"Turfgrasses do not eat cookies – your turf soil solutions"

STMA
WEST Palm Beach
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OBJECTIVES

- Plant Roots and Nutrient Uptake
- All the "3-3-3… Golden Rules" of plant nutrients and nutrient physiology
- Turfgrass Nitrogen
- Fe and Ni Nutrition and Studies at Clemson University

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Plant Roots and Nutrient Uptake

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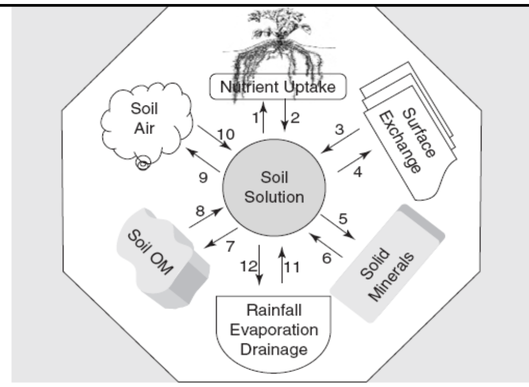

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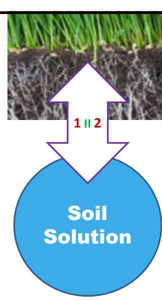

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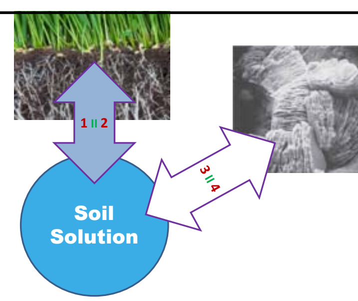

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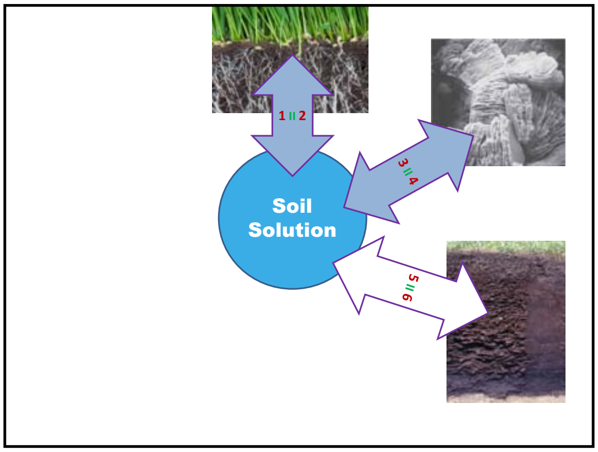

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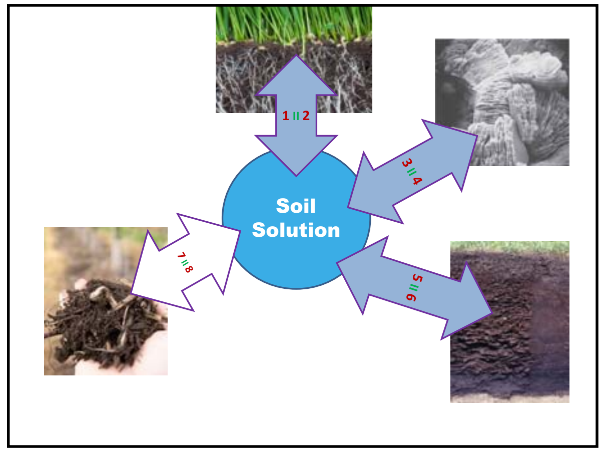

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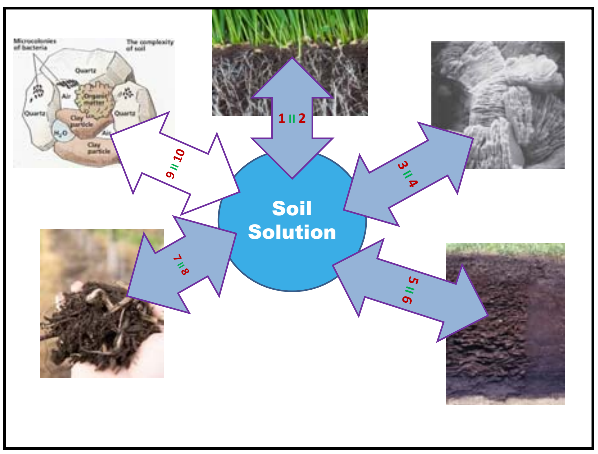

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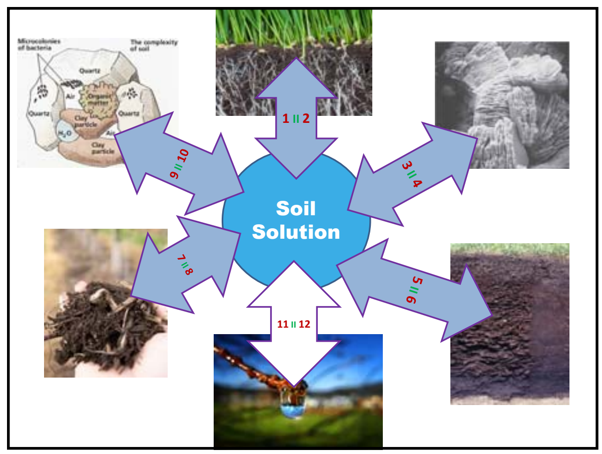

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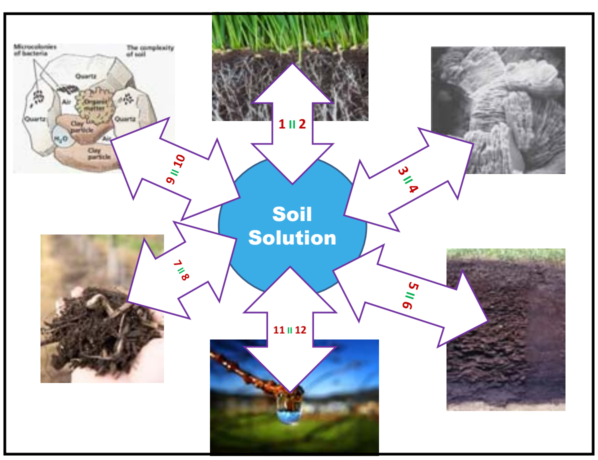

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Part II of Chapter 2
Review of Plant Roots and Nutrient Uptake

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Tap roots of plants

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Fibrous roots of bamboo

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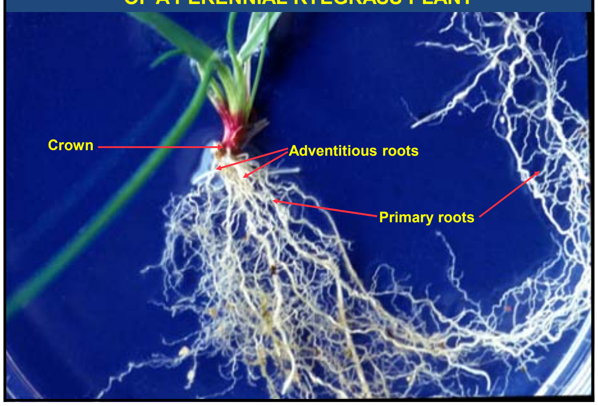
PRIMARY AND DEVELOPING SECONDARY FIBROUS ROOTS OF A PERENNIAL RYEGRASS PLANT



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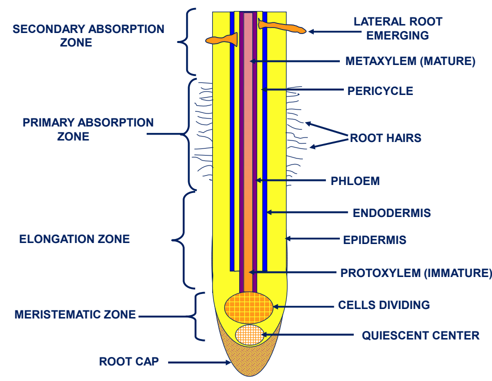
LONGITUDINAL DIAGRAM OF A GRASS ROOT SHOWING ZONES OF GROWTH AND ABSORPTION



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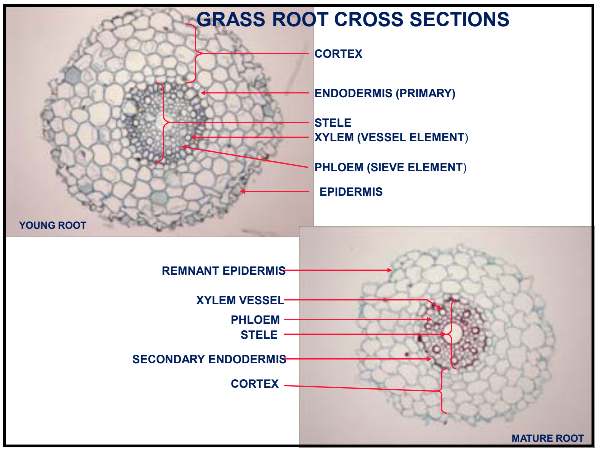
GRASS ROOT CROSS SECTIONS



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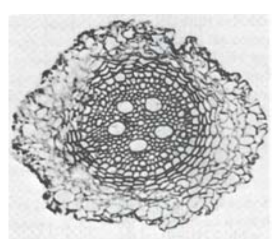
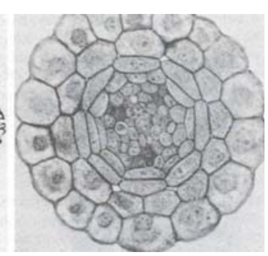


Old Kentucky bluegrass Root



Young creeping bentgrass root

J. Beard, 1973

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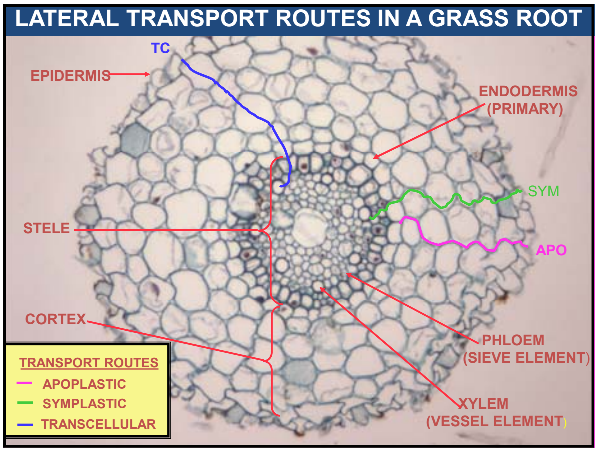

