective method for preventing Mn deficiency on high pH soils is to place S in the furrow together with Mn when planting sugarcane. When soil pH exceeds 6.0, the recommendation is to apply 5 lb Mn/A (for mucks and sandy mucks) and 2.5 lb Mn/A (for mucky sands and sands). The Mn application should be delivered into the furrow at planting.

**Deficiency symptoms:** Since manganese is an immobile nutrient deficiency symptoms typically affect younger leaves first. The deficiency is characterized by pale-green to white interveinal stripes (chlorosis) alternating with normal color from the tip to the middle of the leaf blade. Leaf splitting or fraying may also be apparent.

Silicon (Si)

**General guidelines:** Silicon is considered a “functional” or “beneficial” (rather than essential) plant nutrient. In Florida, Si amendments may increase cane and sugar yields as much as 25% and may support more successful ratoon crops. Calcium silicate slag, a popular Si source, tends to have low solubility under high soil pH conditions. The Everglades Soil Testing Lab offers a Si soil-test but does not offer specific Si application recommendations since the Si soil-test has not been calibrated for sugarcane production. Nonetheless, growers have experimented with Ca-silicate slag applications for many years. This collective experience suggests that when soils test low for acetic acid extractable Si (less than 10 ppm in the soil extract), a 3 ton/A application of Ca-silicate slag will likely support favorable yield improvements over a three-crop cycle. The Si source is generally broadcast applied and disked into the soil prior to planting. Leaf Si analysis is very useful for supplementing soil Si values in determining the need for calcium silicate application.

**Deficiency symptoms:** Si deficiency is characterized by minute, circular white leaf spots (freckles). The freckling is more severe on older leaves. Older leaves may senesce prematurely, and the stools exhibit poor tillering and ratooning characteristics.

Zinc (Zn)

**General guidelines:** Zinc deficiency more commonly occurs on high pH sandy soils. Zn deficiency can be enhanced by high applications of...
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banded P and K, by over-liming, and by high soil pH. The recommended application rate for Zn, based on apparent crop requirement, is 2 lb Zn/A (for mucks and sandy mucks) and 1 lb Zn/A (for mucky sands and sands). The Zn application should be delivered directly into the furrow at planting. No recommendations are made for ratoon cane.

**Deficiency symptoms:** Zinc is considered fairly immobile and thus deficiency symptoms typically appear in younger leaves. Young leaves have light-green stripes that are in the leaf veins, (not between the veins as in a Mn deficient leaf). The leaves are also small and non-symmetrical. Necrosis of the leaf tips may occur when the Zn deficiency is severe. A Zn deficient plant has reduced tillering and ratooning ability.

**pH**

**General guidelines:** Organic soils are highly buffered against pH change. Liming low pH organic soils is not practical due to the enormous quantity of limestone amendment required. Lowering the soil pH of organic soils can be accomplished with S applied in the furrow. This treatment is temporary, localized to the furrow, and expensive. High soil pH (pH > 6.6) levels are often associated with a range of micronutrient deficiencies.

Many sandy soils under sugarcane production have lower than optimum surface soil pH. A yield increase due to liming is expected if soil pH is less than 5.5. Liming with dolomite effectively raises soil pH and supplies plant available Mg. A 2 ton/A application of dolomite (broadcast applied and disk incorporated prior to planting) should increase soil pH by approximately one pH unit.

**References**