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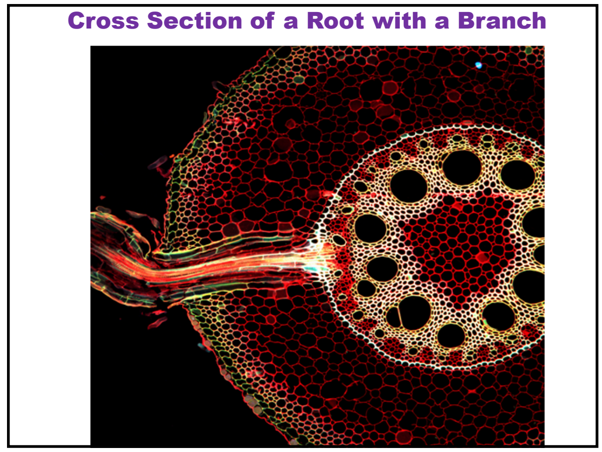

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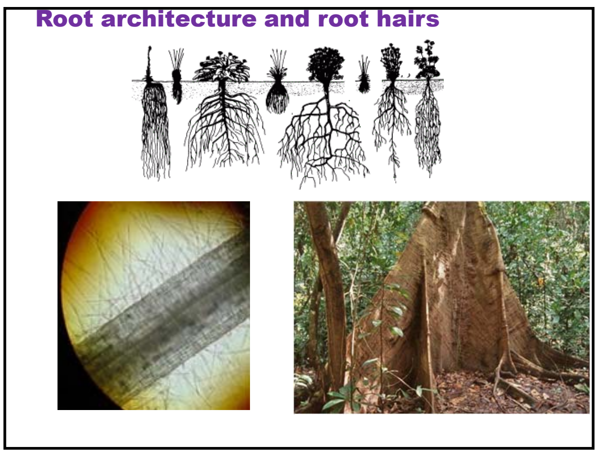

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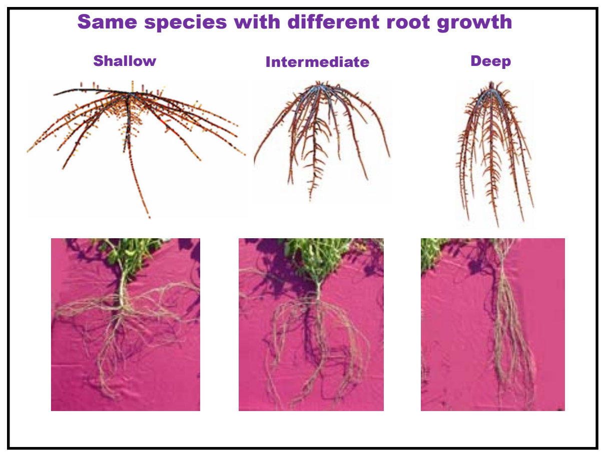

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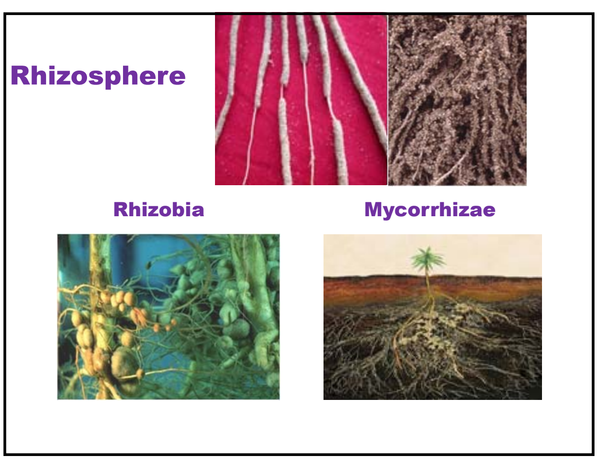

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First Plant Nutrient Rule

- Unreplaceable
- Unique Metabolic and biological functions
- Deficiency symptoms appear under stresses

C, O, H, N, P, K, Ca, Mg, S, Fe, Cl, Mn, B, Cu, Zn, Mo, Ni

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Essential elements

Plants require
17 essential elements:
The 3 elements from air and water

Carbon C
Hydrogen H
Oxygen O

C + H + O = 90-95% of the dry weight

The rest 14 elements are from soils called Plant Nutrients

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Mineral nutrients (14)

Primary nutrients: 0.2 to 6%

N, P, and K

Secondary nutrients: 0.1 to 0.6%

Mg, Ca, and S

Micronutrients: 0.0001 to 0.05%

Fe, Mn, Cu, Zn, B, Mo, Ni, and Cl

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Micronutrients

		Available form being taken up by plants	Dry wt. ppm
Iron	Fe	Fe^{2+}, Fe^{3+}	100-200
Manganese	Mn	Mn^{2+}	30-70
Zinc	Zn	Zn^{2+}	20-50
Copper	Cu	Cu^{2+}	5-20
Nickel	Ni	Ni^{2+}	0.01-10
Chlorine	Cl	Cl^-	100-200
Molybdenum	Mo	MoO_4^{2-}	0.1-2
Boron	B	$B(OH)_3$ (Boric acid)	20-40

The above 8 elements are micro-nutrients for plants.
Crops often do not require application of micro-nutrients

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Micronutrients

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Zinc	Zn	Zn^{2+}	20-50
Copper	Cu	Cu^{2+}	5-20
Molybdenum	Mo	MoO_4^{2-}	0.1-2
Nickel	Ni	Ni^{2+}	0.01-10

The above 8 elements are micro-nutrients for plants.
Crops often do not require application of micro-nutrients

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Micronutrient Concentrations In Plants and Soil

		Dry wt. in plants ppm	In Soils ppm
Iron	Fe	100-200	20,000-50,000
Manganese	Mn	30-70	500-5,000
Chlorine	Cl	100-200	50-500
Boron	B	20-40	20-200
Zinc	Zn	20-50	17-160
Nickel	Ni	0.01-10	5-100
Copper	Cu	5-20	5-50
Molybdenum	Mo	0.1-2	0.1-5.0

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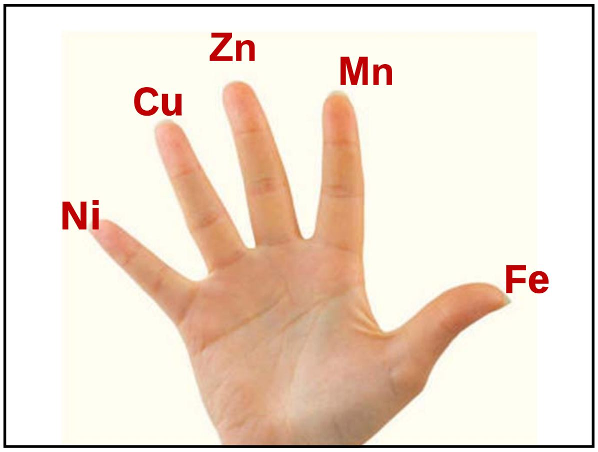

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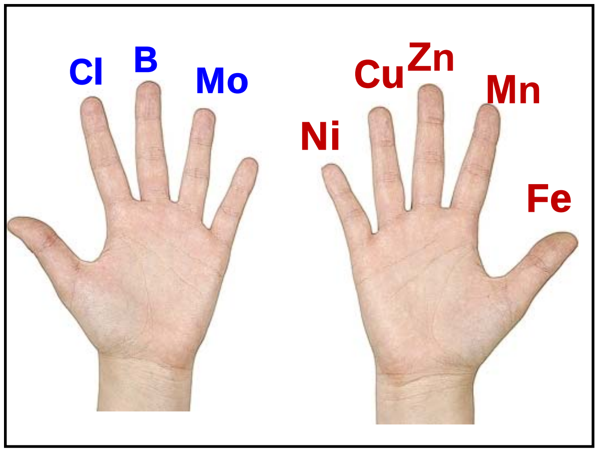

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Movements of Ions from Soils to Roots

-Mass flow

-Diffusion

$NO_3^- > K^+ > H_2PO_4^-$

-Interceptions

<1%

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Plant nutrient uptake

- Active transport cost energy
- Passive transport no energy expenses
- Combination of both

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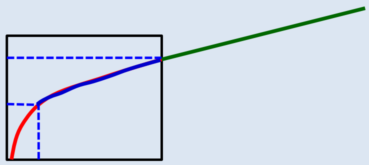
Nutrient Uptake Kinetics

Active uptake   Passive uptake



- Less Genetically controlled
- Genetically controlled
- Most genetically controlled

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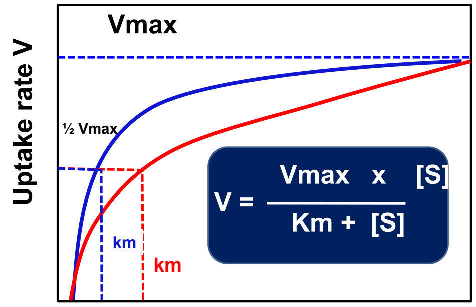
Michaelis Menten Equation



$$V = \frac{Vmax \;\; x \;\; [S]}{Km + [S]}$$

External nutrient concentration S = mM (or uM)
Please notice the differences of two kms.

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The Most Important Law of Plant Nutrition

Law of the Minimum

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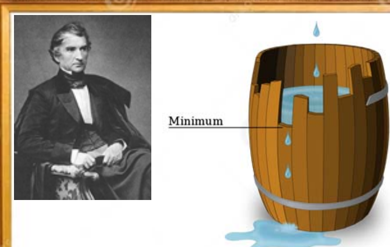

Justus von Liebig (1803 – 1873), German chemist

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Justus von Liebig formulated the *Law of the Minimum*, stating that a plant's development is limited by the one essential mineral that is in the relatively shortest supply, visualized as "Liebig's barrel". This concept is a qualitative version of the principles used to determine the application of fertilizer in modern agriculture.

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Nitrogen:

3 - 6% in dry tissue
NO_3^- , NH_4^+ , and urea
Mobile in turfgrasses and soils
Most frequently deficient
Older leaves turn yellow and reduced shoot growth

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Functions of Nitrogen in Plants

- Chlorophylls
- Proteins
- DNA, RNA
- Hormones
- Secondary metabolites

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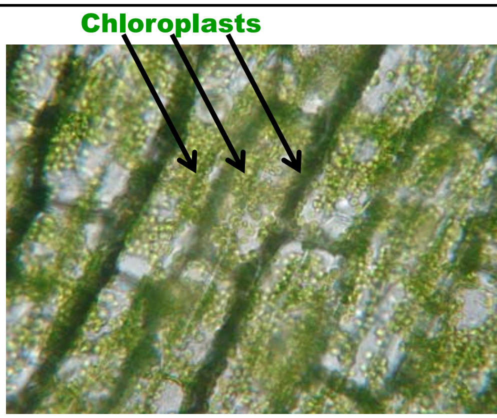

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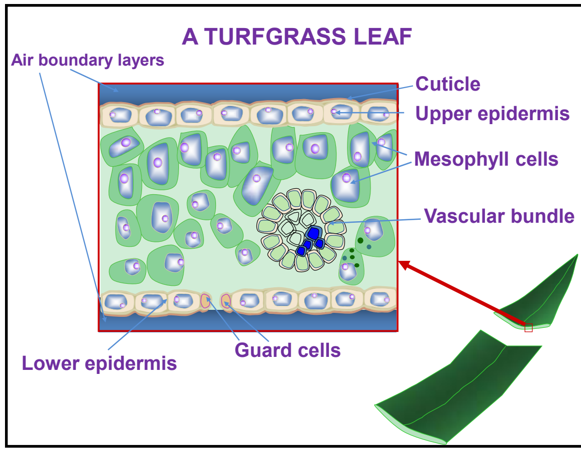

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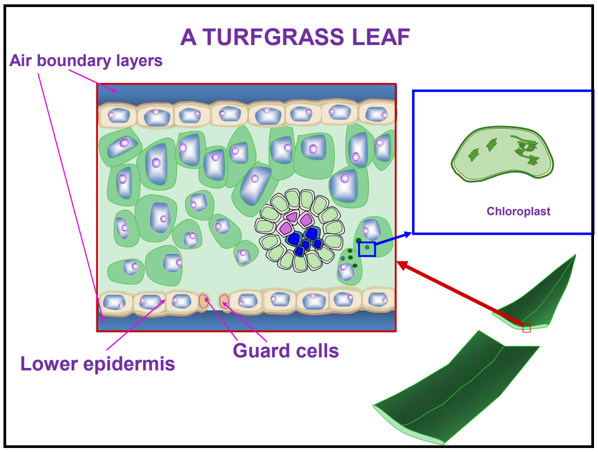

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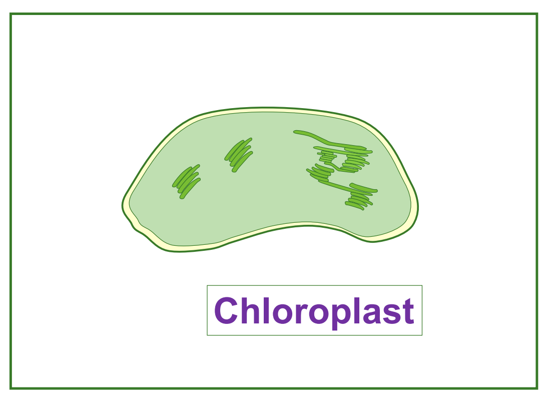

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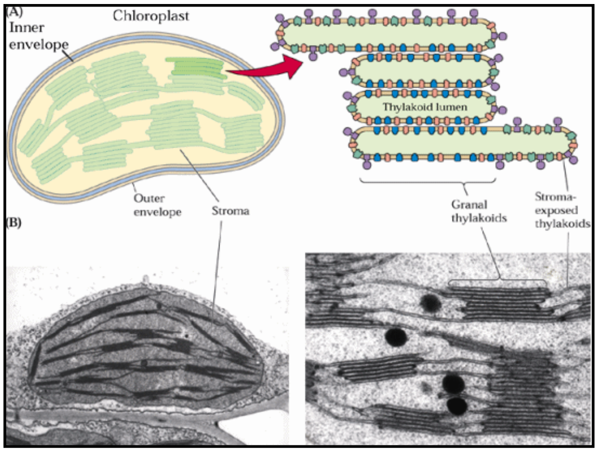

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Symptoms of N Deficiency

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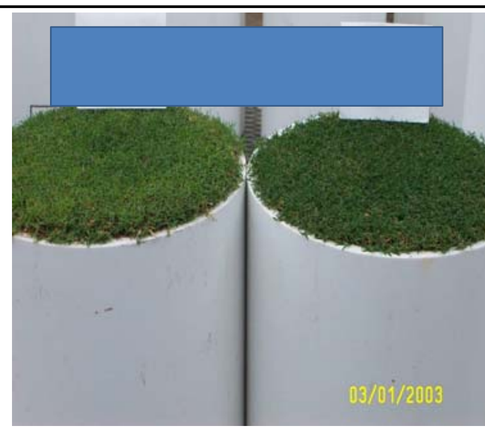

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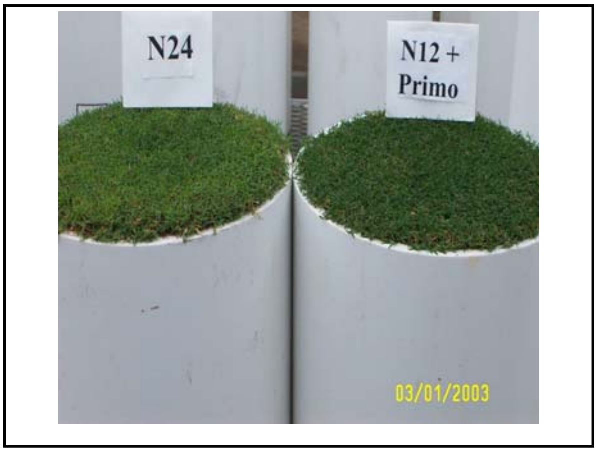

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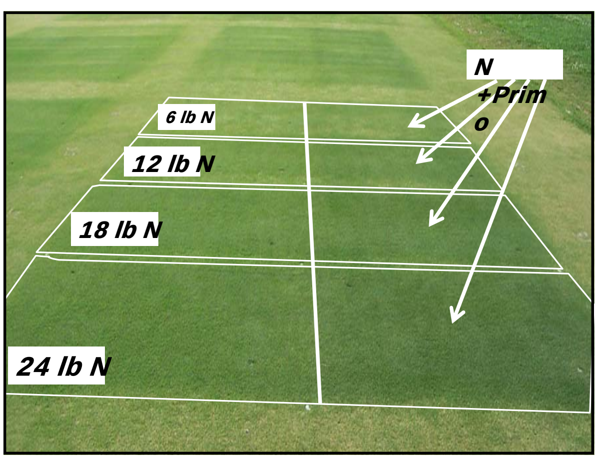

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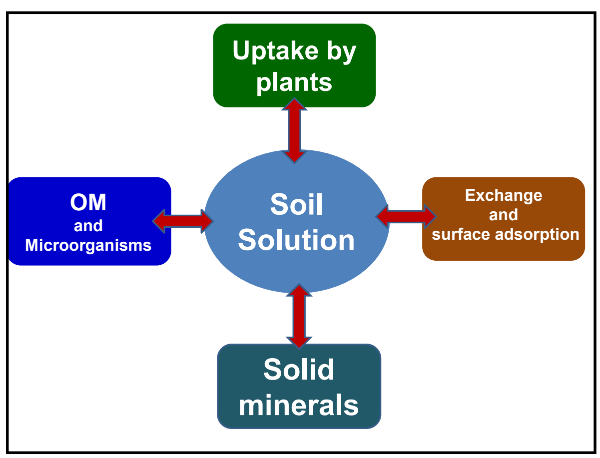

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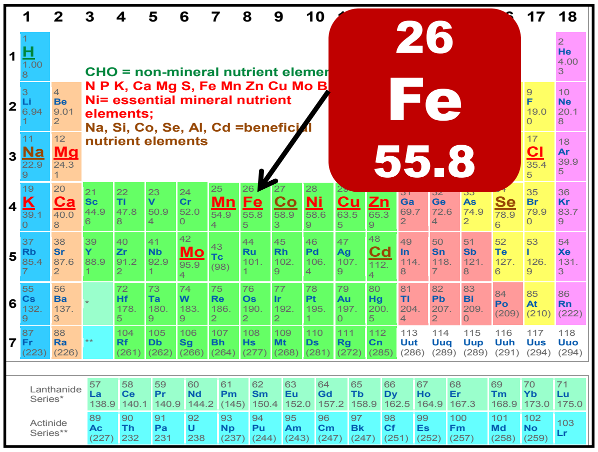

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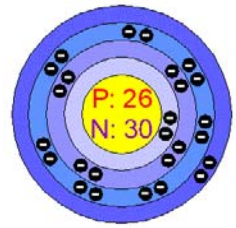
Iron

Symbol: Fe
Atomic Number: 26
Atomic Mass: 55.845 amu
Melting Point: 1535.0 ° C (1808.15 K, 2795.0 ° F)
Boiling Point: 2750.0 ° C (3023.15 K, 4982.0 ° F)
Number of Protons/Electrons: 26
Number of Neutrons: 30
Classification: Transition Metal
Crystal Structure: Cubic
Density @ 293 K: 7.86 g/cm^3
Color: Silvery



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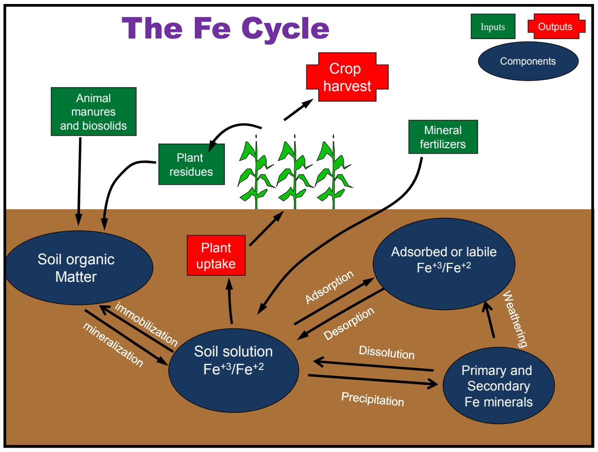
The Fe Cycle



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Iron Functions in Plants

- **Chlorophyll development and function.**
- **It plays a role in energy transfer within the plant.**
- **It is a constituent of certain enzymes and proteins.**
- **Iron functions in plant respiration, and plant metabolism.**
- **It is involved in nitrogen fixation**

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Review | CellPress

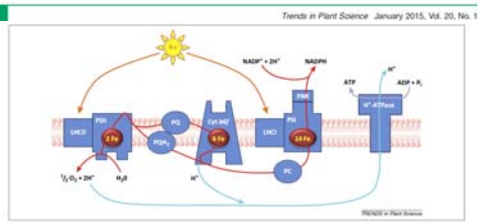
Iron nutrition, biomass production, and plant product quality

Jean-François Briat, Christian Dubos, and Frédéric Gaymard

Biochimie et Physiologie Moléculaire des Plantes, Centre National de la Recherche Scientifique, Institut National de la Recherche

Review

Trends in Plant Science January 2015, Vol. 20, No. 1



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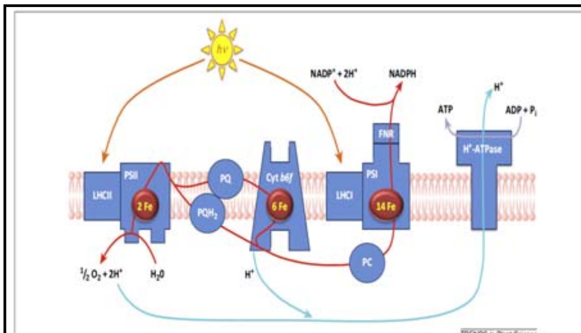

Figure 1. Iron (Fe) is essential for the structure and/or function of the photosynthetic electron transfer chain. Fe is found in the three major complexes of the photosynthetic apparatus (adapted from [19]). Two Fe atoms are present in the photosystem II (PSII) complex: one non-heme Fe is coordinated by four histidines and a bicarbonate, and the second heme Fe is present in cytochrome *b559*. The cytochrome *b6f* (Cyt *b6f*) contains four heme Fe atoms and two Fe atoms in the [2Fe–2S] cluster of the Rieske protein. Three [4Fe–4S] clusters (i.e., 12 Fe atoms) are located in the photosystem I (PSI) complex, and a [2Fe–2S] cluster is found in soluble ferredoxin, and their main function is to carry an electron from the PSI-[Fe–S] complex to the ferredoxin-NADP+ reductase (FNR). The two light-harvesting complexes (LHC) associated with the two photosystems contain chlorophylls, whose synthesis is also Fe-dependent (Box 1). Remodeling of the photosynthetic apparatus according to Fe nutrition conditions has been documented, in particular within the model unicellular green algae *Chlamydomonas reinhardii* (Box 2).

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Two major mechanisms of iron acquisition studied in plants are based either on reduction of organic ferric iron chelates, via a membrane-associated ferrireductase called strategy I plants, mainly broadleaf plants.

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Gramineae (grass) family produces phytosiderophores that bind ferric ions and introduce them into the cell via a special receptor, called strategy II plants.

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What are phytosiderophores?

Phytosiderophores (PS) are organic substances (such as nicotinamine, mugineic acids (MAs) and avenic acid etc) produced by plants under Fe-deficient conditions, which can form organic complexes or chelates with Fe^{3+}, and increase the movement of iron in soil. It is non proteineous, low molecular weight acids released by the graminaceous species under the iron and Zn deficiency stress. The PS mobilize micronutrients Fe, Zn, Mn, and Cu from the soils to plant in deficient conditions.

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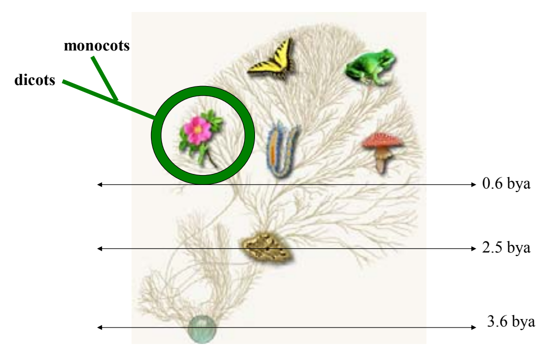
Evolutionary Lineages of Life



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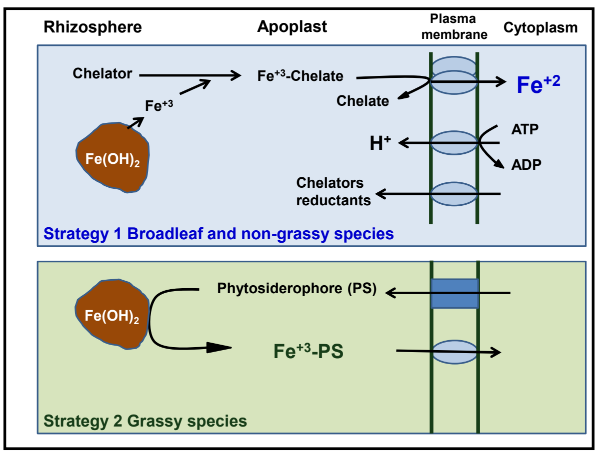

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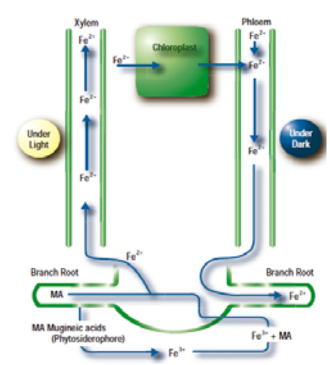
Iron absorption strategy in roots of grasses and transport under light and dark (Schmidt, 2008).



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Factors Affecting Fe Availability

- Soil pH and (HCO_3^-) bicarbonate;
- Water-logging conditions;
- Low soil OM and calcareous soils;
- In most cases, Fe^{3+} is reduced to Fe^{2+} for plant absorption and plants differ in Fe^{2+} absorption;
- Interactions with other nutrients: high Cu and Mn in soils can worsen Fe deficiency;
- NO_3^- can worsen Fe deficiency than NH_4^+.

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Highly Responsive Crops to Fe

Alfalfa, asparagus, barley, beans (white), beets, broccoli, brussel sprouts, cabbage, cauliflower, celery, citrus, grass, oats, peanuts, rye, rice, soybean, sorghum, spinach, strawberry, sudangrass, tomato, and turfgrasses.

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Iron Management for Turfgrasses

- Iron is an essential micronutrient for turfgrasses (.01 to 0.1%).
- Because iron in the soil is often insoluble, it is not always readily available for plant uptake.
- The best responses to iron fertilization occur when the turfgrass is under stress.
- Chelated iron is more readily absorbed by grasses.
- Frequently mowed turf requires more frequent iron applications.

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Iron Deficiency

- Alkaline soils pH > 7.0
- Poor rooting
- Excessive thatch
- Cold, wet soils in spring
- Low organic matter soils
- Heavy metals such as Cu, Zn, Mn,
- Poor irrigation water with high concentrations of HCO_3^-, Ca, P, and other metals

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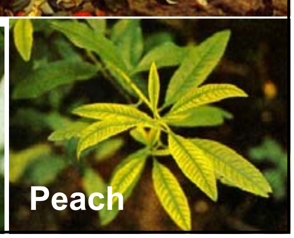
Fruit Tree Iron Deficiencies



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Citrus Iron Deficiency

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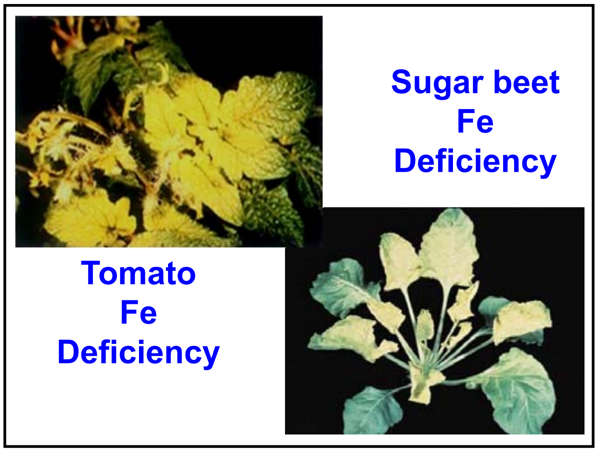

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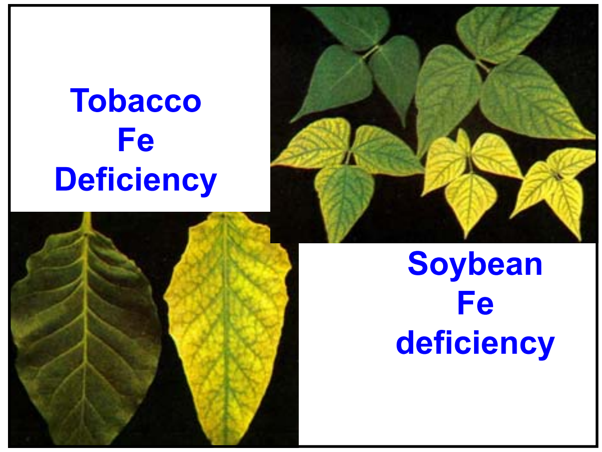

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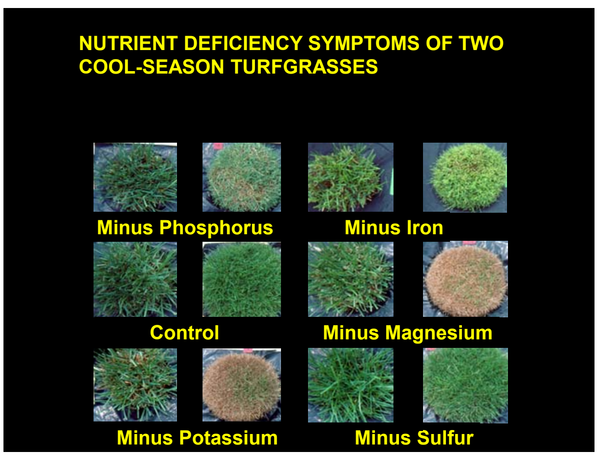

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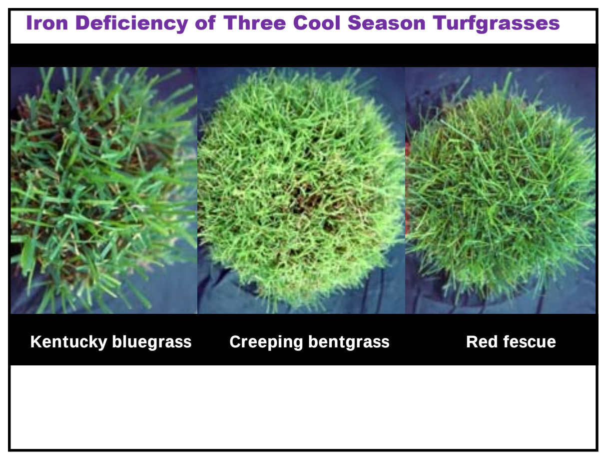

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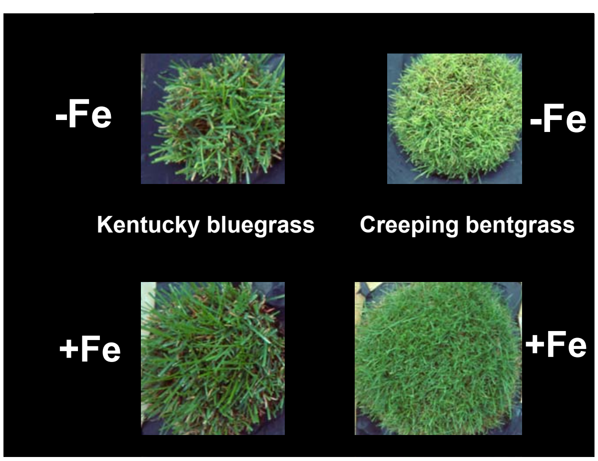

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Iron deficiencies can be corrected by application of numerous iron containing fertilizers. Here Ironite is one of examples.

Most products only last three to four weeks.

91

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Most products only last three to four weeks.

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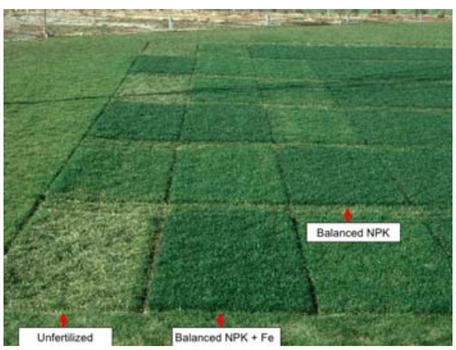
Apply when only needed



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Rice Fe disorders

Deficiency

Toxicity

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Iron Toxicity

- **Blacken the canopy can cause tissue injury or heat stress**
- **High Fe can induce Mn deficiency**
- **Acid and poor drained soils can produce toxic level of Fe.**
- **Black layer for turfgrasses**

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Common Fe Fertilizers

Source	Formula	%Fe
Ferrous sulfate	$FeSO_4 \cdot 7H_2O$	19
Ferric sulfate	$Fe_2(SO_4)_3 \cdot 4H_2O$	23
Ferrous oxide	FeO	77
Ferric oxide	Fe_2O_3	69
Ferrous ammonium phosphate	$Fe(NH_4)PO_4 \cdot H_2O$	29
Ferrous ammonium sulfate	$(NH_4)SO_4FeSO_4 \cdot 6H_2O$	14
Iron ammonium polyphosphate	$Fe(NH_4)HP_2O_7$	22
Iron chelates	NaFeEDTA	5-14
	NaFeEDDHA	6
	NaFeDTPA	10
Natural organic materials		5-10

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Complex

When a metal ion combines with an electron donor, the resulting substance is said to be a complex or a coordination compound.

(CH3)-N-COO-metal

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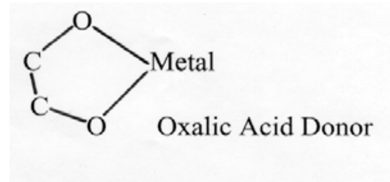
Chelate

The main difference between a metal complex and a chelate is that in the chelate, the donor atoms are attached not only to the metal but also to each other.



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Common Synthetic and Natural Chelate Compounds

Name	*Formula*	*Abbre.*
EthyleneDiamineTetraAcetic acid	$C_{10}H_{16}O_8N_2$	EDTA
DiethyleneTriaminePentaAcetic acid	$C_{14}H_{23}O_{10}N_3$	DTPA
CyclohexaneDiamineTetraAcetic acid	$C_{14}H_{23}O_8N_2$	CDTA
EthyleneDiamineDi-o-HydroxyphenylAcetic acid	$C_{18}H_{20}O_6N_2$	EDDHA
Citric acid	$C_6H_8O_4$	CIT
Oxalic acid	$C_2H_2O_4$	OX
Pyrophosphoric acid	$H_4P_2O_7$	PPA

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Why Use Strong Chelated Products?

- Compatibility and stability mixing with other nutrients and products
- Protects nutrient from falling out (precipitating) and assists in plant uptake and translocation

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Synthetic Chelating Agents

- Synthetic chelates used extensively in turf products:
  - EDTA , DTPA, CDTA, etc.
- Very popular but are effective only on the metal micronutrients.
- Were developed for soils and are not as effective when used as a foliar.
- Are not biodegradable and may remain as a residue in the plant tissue or soil.
- Can be toxic to the plant tissue under some conditions and concentrations.

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Organic Chelating and Complexing Agents

- Organic products used as complexing and chelating agents:
  - humic, citric, fulvic, yucca extracts & amino acids
  - ligno sulfonates
  - wood pulp derivatives & hydrolyzed protein mixes
- Some generally have low stability constants.
  - mixed with phosphate may result in precipitate
- When added to the soil or plant, high organic concentrations are biodegradable.
- Some organics may also provide an energy source to plants and soil microorganisms thus aiding in nutrient availability.

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Products with Organic Chelating Agents

- The difference in the various products on the market is in the type and amount of Organic facilitating agents as well as the type and formulation of nutrients
- Many products are not 100% chelated and although classified by law as a chelate may not be effective
- Even among organics some chelating agents are far superior to others and all are classified as chelating agents by law
- Many products have an improper pH

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Objective

- To determine whether the application of Iron (Fe) will affect Diamond zoysiagrass and 'Tifgrand' bermudagrass under a shaded and full sun environment
- By Improving:
  - Turf color
  - Turf quality
  - Winter dormancy color
  - Spring green-up

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Material and Methods
Field Study

- Testing done on Diamond zoysiagrass and 'Tifgrand' Bermudagrass at Clemson's University's turf research plots
- In full sun and under shaded conditions (60% shade)
- Iron rates of 1, 3, and 5 kg/ha applied every 3 weeks from May to October
- Ferrous sulfate is the iron fertilizer used
- Data will be collected by:
  - Turf quality (Visual Rating)
  - Normal Vegetative Index (NDVI)
  - Chlorophyll Content
  - Turf Clippings/Clipping Nutrients (Fe)

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Materials and Methods

Warm-season Turfgrasses

- ‘Diamond’ Zoysiagrass
  - (Zoysia matrella (L.) Merr)
- ‘Tifgrand’ Bermudagrass
  - (Cynodon transvaalensis Burt-Davy x C. dactylon)

Light Treatments

- Control (full-sun)
- 40% continuous shade

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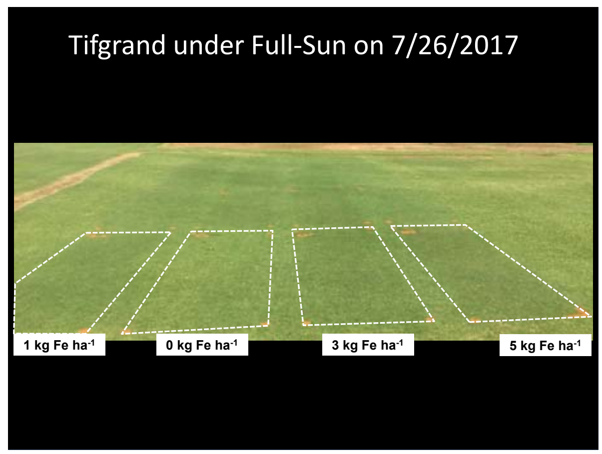

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Utilizing Iron In Turfgrass Management

David Wehner, Ph.D.
University of Illinois, Urbana-Champaign

Dr. George Wallace — a researcher from UCLA, writing in *HortScience* — called the 1980s the decade for advancement in our knowledge of iron (Fe) and plant nutrition.

He states:

"More progress was perhaps made in the 1980s on understanding and management of Fe chlorosis in plants than during the previous 150 years since it became known that Fe deficiency was involved in the chlorosis."

Iron is used in a broad range of applications by turfgrass managers including treating iron deficiencies and using it to enhance color. Although much of the research to which Dr. Wallace was referring was conducted on species other than

Iron deficiency in turfgrass is symptomized by appearance of interveinal chlorosis. As severity increases, entire leaf will become chlorotic and may appear bleached.

listed causes for turfgrass iron deficien-

soil or in irrigation water. Iron availability is greatly reduced at high soil pH which occurs in the presence of calcium carbonate in the soil. This problem is called lime-induced chlorosis. Iron chlorosis also can result from application of irrigation water with a high bicarbonate ion concentration.

In addition, there are several plant-related causes of iron deficiency. Some of the most common causes include:

• A poor root system. Plants that have poor root systems as a result of insects or disease, excessive thatch, improper mowing, excessive N fertilization or other causes may not absorb enough iron, particularly if a soil-related factor also is present.

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Kentucky bluegrass treated with excessive rates of ferrous sulfate (foreground) and iron chelate (background) shows impact 24 hours after application (foliar).

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Nickel

Symbol: Ni
Atomic Number: 28
Atomic Mass: 58.6934 amu
Melting Point: 1453.0 ° C (1726.15 K, 2647.4 ° F)
Boiling Point: 2732.0 ° C (3005.15 K, 4949.6 ° F)
Number of Protons/Electrons: 28
Number of Neutrons: 31
Classification: Transition Metal
Crystal Structure: Cubic
Density @ 293 K: 8.902 g/cm^3
Color: white

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Ni Functions in Plants

- Nickel is a functional constituent of seven enzymes.
- Among the seven, urease (i.e., urea amidohydrolase) is extremely important to N metabolism in plants.
- Urease assists in the hydrolysis of urea.
- Nickel works as a cofactor to enable urease to catalyze the conversion of urea into the ammonium ions, which plants can use as a source of N.

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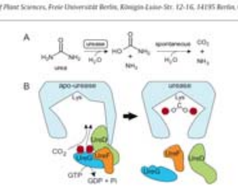
journal homepage: www.elsevier.com/locate/plantsci

Review

Urea metabolism in plants

Claus-Peter Witte*
Department of Plant Biochemistry, Dahlem Centre of Plant Sciences, Freie Universität Berlin, Königin-Luise-Str. 12-16, 14195 Berlin, Germany



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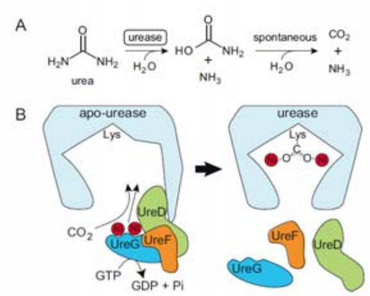

Fig. 1. Urease reaction and model of urease activation. (A) Enzymatic hydrolysis of urea to carbamate catalysed by urease and subsequent non-enzymatic decay of carbamate. Two mol ammonia and one mol carbon dioxide are generated from urea by these reactions. (B) Hypothetical model of plant urease activation involving the binding of the three urease accessory proteins (UreD, UreF and UreG) to apo-urease, covalent modification of an active site lysine by nitrogen carboxylation, and specific incorporation of two nickel ions per active site. The accessory proteins dissociate from urease after activation. Activation may require GTP hydrolysis mediated by UreG.

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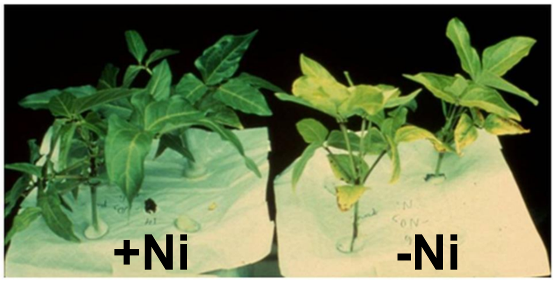

cowpea seedlings

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Pecan Ni deficiency

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Pecan Ni deficiency

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Some Ni Fertilizers

Product	Chemical Formula	B %
Nickel Sulfate	$NiSO_4 \cdot 6H2O$	32.1
Anhydrous nickel sulfate	$NiSO_4$	37.5
Nickel nitrate	$Ni(NO_3)_2 \cdot 6H_2O$	20.2
Nickel Chloride	$NiCl_2 \cdot 6H_2O$	37.2
Sewage sludge	composite	2.4-5.3

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TifEagle bermudagrass growth reduction under four Ni^{2+} levels

Control, 400, 800, 1600 µM

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Diamond zoysiagrass growth reduction under four Ni^{2+} levels

Control, 400, 800, 1600 µM

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TifEagle

Control 400 800 1600 µM Ni^{2+}

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Diamond

Control 400 800 1600 µM Ni^{2+}

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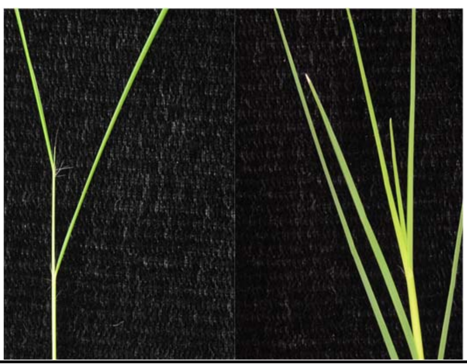
Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under control treatments.



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Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under 400 µM Ni treatments.

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Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under 800 µM Ni treatments.

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Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under 1600 µM Ni treatments.

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Thank you and any questions!

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Foliar Fertilization

Plant Leaf Morphological Characteristics Related to Hydrophobicity and Foliar Absorption

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Team Work Efforts

- Investigators
  - Dr, Haibo Liu
  - Dr. Frank Bethea
- Team Members
  - Dr. Terri Bruce
  - Dr. Hong Luo
  - Dr. William Bridges
- Turf Team Members
  - Dr. Bert McCarty
  - Dr. Dara Park
  - Dr. Bruce Martin
  - Dr. JC Chong
  - Mr. Don Garrett
  - Mr. Mike Echols
- Clemson Light Microscopy Facility
  - Rhonda Powell
  - Sarah Howell
  - Caroline Ashworth
- Clemson EM Facility
- Clemson Multiuser Analytical Lab
- Other Colleagues and Students
  - Dr. Bob Cross
  - Dr. Jeff Atkinsion
  - Mr. Nate Gambrell
  - Dr. Brad Shaver
  - Dr. Nick Menchyk
  - Mr. Nickles Mirmow
  - Dr. Zhigang Li
  - Ms. Emily Wakefield

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Overall Goal

- Investigate the factors affecting foliar absorption
  - Solution chemistry
  - Solution concentration
  - Plant leaf surface characteristics
    - Leaf morphology
    - Cuticle analysis
    - Stomata size and density
    - Leaf hydrophobicity
  - Solution uptake

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When it is too hot, potential of chemical burn is high

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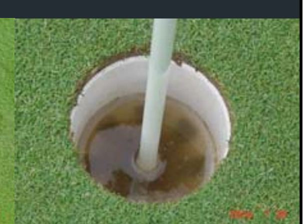
When plant, environmental, and soil conditions are restricted or less favorable for root nutrient absorption

Severe lose of roots during the summer



Poor drainage or high soil moisture content

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Turfgrass Industry

- Economic Impact
  - $40 Billion estimated value annually in US
  - ~ 50 million acres of land in US
- South Carolina
  - $2.7 billion from golf courses alone (Jackson, 2012)
  - Golf Course Expenses ~ $540 m
  - $27 million in fertilizers
    - 5% Course operating expenses

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Turfgrass Management

- Foliar Fertilizer Applications
  - Benefits:
    - Quick Response
    - Reduce growth surge
    - Accurate application
      - Environmental friendly
    - Labor Efficient
      - Tank Mixing
- Major Problem
  - Inconsistent uptake (10-60%)

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Solution Chemistry

- Foliar Fertilizer and Pesticide Applications
- Products
  - Fertilizers
  - Adjuvants
  - Pesticides
- Rates/Carrier Volumes
  - Product variation
  - Area applied

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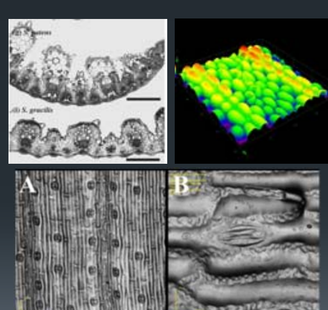
Plant Leaf Surface

- Leaf Size
- Leaf Morphology
  - Cell Structure and Orientation
  - Surface Roughness
- Stomata
  - Size (µm)
  - Stomata mm^{-2}



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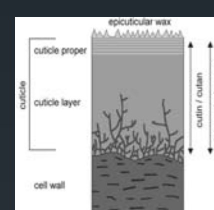
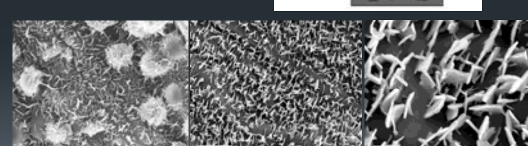
Plant Leaf Cuticle

- Cuticle Composition
  - Protective waxy coating
  - Long chain carbon compounds
- Cuticle Morphology
  - Crystalloids





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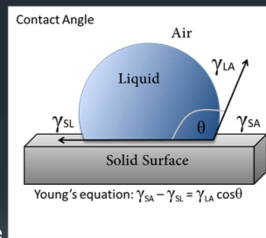
Plant Leaf Hydrophobicity

- Contact Angle
  - solid, liquid and air interface
- Hydrophobic
  - > 90°
- Hydrophilic
  - < 90°
- Quantifies the wettability of a surface
- Surface chemistry and surface roughness



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Question: Does solution hydrophobicity differ among plant species?

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Part I: Solution Hydrophobicity Interaction with Three Plant Leaf Surfaces

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Part I: Objective

- To determine the hydrophobicity of various popular foliar application products (fertilizers, adjuvants, and pesticides) on three plant species (St. Augustinegrass, tall fescue and white clover)

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Part I: Materials and Methods

Purpose	Product	Concentration	Rate
Fertilizer			
	$CO(NH_2)_2$	0.63 M	561 L ha^{-1}
		1.26 M	280 L ha^{-1}
	NH_4NO_3	0.62 M	561 L ha^{-1}
		1.24 M	280 L ha^{-1}
	KNO_3	0.22 M	561 L ha^{-1}
		0.44 M	280 L ha^{-1}
	KH_2PO_4	0.22 M	561 L ha^{-1}
		0.44 M	280 L ha^{-1}
Adjuvant			
	Non-ionic surfactant	2.5 ml	0.250% (v/v)
	Drift Reducer	2.5 ml	0.250% (v/v)
	Nonionic Siloxane Surfactant	2.5 ml	0.250% (v/v)
Fungicide			
	Chlorothalonil	17.5 g L^{-1}	561 L ha^{-1}
		35.0 g L^{-1}	280 L ha^{-1}
Growth Regulator			
	Trinexapac-ethyl	0.78 ml L^{-1}	561 L ha^{-1}
		1.56 ml L^{-1}	280 L ha^{-1}

- Plants Species
  - St. Augustinegrass
  - Tall Fescue
  - White Clover

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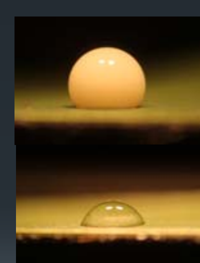
Objective I: Materials and Methods

- Hydrophobicity Test
  - 1µl droplet
  - Adaxial leaf side
  - Beattie and Marcell (2002)



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Part I: Results

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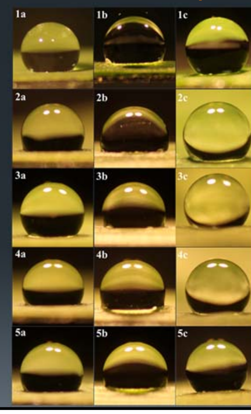
Fertilizer Group



Main Effect	Contact Angle (°)
Plant Species	‡
White Clover	129.2a
St. Augustinegrass	123.9b
Tall Fescue	112.0c
Fertilizer	
Water	126.8a
NH_4NO_3	122.4b
KNO_3	121.8b
KPO_4	120.5b
$CO(NH_2)_2$	117.1c
Concentration	
Water	126.8a
NH_4NO_3	
561 L ha^{-1}	123.6ab
280 L ha^{-1}	121.4bc
KNO_3	
561 L ha^{-1}	121.0bc
280 L ha^{-1}	122.7bc
KPO_4	
561 L ha^{-1}	119.5c
280 L ha^{-1}	121.5bc
$CO(NH_2)_2$	
561 L ha^{-1}	119.7c
280 L ha^{-1}	114.3d
ANOVA	
Main Effect	**p-value**
Plant Species	<0.0001
Fertilizer	<0.0001
Concentration	0.0098
Plant × Fertilizer	0.0932
Plant × Concen.	0.1091

‡ Means connected by the same letter within main effects are not significantly different according to Students t test $\alpha = 0.05$

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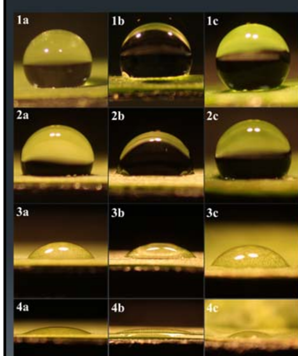
Adjuvant Group



Main Effect	Contact Angle (°)
Plant Species	‡
White Clover	80.3a
St. Augustinegrass	77.6a
Tall Fescue	61.3b
Adjuvant	
Water	126.8a
Drift Reducer	104.9b
Nonionic Surf.	44.7c
Nonionic Siloxane	15.8d
ANOVA	
Main Effect	**p-value**
Plant Species	<0.0001
Adjuvant	<0.0001
Plant × Adjuvant	<0.0001

‡ Means connected by the same letter within main effects are not significantly different according to Students t test $\alpha = 0.05$

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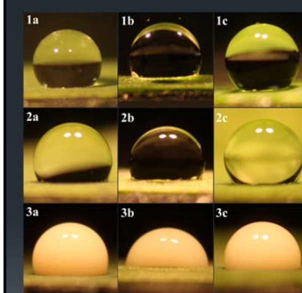
Pesticide Group



Main Effect	Contact Angle (°)
Plant Species	‡
White Clover	126.3a
St. Augustinegrass	121.2b
Tall Fescue	102.9c
Product	
Water	126.9a
Trinexapac-ethyl	117.1b
Chlorothalonil	106.4c
Concentration	
Water	126.9a
Trinexapac-ethyl	
561 L ha^{-1}	116.0bc
280 L ha^{-1}	117.3b
Chlorothalonil	
561 L ha^{-1}	112.5c
280 L ha^{-1}	100.3d
ANOVA	
Main Effects	**p-value**
Plant Species	<0.0001
Product	<0.0001
Concentration	<0.0001
Plant × Product	<0.0001
Plant × Concen.	0.1108

‡ Means connected by the same letter within main effects are not significantly different according to Students t test $\alpha = 0.05$

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Conclusions

- Products significantly reduce hydrophobicity
  - Urea reduced hydrophobicity the most by the fertilizer group
  - Chlorothalonil significantly reduced hydrophobicity, compared to trinexapac-ethyl
  - Nonionic siloxane surfactant had the lowest average contact angle
- Plant species differed in hydrophobicity
  - White clover consistently had the highest contact angle, with St. Augustinegrass and tall fescue were second and third, respectively

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Question: What plant leaf surface characteristics influence leaf hydrophobicity?

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Part II: Plant Leaf Surface Characteristics related to Leaf Hydrophobicity

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Part II: Objective

- The objectives were to investigate the plant leaf surface characteristics that influence the hydrophobicity of the leaf surface

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Part II: Materials and Methods

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Plant Species

- 15 different species
  - 5 species with multiple cultivars
  - 32 different plants
  - C_3 & C_4 plants
  - Various applications
    - Golf Courses
    - Sports Fields
    - Home Lawns
    - Utility Areas
    - Weed Species


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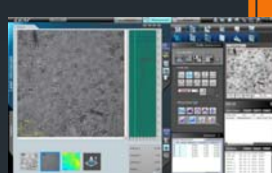
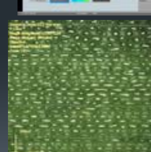
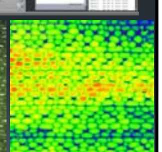
Plant Leaf Morphology

- LEXT OLS4000 Optical Profiler
  - 20X Objective
- Imaging:
  - Color
  - Laser
  - Topographical Map
- Surface Roughness (S_R)
  - Average of the deviation distances from the Mean Line of all the Peaks to all the Valleys with in a sample

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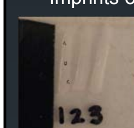
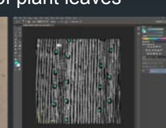
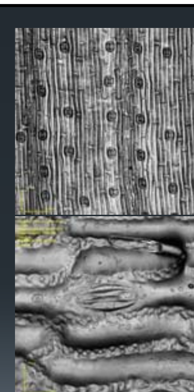
Plant Stomata

- Imprints of plant leaves



- Size
  - µm
- Stomata density
  - stomata mm^{-2} leaf area

(Radoglou and Jarvis, 1990)

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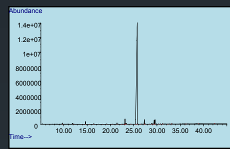
Plant Cuticle Analysis

- Cuticle Composition and Quantification
  - Jenks et al. (1995)
  - Cuticle extracted in hexane
  - Gas Chromatography/Mass Spectroscopy



- Cuticle Morphology
  - Hitachi S4800 (SEM)
  - Air-dried (Pathen et al., 2008)
  - Sputter-coated with platinum

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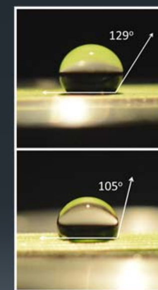
Plant Leaf Hydrophobicity

- Contact Angle measurement
  - 1 µl water droplet
  - Horizontal Stereoscope
  - Beattie and Marcell (2002)



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Part II: Results

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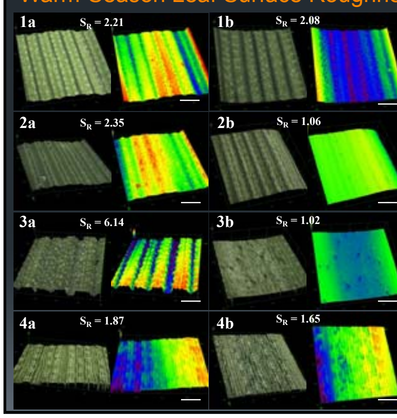
Warm-Season Leaf Surface Roughness



- Examples of 3-D light micrographs of four warm-season grass species leaf surface roughness. 1-4) Ultradwarf bermudagrass, zoysiagrass, seashore paspalum, centipedegrass, respectively. a-b) Adaxial and (Scale Bars = 150µm)
- Averages:
  - Adaxial: 2.42
  - Abaxial: 1.44

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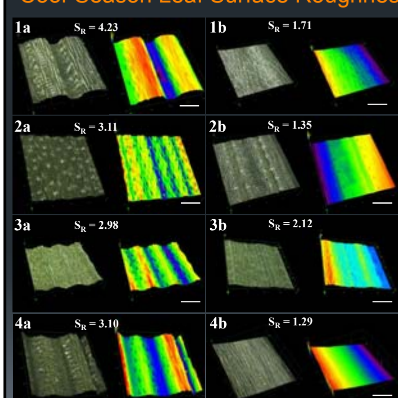
Cool-Season Leaf Surface Roughness



- Examples of 3-D light micrographs of four cool-season grass species leaf surface roughness. 1-4) Tall fescue, Kentucky bluegrass, creeping bentgrass, perennial ryegrass, respectively. a-b) Adaxial and abaxial (Scale Bars = 150µm)
- Averages:
  - Adaxial: 3.14
  - Abaxial: 1.71

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Stomata Analysis

Warm-season

- Adaxial
  - Mean Stomata mm^{-2} : 28.3
    - Range: 9.0-65.1
  - Mean Stomata size : 18.1 um
    - Range: 14.7-36.9
- Abaxial
  - Mean Stomata mm^{-2} : 23.0
    - Range: 5.15-40.4
  - Mean Stomata size: 23.0 um
    - Range: 15.2-40.7

Cool-Season

- Adaxial
  - Mean Stomata mm^{-2} : 11.9
    - Range: 0-18.3
  - Mean Stomata size: 25.8 um
    - Range: 0-36.9
- Abaxial
  - Mean Stomata mm^{-2} : 6.0
    - Range: 0-17.5
  - Mean Stomata Size: 28.9 um
    - Range: 0-36.9

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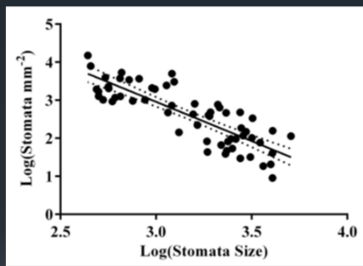
Stomata Analysis



- Log transformed relationship among plant leaf stomata size and plant leaf stomata density. Each point represents the adaxial or abaxial means or stomata size and density. (R^2=0.7009, p = <0.0001)

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Cuticle Analysis: Warm-Season

Species / Cultivar	Total Wax	Primary Alcohol	Fatty Acid	Alkane	Unknown
	µg dm^{-2}				
Cynodon dactylon					
Celebration	28.44	20.65	1.08	4.84	1.60
Cynodon dactylon × C. transvaalensis					
Tifway 419	21.73	15.16	0.88	3.73	1.68
TifEagle	11.08	7.93	0.57	1.99	0.51
MiniVerde	12.47	9.53	0.47	1.76	0.65
Champion	17.35	13.07	1.33	2.07	0.84
Zoysia japonica					
Meyer	4.49	0.91	0.58	0.80	2.18
Palisades	5.50	1.16	0.32	1.32	2.65
Zoysia matrella					
Diamond	7.40	1.60	0.53	2.50	2.72
Zorro	8.05	1.17	0.88	2.04	3.89
Cavalier	16.00	3.27	0.93	3.09	8.65
Eremochloa ophiuroides					
TifBlair	6.83	0.67	0.44	2.46	3.24
Stenotaphrum secondatum					
Palmetto	5.99	0.60	0.56	4.26	0.54
Paspalum notatum					
Unknown	8.83	2.85	1.01	4.27	0.69
Buchloe dactyloides					
Unknown	9.08	3.94	1.36	2.13	1.62
Paspalum vaginatum					
SeaDwarf	15.74	2.35	1.49	8.03	3.74
LSD	7.02[a]	4.80	0.41	2.24	2.52

[a]Fisher's least significant difference (LSD) for cultivar means within columns.

- Celebration had highest amount of total wax and primary alcohols
- Primary alcohol content was variable for species
- Composition revealed multiple compounds comprise the primary alcohol group contrary to cool-season

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Cuticle Analysis: Cool-Season

Species / Cultivar	Total Wax	Primary Alcohol	Fatty Acid	Alkane	Unknown
	µg dm^{-2}				
Poa pratensis					
Abbey	56.30	37.20	1.44	16.97	0.69
Avalanche	28.11	14.59	0.65	12.50	0.36
Gaelic	31.18	18.45	0.68	11.59	0.46
Julia	42.64	33.72	0.65	7.68	0.60
Midnight	39.78	27.52	0.54	11.19	0.53
Noble	42.37	38.43	0.48	3.28	0.17
Lolium perenne					
Citation	50.02	44.01	2.03	2.99	0.98
Agrostis stolonifera					
Penn-A1	51.62	49.32	1.40	0.29	0.60
237-1	29.53	28.12	0.59	0.61	0.19
237-2	37.04	32.88	1.36	2.26	0.53
237-4	53.24	49.49	0.66	1.59	1.51
239-7	55.48	53.21	1.16	0.80	0.29
239-14	40.48	38.05	0.77	1.09	0.17
Festuca arundinacea					
Faith	55.69	44.92	1.24	9.18	0.34
Trifolium repens					
wildtype	16.22	13.48	0.53	0.76	1.46
LSD	14.49	13.73	0.64	3.36	0.99

[a]Fisher's least significant difference (LSD) for cultivar means within columns.

- Abbey, Kentucky bluegrass had the most total wax
- Considerably more total wax for cool-season that warm-season
- Primary alcohol content mostly composed of one compound, C_{26}, hexacosanol

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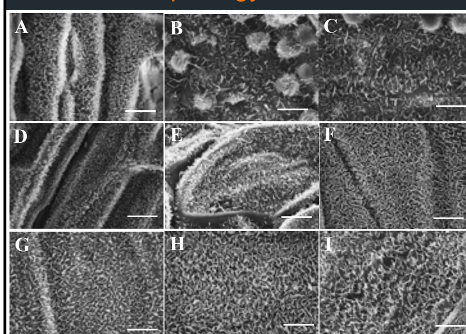
Cuticle Morphology



Examples of scanning electron micrographs of grass species cuticle morphology . A-I) Bermudagrass, zoysiagrass, Diamond, Meyer, seashore paspalum, St. Augustinegrass, Kentucky bluegrass, perennial ryegrass, tall fescue and creeping bentgrass respectively. Images were acquired at 5,000X, Scale Bar = 5µm.

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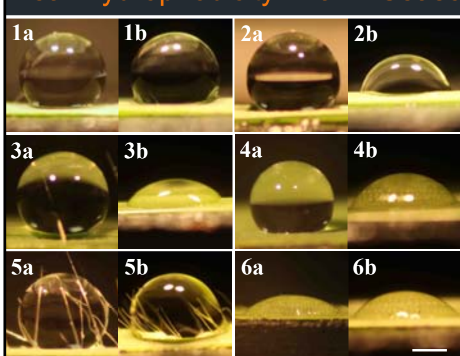
Leaf Hydrophobicity: Warm-Season



Light micrographs of water droplet on six warm-season grass species used to test hydrophobicity. 1-6) Common bermudagrass, zoyisagrass seashore paspalum, St. Augustinegrass, buffalograsa, centipedegrass, respectively. a-b) adaxial and abaxial (Scale Bar = 500 µm)

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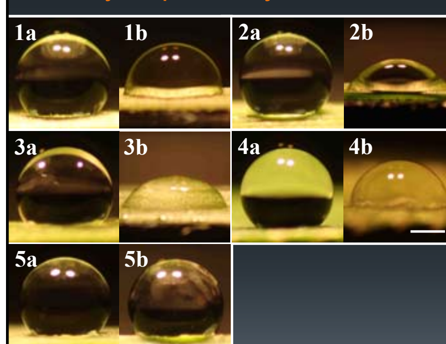
Leaf Hydrophobicity: Cool-Season



Light micrographs of water droplet on six cool-season grass species used to test hydrophobicity. 1-5) Kentucky bluegrass, perennial ryegrass, tall fescue, white clover, and creeping bentgrass respectively. a-b) adaxial and abaxial (Scale Bar = 500 µm)

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Correlation Analysis
Cool-Season Adaxial

Parameter	Contact Angle	Stomata Size	Stomata Density	Leaf Surface Area	Leaf Surface Roughness	Total Cuticle Wax	Primary Alcohol Content
Contact Angle		[a]-0.6003	-0.1092	-0.6210	-0.7958	-0.6487	-0.6967
Stomata Size	[b]0.0301*		0.5001	0.0650	0.6038	0.6320	0.5134
Stomata Density	0.7226	0.0818		-0.1267	-0.1990	0.0780	-0.0809
Leaf Surface Area	0.0311*	0.8408	0.6955		0.3148	0.2395	0.4750
Leaf Surface Roughness	0.0011*	0.0289*	0.5146	0.3189		0.7040	0.6744
Total Cuticle Wax	0.0225*	0.0275*	0.8097	0.4534	0.0106*		0.9405
Primary Alcohol Content	0.0118*	0.0878	0.8026	0.1186	0.0162*	<0.0001*	

[a] Correlation coefficients for plant leaf characteristics for adaxial leaf side of cool-season plants
[b] Correlation probability for plant leaf characteristics for adaxial leaf side of cool-season plants

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Correlation Analysis
Warm-Season Adaxial

Parameter	Contact Angle	Stomata Size	Stomata Density	Leaf Surface Area	Leaf Surface Roughness	Total Cuticle Wax	Primary Alcohol Content
Contact Angle		[a]-0.1830	-0.0464	-0.3210	0.6696	0.7309	0.6976
Stomata Size	[b]0.5902		-0.3143	-0.5035	-0.1463	0.1693	-0.0638
Stomata Density	0.8923	0.3466		0.5304	-0.3779	-0.5542	-0.5417
Leaf Surface Area	0.3358	0.1144	0.0932		-0.6228	-0.4516	-0.1842
Leaf Surface Roughness	0.0242*	0.6678	0.2518	0.0407*		0.4693	0.4268
Total Cuticle Wax	0.0106*	0.6186	0.0769	0.1632	0.1453		0.9297
Primary Alcohol Content	0.0170*	0.8522	0.0852	0.5876	0.1905	<0.0001*	

[a] Correlation coefficients for plant leaf characteristics for adaxial leaf side of warm-season plants
[b] Correlation probability for plant leaf characteristics for adaxial leaf side of warm-season plants

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Conclusions

- Leaf surface roughness
  - Variable for species and leaf side
  - Correlated with contact angle
- Cuticle Analysis
  - Variable for species
  - Cool-season vs. warm-season plants
  - Primary alcohol related to cuticle morphology
  - TCW and PA correlate with hydrophobicity
- Stomata analysis
  - Stomata density: Adaxial > Abaxial
    : Warm-season > Cool-Season
  - Stomata size negative relationship with stomata density

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Question: Do the plant leaf surface characteristics influence actual foliar N absorption?

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Part III: Microscopic Investigation of Solution Uptake and ^{15}N Absorption

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Part III: Objective

- The objectives were to investigate urea fertilizer solution uptake using confocal microscopy and to determine ^{15}N absorption on various warm- and cool-season plant species.

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Part III: Materials and Methods

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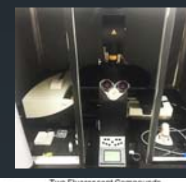
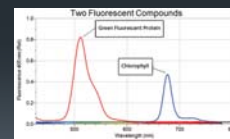
Plant Leaf Solution Uptake

- Solution Uptake
  - FITC (Fluorescein isothiocyanate) or HCCA (7-hydroxycoumarin-3-carboxylic acid) was added to the solution (2mg/ml)
  - Harvested 24h after application
  - Leaves fixed with 50/50 (v/v) 1X PBS/glycerol
  - Leica SP8X-MP (63X oil immersion objective, 2X zoom)





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^{15}N Solution Absorption and Stomata Uptake

- ^{15}N-labeled urea solution
  - 0.98 g N m^{-2}
  - 2.67 % atom ^{15}N in fertilizer
  - Applied with spray chamber
  - 280 L ha^{-1} carrier volume

- Stomata Involvement
  - ^{15}N-labeled urea solution
  - applied with brush
  - adaxial vs. abaxial leaf sides

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Part III: Results

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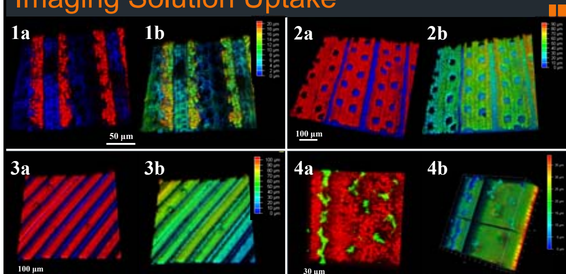
Imaging Solution Uptake



- Examples confocal microscopy images demonstrating foliar applied solution deposition and penetration on four grass species 1-4) Celebration, common bermudagrass, Abbey, Kentucky bluegrass, Palisades, zoysiagrass, Palmetto, St. Augustinegrass, respectively a-b) Overlay of red and blue channels and depth coding imaging, respectively.

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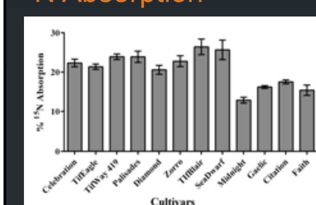
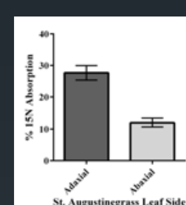
^{15}N Absorption



- Percent ^{15}N-labeled urea absorption 24 h after foliar application by 12 turfgrass cultivars
- Mean ^{15}N Absorption: 20.7 %
- Warm-Season: 23.3%
- Cool-Season: 15.8%



Percent ^{15}N-labeled urea absorption 24 h after application to the adaxial or abaxial leaf side of St. Augustinegrass

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^{15}N Absorption Correlation

Parameter	% ^{15}N Absorption	Leaf Surface Roughness	Total Cuticle Wax	Primary Alcohol Content
% ^{15}N Absorption		[a]-0.6886	-0.7716	-0.7457
Leaf Surface Roughness	[b]0.0191*		0.8308	0.8116
Total Cuticle Wax	0.0033*	0.0008*		0.9864
Primary Alcohol Content	0.0054*	0.0013*	<0.0001*	

[a] Correlation Coefficients for % ^{15}N Urea Absorption and Adaxial Plant Leaf Characteristics
[b] Correlation Probability for % ^{15}N Urea Absorption and Adaxial Plant Leaf Characteristics

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^{15}N Absorption Multiple Regression Model

Summary of Fit

Model	r	R^2	Adj. R-squared	RMSE
1	0.9888	0.9779	0.9229	1.1736

ANOVA

Source	df	Sum of Squares	Mean Squares	F Ratio	P-value
Model	7	174.51	24.93	18.10	0.0185*
Error	3	4.13	1.38		
Total	10	178.64			

Coefficients

Model	Unstandardized Coefficients		Standardized Coefficients		
	B	Standard Error	Beta	t Ratio	P-Value
Intercept	54.024	4.885		11.06	0.0016*
SS	-0.326	0.088	-0.693	-3.70	0.0344*
S_R	-8.548	1.524	-1.558	-5.61	0.0112*
TCW	-0.179	0.059	-0.772	-2.98	0.0586
(SS-22.89)*(S_R-2.59)	1.700	0.424	2.197	4.00	0.0279*
(SS-2.89)*(TCW-24.16)	-0.034	0.009	-1.203	-3.94	0.0291*
(S_R-2.59)*(TCW-24.16)	-0.401	0.098	-1.498	-4.10	0.0263*
(SS-22.89)*(S_R-2.59)*(TCW-24.16)	0.089	0.0147	2.745	6.05	0.0090*

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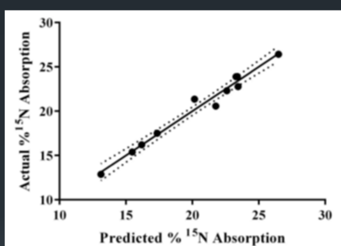
Regression Model Accuracy



- Regression analysis for actual % ^{15}N absorption by predicted % ^{15}N absorption.
- R^2=0.9769, p = <0.0001

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Conclusions

- Solution Uptake
  - Collected at and around stomata complexes and valleys
  - Penetration maybe be occurring at stomata, not conclusive
- ^{15}N Absorption
  - Variation between species
  - Warm- season greater uptake than cool-season grasses
  - Stomata density increased foliar uptake for St. Augustinegrass
  - Correlated with surface roughness, total cuticle wax and primary alcohol
- Multiple Regression Analysis
  - Stomata size, leaf surface roughness and total cuticle
  - As predictors increase, ^{15}N absorption decreases
    - Complex process

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Summary and Perspectives

- Part I:
  - Solution chemistry and concentration affect hydrophobicity
  - Determine solution and plant species interaction
- Part II:
  - Plant leaf surface characteristics influence hydrophobicity (S_R, TCW, PA)
  - Stomata density greater for adaxial leaf side and warm-season plants

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Summary and Perspectives

- Part III:
  - Plant leaf surface characteristics = good predictors of foliar ^{15}N absorption
  - Stomata, leaf surface roughness and total cuticle wax
- Future Research:
  - Expand species (broad leaf) and solution uptake
  - Application timing
  - Identify second chance uptake

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Funding Sources

USDA

USGA

The Cliffs

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Thank You

Questions?

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